The Ministry of Health, Labour and Welfare Ministerial Notification No. 65

Pursuant to Paragraph 1, Article 41 of the Pharmaceutical Affairs Law (Law No. 145, 1960), the Japanese Pharmacopoeia (hereinafter referred to as “new Pharmacopoeia”), which has been established as follows*, shall be applied on April 1, 2011. However, in the case of drugs which are listed in the Pharmacopoeia (hereinafter referred to as “previous Pharmacopoeia”) [limited to those listed in the Japanese Pharmacopoeia whose standards are changed in accordance with this notification (hereinafter referred to as “new Pharmacopoeia’’)] and drugs which have been approved as of April 1, 2011 as prescribed under Paragraph 1, Article 14 of the same law [including drugs the Minister of Health, Labour and Welfare specifies (the Ministry of Health and Welfare Ministerial Notification No. 104, 1994) as those exempted from marketing approval pursuant to Paragraph 1, Article 14 of the Pharmaceutical Affairs Law (hereinafter referred to as “drugs exempted from approval’’)], the Name and Standards established in the previous Pharmacopoeia (limited to part of the Name and Standards for the drugs concerned) may be accepted to conform to the Name and Standards established in the new Pharmacopoeia before and on September 30, 2012. In the case of drugs which are listed in the new Pharmacopoeia (excluding those listed in the previous Pharmacopoeia) and drugs which have been approved as of April 1, 2011 as prescribed under Paragraph 1, Article 14 of the same law (including those exempted from approval), they may be accepted as those being not listed in the new Pharmacopoeia before and on September 30, 2012.

Ritsuo Hosokawa
The Minister of Health, Labour and Welfare

March 24, 2011

(The text referred to by the term “as follows” are omitted here. All of them are made available for public exhibition at the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, at each Regional Bureau of Health and Welfare, and at each Prefectural Office in Japan).

*The term “as follows” here indicates the contents of the Japanese Pharmacopoeia Sixteenth Edition from General Notices to Ultraviolet-visible Reference Spectra (pp. 1 - 2131).
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In July 2006, the Committee on JP established the basic principles for the preparation of the JP 16th Edition, setting out the roles and characteristics of the JP, the definite measures for the revision, and the date of the revision.

At the Committee, the five basic principles of JP, which we refer to as the “five pillars”, were established as follows: 1) Including all drugs which are important from the viewpoint of health care and medical treatment; 2) Making qualitative improvement by introducing the latest science and technology; 3) Promoting internationalization; 4) Making prompt partial revision as necessary and facilitating smooth administrative operation; and 5) Ensuring transparency regarding the revision, and disseminating the JP to the public. It was agreed that the Committee on JP should make efforts, on the basis of these principles, to ensure that the JP is used more effectively in the fields of health care and medical treatment by taking appropriate measurements, including getting the understanding and cooperation of other parties concerned.

It was agreed that the JP should provide an official standard, being required to assure the quality of medicines in Japan in response to the progress of science and technology and medical demands at the time. It should define the standards for specifications, as well as the methods of testing to assure overall quality of all drugs in principle, and it should have a role in clarifying the criteria for quality assurance of drugs that are recognized to be essential for public health and medical treatment.

The JP has been prepared with the aid of the knowledge and experience of many professionals in the pharmaceutical field. Therefore, the JP should have the characteristics of an official standard, which might be widely used by all parties concerned. It should provide information and understanding about the quality of drugs to the public, and it should be conducive to smooth and effective regulatory control of the quality of drugs, as well as promoting and maintaining international consistency and harmonization of technical requirements.

It was also agreed that JP articles should cover drugs, which are important from the viewpoint of health care and medical treatment, clinical results and frequency of use, as soon as possible after they reach the market.

The target date for the publication of JP 16th Edition (the Japanese edition) was set as April 2011.

JP Expert Committees are organized with the following panels: Panel on the Principles of Revisions; Sub-committee on the Principles of Revisions; Panel on Medicinal Chemicals; Panel on Antibiotics; Panel on Biologicals; Panel on Crude Drugs; Panel on Pharmaceutical Excipients; Panel on Physico-Chemical Methods; Panel on Preparations; Panel on Physical Methods; Panel on Biological Tests; Panel on Nomenclature; Panel on International Harmonization; Panel on Pharmaceutical Water; and Panel on Reference Standards. Furthermore, working groups are established under the Panel on Physico-Chemical Methods, Panel on Preparations and Panel on Biological Tests to expedite discussion on revision drafts.

In the Committee on JP, Takao Hayakawa took the role of chairman from July 2003 to December 2010, and Mitsuru Hashida from January 2011 to March 2011.

In addition to the regular revision every five years in line with the basic principles for the preparation of the JP it was agreed that partial revision should be done as necessary to take account of recent progress of science and in the interests of international harmonization.

In accordance with the above principles, the panels initiated deliberations on selection of articles, and on revisions for General Notices, General Rules for Crude Drugs, General Rules for Preparations, General Tests, Monographs and so on.

Draft revisions covering subjects in General Notices, General Rules for Crude Drugs, General Rules for Preparations, General Tests and Monographs, for which discussions were finished between September 2005 and March 2007, were prepared for a supplement to the JP 15. They were examined by the Committee on JP in April 2007, followed by the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in June 2007, and then submitted to the Minister of Health, Labour and Welfare. The supplement was named “Supplement I to the JP 15th Edition”, promulgated on September 28, 2007 by Ministerial Notification No. 316 of MHLW, and became effective on October 1,
2007.

Numbers of discussions in the panels to prepare the supplement drafts were as follows: Panel on Principles of Revisions (7); Sub-panel on Principles of Revision (6), Panel on Medicinal Chemicals (33, including the working group); Panel on Antibiotics (9); Panel on Biologicals (8); Panel on Crude Drugs (17); Panel on Pharmaceutical Excipients (7); Panel on Physico-Chemical Methods (12); Panel on Preparations (10); Panel on Physico-Chemical Methods (8); Panel on Biological Tests (7); Panel on Nomenclature (9); Panel on International Harmonization (2); and Panel on Pharmaceutical Water (7).

It should be noted that in the preparation of the drafts for the supplement, generous cooperation was given by the Technical Committee of the Pharmaceutical Manufacturer’s Association of Osaka and of Tokyo, the Tokyo Crude Drugs Association, the Japan Pharmaceutical Excipients Council, the Japan Kampo Medicine Manufacturers’ Association, the Japan Flavor and Fragrance Materials Association, the Japan Medical Plants Federation, the Japan Pharmaceutical Manufacturers Association, and the Japan Oilseeds Processors Association.

In consequence of this revision, the JP 15th Edition carries 1567 articles, owing to the addition of 90 articles and the deletion of 6 articles.

Draft revisions covering subjects, the addition of specification “Diethylene glycol and related substances” to the Purity of both monographs Glycerin and Concentrated Glycerin was examined by the Committee on JP in September 2007, followed by PAFSC in October 2007, and then submitted to the Minister of Health, Labour and Welfare.

This revision was promulgated on February 21, 2008 by Ministerial Notification No. 32 of MHLW, and became effective.

Draft revisions covering subjects, the addition of specification “Over-sulfated chondroitin sulfate” to the Purity of monograph Heparin Sodium and the resultant addition of Over-sulfated Chondroitin Sulfate Reference Standard to the list of Reference Standards in the General Tests were examined by the Committee on JP in July 2008, followed by PAFSC in October 2008, and then submitted to the Minister of Health, Labour and Welfare.

This revision was promulgated on March 31, 2009 by Ministerial Notification No. 190 of MHLW, and became effective.

Draft revisions covering subjects in General Rules for Crude Drugs, General Tests and Monographs, for which discussions were completed between April 2007 and March 2009, were prepared for a supplement to the JP 15. They were examined by the Committee on JP in April 2009, followed by PAFSC in June 2009, and then submitted to the Minister of Health, Labour and Welfare.

The supplement was named “Supplement II to JP 15th Edition” and promulgated on September 30, 2009 by Ministerial Notification No. 425 of MHLW, and became effective on October 1, 2009.

Numbers of discussions in the panels to prepare the revision drafts were as follows: Panel on Principles of Revisions (3); Panel on Medicinal Chemicals (23); Panel on Antibiotics (8); Panel on Biologicals (8); Panel on Crude Drugs (21); Panel on Pharmaceutical Excipients (10); Panel on Physico-Chemical Methods (11, including the working group); Panel on Preparations (19, including the working group); Panel on Physico-Chemical Methods (9); Panel on Biological Tests (9); Panel on Nomenclature (6); Panel on International Harmonization (3); and Panel on Pharmaceutical Water (8).

It should be noted that in the preparation of the drafts for the supplement, generous cooperation was given by the Technical Committee of the Pharmaceutical Manufacturer’s Association of Osaka and of Tokyo, the Tokyo Crude Drugs Association, the Japan Pharmaceutical Excipients Council, the Home Medicine Association of Japan, the Japan Kampo Medicine Manufacturers’ Association, the Japan Flavor and Fragrance Materials Association, the Japan Medical Plants Federation, the Japan Parental Drug Association, the Japan Reagent Association, the Japan Oilseeds Processors Association, and the Association of Membrane Separation Technology of Japan.

In consequence of this revision, the JP 15th Edition carries 1673 articles, owing to the addition of 106 articles and the deletion of 1 article.

Draft revisions covering subjects, the revision of the General Tests connected with the harmonization between the three pharmacopoeias, JP, EP and USP, the revision of the specification of monograph Longgu and the addition of a monograph Powdered Longgu were examined by the Committee on JP in December 2008, followed by PAFSC in March 2009, and then submitted to the Minister of Health, Labour and Welfare.

This revision was promulgated on March 31, 2009 by Ministerial Notification No. 190 of MHLW, and became effective.

Draft revisions covering subjects in General Rules for Crude Drugs, General Tests and Monographs, for which discussions were completed between April 2007 and March 2009, were prepared for a supplement to the JP 15. They were examined by the Committee on JP in April 2009, followed by PAFSC in June 2009, and then submitted to the Minister of Health, Labour and Welfare.

The supplement was named “Supplement II to JP 15th Edition” and promulgated on September 30, 2009 by Ministerial Notification No. 425 of MHLW, and became effective on October 1, 2009.

Numbers of discussions in the panels to prepare the revision drafts were as follows: Panel on Principles of Revisions (3); Panel on Medicinal Chemicals (23); Panel on Antibiotics (8); Panel on Biologicals (8); Panel on Crude Drugs (21); Panel on Pharmaceutical Excipients (10); Panel on Physico-Chemical Methods (11, including the working group); Panel on Preparations (19, including the working group); Panel on Physico-Chemical Methods (9); Panel on Biological Tests (9); Panel on Nomenclature (6); Panel on International Harmonization (3); and Panel on Pharmaceutical Water (8).

It should be noted that in the preparation of the drafts for the supplement, generous cooperation was given by the Technical Committee of the Pharmaceutical Manufacturer’s Association of Osaka and of Tokyo, the Tokyo Crude Drugs Association, the Japan Pharmaceutical Excipients Council, the Home Medicine Association of Japan, the Japan Kampo Medicine Manufacturers’ Association, the Japan Flavor and Fragrance Materials Association, the Japan Medical Plants Federation, the Japan Parental Drug Association, the Japan Reagent Association, the Japan Oilseeds Processors Association, and the Association of Membrane Separation Technology of Japan.

In consequence of this revision, the JP 15th Edition carries 1673 articles, owing to the addition of 106 articles and the deletion of 1 article.

Draft revisions covering subjects, the revision of the General Tests connected with the harmonization between the three pharmacopoeias, JP, EP and USP, the revision of the specification of monograph Longgu and the addition of a monograph Powdered Longgu were examined by the Committee on JP in December 2008, followed by PAFSC in March 2009, and then submitted to the Minister of Health, Labour and Welfare.

This revision was promulgated on March 31, 2009 by Ministerial Notification No. 190 of MHLW, and became effective.
revision in the Purity of two monographs Heparin Calcium and Heparin Sodium, and the several additions to the Reference Standards and the Reagents, Test Solutions were examined by the Committee on JP in August 2009, followed by PAFSC in September 2009, and then submitted to the Minister of Health, Labour and Welfare.

This revision was promulgated on July 30, 2010 by Ministerial Notification No. 322 of MHLW, and became effective.

Draft revisions covering subjects in General Notices, General Rules for Crude Drugs, General Rules for Preparations, General Tests and Monographs, for which discussions were completed between April 2009 and March 2010, were prepared for JP 16. They were examined by the Committee on JP in September 2010, followed by PAFSC in October 2010, and then submitted to the Minister of Health, Labour and Welfare.

Numbers of discussions in the panels to prepare the revision drafts were as follows: Panel on Principles of Revisions (3); Panel on Medicinal Chemicals (20); Panel on Antibiotics (5); Panel on Biologicals (2); Panel on Crude Drugs (10); Panel on Pharmaceutical Excipients (5); Panel on Physico-Chemical Methods (10, including the working group); Panel on Preparations (10, including the working group); Panel on Physico-Chemical Methods (8); Panel on Biological Tests (9, including the working group); Panel on Nomenclature (3); Panel on International Harmonization (1); and Panel on Pharmaceutical Water (4).

It should be noted that in the preparation of the drafts, generous cooperation was given by the Technical Committee of the Pharmaceutical Manufacturer’s Association of Osaka and of Tokyo, the Tokyo Crude Drugs Association, the Japan Pharmaceutical Excipients Council, the Home Medicine Association of Japan, the Japan Kampo Medicine Manufacturers’ Association, the Japan Flavor and Fragrance Materials Association, the Japan Medical Plants Federation, the Japan Parental Drug Association, the Japan Reagent Association, the Japan Oilseeds Processors Association, and the Association of Membrane Separation Technology of Japan.

In consequence of this revision, the JP 16th Edition carries 1764 articles, owing to the addition of 106 articles and the deletion of 15 articles.

The principles of description and the salient points of the revision in this volume are as follows:

1. The JP 16th Edition comprises the following items, in order: Notification of MHLW; Contents; Preface; General Notices; General Rules for Crude Drugs; General Rules for Preparations; General Tests, Processes and Apparatus; Official Monographs; then followed by Infrared Reference Spectra and Ultraviolet-visible Reference Spectra; General Information; Table of Standard Atomic Weights 2010 as an appendix; and a Cumulative Index.

2. The articles in General Rules for Preparations, Official Monographs, Infrared Reference Spectra and Ultraviolet-visible Reference Spectra are respectively placed in alphabetical order in principle.

3. The following items in each monograph are put in the order shown below, except that unnecessary items are omitted depending on the nature of the drug:

   (1) English title
   (2) Commonly used name(s)
   (3) Latin title (only for crude drugs)
   (4) Title in Japanese
   (5) Structural formula or empirical formula
   (6) Molecular formula and molecular mass
   (7) Chemical name
   (8) CAS Registry Number
   (9) Origin
   (10) Limits of the content of the ingredient(s) and/or the unit of potency
   (11) Labeling requirements
   (12) Method of preparation
   (13) Description/Description of crude drugs
   (14) Identification tests
   (15) Specific physical and/or chemical values
   (16) Purity tests
   (17) Loss on drying or Ignition, or Water
   (18) Residue on ignition, Total ash or Acid-insoluble ash
   (19) Tests being required for pharmaceutical preparations and other special tests
   (20) Isomer ratio
   (21) Assay or the content of the ingredient(s)
   (22) Containers and storage
   (23) Expiration date
   (24) Others

4. In each monograph, the following physical and chemical values representing the properties and quality of the drug are given in the order indicated below, except that unnecessary items are omitted depending on the nature of drug:

   (1) Alcohol number
   (2) Absorbance
   (3) Congealing point
   (4) Refractive index
   (5) Osmolarity
   (6) Optical rotation
   (7) Viscosity
(8) pH
(9) Specific gravity
(10) Boiling point
(11) Melting point
(12) Acid value
(13) Saponification value
(14) Ester value
(15) Hydroxyl value
(16) Iodine value

5. Identification tests comprise the following items, which are generally put in the order given below:

(1) Coloration reactions
(2) Precipitation reactions
(3) Decomposition reactions
(4) Derivatives
(5) Infrared and/or ultraviolet-visible absorption spectrometry
(6) Special reactions
(7) Cations
(8) Anions

6. Purity tests comprise the following items, which are generally put in the order given below, except that unnecessary items are omitted depending on the nature of drug:

(1) Color
(2) Odor
(3) Clarity and/or color of solution
(4) Acidity or alkalinity
(5) Acidity
(6) Alkalinity
(7) Chloride
(8) Sulfate
(9) Sulfite
(10) Nitrate
(11) Nitrite
(12) Carbonate
(13) Bromide
(14) Iodide
(15) Soluble halide
(16) Thiocyanate
(17) Selenium
(18) Cationic salts
(19) Ammonium
(20) Heavy metals
(21) Iron
(22) Manganese
(23) Chromium
(24) Bismuth
(25) Tin
(26) Aluminum
(27) Zinc
(28) Cadmium
(29) Mercury
(30) Copper
(31) Lead
(32) Silver
(33) Alkaline earth metals
(34) Arsenic
(35) Foreign matters
(36) Related substances
(37) Residual solvent
(38) Other impurities
(39) Readily carbonizable substances

7. The following paragraphs of General Notices were revised:

(1) Paragraph 3: The sentence “The distinction of the preparations name of Fine Granules and Powders follows according to the definition in the section of "Powders" of General Rules for Preparations.” was deleted according to the revision of General Rules for Preparations.

(2) Paragraph 4: Exampled name of preparations was revised in accordance with the current status of listed monographs.

(3) Paragraph 8: Atomic masses the JP refers to was revised to the table of “Standard Atomic Weights 2010”.

(4) Paragraph 9: Being often used two units, pS cm\(^{-1}\) and CFU, were added.

(5) Paragraph 16: The definition of water used to measure the number of drops of a dropping device was revised in accordance with the revision of Paragraph 20.

(6) Paragraph 20: The provision of water used for the test of drugs was revised according to the revision of monograph Purified Water.

(7) Other descriptions were improved.

8. To Paragraph 1 of General Rules for Crude Drugs the following items were added:

(1) Aluminum Silicate Hydrate with Silicon Dioxide
(2) Brown Rice
(3) Koi
(4) Sesame

9. The General Rules for Preparations was revised as follows in general:

The addition of dosage forms which had not have been prescribed, the classification of dosage forms based on the route and/or site of administration, and the definition of individual dosage forms and the tests to be applied to them.

10. The following items in General Tests, Processes and Apparatus were revised:
(1) 2.01 Liquid Chromatography
(2) 2.46 Residual Solvents Test
(3) 2.51 Conductivity Measurement
(4) 2.54 pH Determination
(5) 2.58 X-Ray Powder Diffraction Method
(6) 3.01 Determination of Bulk and Tapped Densities
(7) 4.01 Bacterial Endotoxins Test
(8) 4.05 Microbial Limit Test
(9) 4.06 Sterility Test
(10) 5.02 Microbial Limit Test for Crude Drugs
(11) 6.03 Particle Size Distribution Test for Preparations
(12) 6.07 Insoluble Particulate Matter Test for Injections
(13) 6.08 Insoluble Particulate Matter Test for Ophthalmic Solutions
(14) 7.02 Test Methods for Plastic Containers
(15) 8.01 Sterilization and Aseptic Manipulation

11. The title of the following item in General Tests, Processes and Apparatus was revised: 8.01 Sterilization and Aseptic Manipulation

12. The following Reference Standards were added:
   - Atorvastatin Calcium
   - Alendronate Sodium
   - Glimepiride
   - Sarpogrelate Hydrochloride
   - Donepezil Hydrochloride
   - Trehalose
   - Nateglinide
   - Fexofenadine Hydrochloride
   - Fluvoxamine Maleate
   - Propiverine Hydrochloride
   - Pemirolast Potassium
   - Rabeprazole Sodium
   - Risedronic Acid

13. The following Reference Standard was revised in Japanese title:
   - Tyrosine

14. The following Reference Standards were deleted from the list of 9.01 Reference Standards:
   - Astromicin Sulfate
   - Insulin
   - Sisomicin Sulfate
   - Cefapirin Sodium
   - Cefuroxime Sodium
   - Netilmicin Sulfate

15. The intended use of each individual Reference Standard was deleted from the list of 9.01 Reference Standards.

16. Some of the names of the reagents or test solutions under 9.41 Reagents, Test Solutions were maintained.

17. To the each individual item in the General Tests, Processes and Apparatus, chapter and section numbers were putted.

18. The following substances were newly added to the Official Monographs:
   - Aciclovir Syrup
   - Aciclovir Injection
   - Aciclovir for Syrup
   - Acetylcysteine
   - Atorvastatin Calcium Hydrate
   - Atorvastatin Calcium Tablets
   - Alendronate Sodium Sulfate for Injection
   - Alendronate Sodium Tablets
   - Alendronate Sodium Tablets
   - L-Isoleucine, L-Leucine and L-Valine Granules
   - Ebastine
   - Ebastine Orally Disintegrating Tablets
   - Ebastine Tablets
   - Carvedilol
   - Carvedilol Tablets
   - Candesartan Cilexetil
   - Candesartan Cilexetil Tablets
   - Quinapril Hydrochloride
   - Quinapril Hydrochloride Tablets
   - Glimepiride
   - Glimepiride Tablets
   - L-Glutamic acid
   - Sarpogrelate Hydrochloride
   - Sarpogrelate Hydrochloride Fine Granules
   - Sarpogrelate Hydrochloride Tablets
   - Diazepam Tablets
   - Purified Water in Containers
   - Sterile Water for Injection in Containers
   - Spironolactone Tablets
   - Zolpidem Tartrate Tablets
   - Tamsulosin Hydrochloride Extended-release Tablets
   - Tamoxifen Citrate
   - Precipitated Calcium Carbonate Fine Granules
   - Precipitated Calcium Carbonate Tablets
   - Temocapril Hydrochloride
   - Temocapril Hydrochloride Tablets
   - Terbinafine Hydrochloride
   - Terbinafine Hydrochloride Cream
   - Terbinafine Hydrochloride Solution
   - Terbinafine Hydrochloride Spray
   - Doxazosin Mesilate Tablets
Donepezil Hydrochloride
Donepezil Hydrochloride Fine Granules
Donepezil Hydrochloride Tablets
Trehalose Hydrate
Nateglinide
Nateglinide Tablets
L-Lactic Acid
Sodium L-Lactate Solution
Halooperidol Fine Granules
Pioglitazone Hydrochloride Tablets
L-Histidine
L-Histidine Hydrochloride Hydrate
Famotidine Injection
Fexofenadine Hydrochloride
Butenafine Hydrochloride
Butenafine Hydrochloride Cream
Butenafine Hydrochloride Solution
Butenafine Hydrochloride Spray
Pravastatin Sodium Fine Granules
Pravastatin Sodium Solution
Pravastatin Sodium Tablets
Fluconazole
Fluvoxamine Maleate
Fluvoxamine Maleate Tablets
Flecainide Acetate
Flecainide Acetate Tablets
Propiverine Hydrochloride
Propiverine Hydrochloride Tablets
Probucol Fine Granules
Probucol Tablets
L-Proline
Betamipron
Pemirolast Potassium
Pemirolast Potassium for Syrup
Pemirolast Potassium Tablets
Beraprost Sodium
Beraprost Sodium Tablets
Mupirocin Calcium Ointment
Methotrexate Capsules
Mosapride Citrate Powder
Rabeprazole Sodium
Risperidone
Risperidone Fine Granules
Risperidone Oral Solution
Risperidone Tablets
Sodium Risedronate Hydrate
Sodium Risedronate Tablets
Roxatidine Acetate Hydrochloride Extended-release Tablets
Roxatidine Acetate Hydrochloride for Injection
Orenegedokuto Extract
Aluminum Silicate Hydrate with Silicon Dioxide
Koi
Brown Rice
Sesame
Saikokeishito Extract
Saibokuto Extract
Shakuyakukanzoto Extract
Juzentaihoto Extract
Shosaikoto Extract
Shoisyryuto Extract
Muko-Daikenchuto Extract
Chutosan Extract
Bakumondoto Extract
Rikkunshito Extract

19. The following monographs were revised:
Zinc Oxide Ointment
Ajmaline Tablets
Ascorbic Acid Powder
Ascorbic Acid Injection
Aspirin Tablets
Acetylcholine Chloride for Injection
Azelaic Acid Hydrochloride Granules
Adrenalin
Adrenalin Solution
Opium Tincture
Opium Alkaloids Hydrochloride Injection
Opium Alkaloids and Atropine Injection
Opium Alkaloids and Scopolamine Injection
Weak Opium Alkaloids and Scopolamine Injection
Meglumine Sodium Amidotrizoate Injection
Amitriptyline Hydrochloride Tablets
Aminophylline Injection
L-Alanine
L-Arginine Hydrochloride Injection
Allopurinol
Sulfur and Camphor Lotion
Sodium Iotalamate Injection
Meglumine Iotalamate Injection
Isoniazid Injection
Isoniazid Tablets
Idoxuridine Ophthalmic Solution
Imipramine Hydrochloride
Irsgladine Maleate Fine Granules
Indigocarmine Injection
Insulin Human (Genetical Recombination)
Indometacin Capsules
Indometacin Suppositories
Ursodeoxycholic Acid Granules
Ecabet Sodium Granules
Estradiol Benzoate Injection
Estradiol Benzoate Injection (Aqueous Suspension)
Estriol Tablets
Estriol Injection (Aqueous Suspension)
Etacrynic Acid Tablets
Ethanol for Disinfection
Etizolam Fine Granules
Ethinyleradiol
Ethinyleradiol Tablets
Etilefrine Hydrochloride Tablets
Edrophonium Chloride Injection
Ephedrine Hydrochloride Injection
Ephedrine Hydrochloride Tablets
10% Ephedrine Hydrochloride Powder
Ergometrine Maleate Hydrochloride Injection
Ergometrine Maleate Hydrochloride Tablets
10% Sodium Chloride Injection
Hydrochloric Acid Lemonade
Compound Oxycodone Injection
Compound Oxycodone and Atropine Injection
Fructose Injection
Potash Soap
Carmellose
Carmellose Calcium
Carmellose Sodium
Xylitol Injection
Diagnostic Sodium Citrate Solution
Sodium Citrate Injection for Transfusion
Glycerin and Potash Solution
Absorptive Cream
Hydrophilic Cream
Clindamycin Hydrochloride
Cresol Solution
Saponated Cresol Solution
Clobifbrate Capsules
Clomifene Citrate Tablets
Chlordiazepoxide Powder
Chlordiazepoxide Tablets
Chlorpheniramine Maleate Powder
Chlorpheniramine Maleate Tablets
Chlorpropamide Tablets
Chlorpromazine Hydrochloride Injection
Ketoconazole Solution
Ketoconazole Cream
10% Codeine Phosphate Powder
Codeine Phosphate Tablets
Compound Salicylic Acid Spirit
Oxygen
Diazepam
Digitoxin Tablets
Diclofenamide Tablets
Distigmine Bromide Tablets
Dydrogesterone Tablets
Zinostatin Stimalamer
1% Dihydrocodeine Phosphate Powder
Dimenhydrinate Tablets
Silver Nitrate Ophthalmic Solution
Water
Purified Water
Sterile Purified Water in Containers
Water for Injection
Dried Aluminum Hydroxide Gel Fine Granules
Suxamethonium Chloride Injection
Suxamethonium Chloride for Injection
Spironolactone
Sulpyrine Injection
Sulfobromophthalein Sodium Injection
Isotonic Sodium Chloride Solution
Cefaclor
Cefaclor Compound Granules
Cefaclor Fine Granules
Cefazolin Sodium Hydrate
Cefatrizine Propylene Glycolate for Syrup
Cefalexin
Cefalexin for Syrup
Cefalotin Sodium
Cefixime Hydrate
Cefepime Dihydrochloride Hydrate
Cefcapene Pivoxil Hydrochloride Hydrate
Cefcapene Pivoxil Hydrochloride Fine Granules
Cefitoren Pivoxil Fine Granules
Cefdinir Fine Granules
Ceftibuten Hydrate
Cefteram Pivoxil Fine Granules
Cefpodoxime Proxetil
Cefroxadine for Syrup
Sevoflurane
D-Sorbitol Solution
Tacrolimus Hydrate
Talc
Sodium Bicarbonate Injection
Simple Syrup
Thiamazole Tablets
Thiamine Chloride Hydrochloride Powder
Thiamine Chloride Hydrochloride Injection
Thiopental Sodium for Injection
Sodium Thiosulfate Injection
Nitrogen
Tipepidine Hibenzate Tablets
L-Tyrosine
Testosterone Enanthate Injection
Deslanoside Injection
Dehydrocholic Acid Injection
Dopamine Hydrochloride Injection
Triclofos Sodium Syrup
Trihexyphenidyl Hydrochloride Tablets
Trimethadione Tablets
Tolnaftate Solution
Tolbutamide Tablets
Droxidopa Fine Granules
Troxipide Fine Granules
Rape Seed Oil
Naphazoline and Chlorpheniramine Solution
Nicardipine Hydrochloride Injection
Nicotinic Acid Injection
Nicomol Tablets
Carbone Dioxide
Nicergoline Powder
Nitroglycerin Tablets
Neostigmine Methylsulfate Injection
Noradrenaline Injection
Norgestrel and Ethinylestradiol Tablets
Baclofen Tablets
Papaverine Hydrochloride Injection
Calcium Paraaminosalicylate Granules
Busacodyl Suppositories
Hydralazine Hydrochloride Powder
Hydralazine Hydrochloride Tablets
Hydralazine Hydrochloride for Injection
Piperazine Phosphate Tablets
Famotidine Powder
Faropenem Sodium for Syrup
Phenytoin Powder
Phenytoin Tablets
Phenobarbital
10% Phenobarbital Powder
Liquefied Phenol
Phenolated Water
Phenolated Water for Disinfection
Phenol and Zinc Oxide Liniment
Phenolsulfonphthalein Injection
Glucose Injection
Prazepam Tablets
Flurazepam Capsules
Prednisolone Tablets
Prednisolone Sodium Succinate for Injection
Procaine Hydrochloride Injection
Prochlorperazine Maleate Tablets
Propylthiouracil Tablets
Flopropione Capsules
Probenecid
Probenecid Tablets
Flomoxef Sodium
Betamethasone Valerate and Gentamicin Sulfate Cream
Betamethasone Sodium Phosphate
Pethidine Hydrochloride Injection
Perphenazine Tablets
Perphenazine Maleate Tablets
Benzalkonium Chloride Solution
Benyl Alcohol
Benzethonium Chloride Solution
Formalin Water
Mercurochrome Solution
D-Mannitol Injection
Yellow Beeswax
Minocycline Hydrochloride
Minocycline Hydrochloride Tablets
Minocycline Hydrochloride for Injection
Alum Solution
10% dl-Methylephedrine Hydrochloride Powder
Methyltestosterone Tablets
Metyldopa Tablets
Metenolone Enanthate Injection
Metronidazole Tablets
Mepivacaine Hydrochloride
Mefruside Tablets
Meropenem Hydrate
Morphine Hydrochloride Tablets
Morphine Hydrochloride Injection
Morphine and Atropine Injection
Folic Acid Tablets
Folic Acid Injection
Meglumine Sodium Iodamide Injection
Iodine Tincture
Dilute Iodine Tincture
Dental Iodine Glycerin
Compound Iodine Glycerin
Iodine, Salicylic Acid and Phenol Spirit
Latomoxef Sodium
Lanatoside C Tablets
Liothyronine Sodium Tablets
L-Lysine Hydrochloride
L-Lysine Acetate
Lidocaine Injection
Rifampicin Capsules
Riboflavin Powder
Riboflavin Sodium Phosphate Injection
Zinc Sulfate Ophthalmic Solution
Magnesium Sulfate Mixture
Magnesium Sulfate Injection
Ringer’s Solution
Dibasic Sodium Phosphate Hydrate
Reserpine Tablets
Reserpine Injection
Levallorphan Tartrate Injection
Levothyroxine Sodium Tablets
Sweet Hydrangea Leaf
Aloe
Powdered Aloe
Foeniculated Ammonia Spirit
Epimedium Herb
Fennel Oil
Turmeric
Powdered Turmeric
Bearberry Leaf
Uva Ursi Fluidextract
Corydalis Tuber
Powdered Corydalis Tuber
Polygala Root
Powdered Polygala Root
Pueraria Root
Kamishoyosan Extract
Glycyrrhiza Extract
Crude Glycyrrhiza Extract
Agar
Platycodeon Fluidextract
Catalpa Fruit
Apricot Kernel
Apricot Kernel Water
Bitter Tincture
Keishibukuryogan Extract
Safflower
Red Ginseng
Magnolia Bark
Powdered Magnolia Bark
Goshajinkigan Extract
Euodia Fruit
Condurango
Condurango Fluidextract
Bupleurum Root
Saireito Extract
Saffron
Gardenia Fruit
Powdered Gardenia Fruit
Cornus Fruit
Jujube Seed
Eleutherococcus Senticosus Rhizome
Sodium Bicarbonate and Bitter Tincture Mixture
Cimicifuga Rhizome
Magnolia Flower
Shimbuto Extract
Senega Syrup
Toad Venom
Atractylodes Lancea Rhizome
Perilla Herb
Alisma Rhizome
Powdered Alisma Rhizome
Uncaria Hook
Powdered Polyporus Sclerotium
Citrus Unshiu Peel
Capsicum
Powdered Capsicum
Capsicum Tincture
Peach Kernel
Powdered Peach Kernel
Orange Peel Syrup
Orange Peel Tincture
Ipecac
Powdered Ipecac

Ipecac Syrup
Eucommia Bark
Ginseng
Powdered Ginseng
Hachimijogion Extract
Honey
Mentha Water
Glehnia Root and Rhizome
Hangekobokuto Extract
Angelica Dahurica Root
Atractylodes Rhizome
Poria Sclerotium
Belladonna Extract
Sinomenium Stem and Rhizome
Saposhnikovia Root and Rhizome
Moutan Bark
Powdered Moutan Bark
Hochuekkito Extract
Nux Vomica Extract
Nux Vomica Extract Powder
Nux Vomica Tincture
Oyster Shell
Powdered Oyster Shell
Ryokejutsukanto Extract
Scopolia Extract
Scopolia Extract Powder
Royal Jelly

20. The following monographs were deleted:
Astromicin Sulfate
Isophane Insulin Injection (Aqueous Suspension)
Insulin
Insulin Injection
Insulin Zinc Injection (Aqueous Suspension)
Insulin Zinc Protamine Injection (Aqueous Suspension)
Crystalline Insulin Zinc Injection (Aqueous Suspension)
Amorphous Insulin Zinc Injection (Aqueous Suspension)
Sisomicin Sulfate
Cefapirin Sodium
Cefuroxime Sodium
Netilmicin Sulfate
Bufexamac
Bufexamac Cream
Bufexamac Ointment

21. The following monographs were changed in Japanese title:
Absorptive Cream
Hydrophilic Cream
Sterile Purified Water in Containers
Cefixime Hydrate
L-Tyrosine
L-Lysine Hydrochloride
L-Lysine Acetate

22. The following monographs were revised in the content limit more precise:
Ajmaline Tablets
Ascorbic Acid Powder
Ascorbic Acid Injection
Aspirin Tablets
Acetylcholine Chloride for Injection
Meglumine Sodium Amidotrizoate Injection
Amitriptyline Hydrochloride Tablets
Aminophylline Injection
Sodium Iotalamate Injection
Meglumine Iotalamate Injection
Amitrazine Tablets
Isoniazid Tablets
Isoniazid Injection
Idoxuridine Ophthalmic Solution
Imipramine Hydrochloride
Indigocarmine Injection
Indomethacin Capsules
Indomethacin Suppositories
Estradiol Benzoate Injection
Estradiol Benzoate Injection (Aqueous Suspension)
Estradiol Tablets
Estradiol Injection (Aqueous Suspension)
Etacrynic Acid Tablets
Ethylestradiol Tablets
Edrophonium Chloride Injection
Ephedrine Hydrochloride Tablets
Ephedrine Hydrochloride Injection
Ergometrine Maleate Tablets
Ergometrine Maleate Injection
Fructose Injection
Xylitol Injection
Clofibrate Capsules Extract
Clomifene Citrate Tablets
Chlordiazepoxide Powder
Chlordiazepoxide Tablets
Chlorpropamide Tablets
Chlorpromazine Hydrochloride Injection
Codeine Phosphate Tablets
Digitoxin Tablets
Diclofenamide Tablets
Distigmine Bromide Tablets
Dimenhydrinate Tablets
Dydrogesterone Tablets
Suxamethonium Chloride Injection
Suxamethonium Chloride for Injection
Sulpyrine Injection
Sulphotolomphthalein Sodium Injection
α-Sorbitol Solution
Sodium Bicarbonate Injection
Thiamazone Tablets
Thiamine Chloride Hydrochloride Powder
Thiamine Chloride Hydrochloride Injection
Thiopental Sodium for Injection
Sodium Thiosulfate Injection
Tipepide Hifenate Tablets
Testosterone Enanthate Injection
Deslanoside Injection
Dehydrocholic Acid Injection
Dopamine Hydrochloride Injection
Triclofos Sodium Syrup
Trihexyphenidyl Hydrochloride Tablets
Trimethadione Tablets
Tolnaftate Solution
Tolbutamide Tablets
Nicardipine Hydrochloride Injection
Nicotinic Acid Injection
Nicomol Tablets
Nitroglycerin Tablets
Neostigmine Methylsulfate Injection
Noradrenaline Injection
Norgestrel and Ethinylestradiol Tablets
Baclofen Tablets
Papaverine Hydrochloride Injection
Bisacodyl Suppositories
Hydralazine Hydrochloride Powder
Hydralazine Hydrochloride Tablets
Hydralazine Hydrochloride for Injection
Piperazine Phosphate Tablets
Glucose Injection
Prazepam Tablets
Flurazepam Capsules
Prednisolone Tablets
Prednisolone Sodium Succinate for Injection
Procaine Hydrochloride Injection
Prochlorperazine Maleate Tablets
Propylthiouracil Tablets
Probenecid Tablets
Pethidine Hydrochloride Injection
Perphenazine Tablets
Perphenazine Maleate Tablets
Benzalkonium Chloride Solution
Benztethonium Chloride Solution
α-Mannitol Injection
Methylclopamide Tablets
Menofenone Enantame Injection
Mefruside Tablets
Morphine Hydrochloride Tablets
Morphine Hydrochloride Injection
Folic Acid Tablets
Folic Acid Injection
Lanatoside C Tablets
Liothyronine Sodium Tablets  
Lidocaine Injection  
Riboflavin Powder  
Riboflavin Sodium Phosphate Injection  
Magnesium Sulfate Injection  
Reserpine Tablets  
Reserpine Injection  
Levallorphan Tartrate Injection  
Levothyroxine Sodium Tablets  

23. The monographs which ‘Method of preparation’ was revised according to the revision of General Rules for Preparations were as follows:

Ascorbic Acid Powder  
Irsogladine Maleate Fine Granules  
Etizolam Fine Granules  
10% Ephedrine Phosphate Powder  
Chlordiazepoxide Powder  
Chlorpheniramine Maleate Powder  
Ketoconazole Solution  
Ketoconazole Cream  
1% Codeine Phosphate Powder  
1% Dihydrocodeine Phosphate Powder  
Dried Aluminum Hydroxide Gel Fine Granules  
Cefaclor Fine Granules  
Ceftriazine Propylene Glycolate for Syrup  
Cefalexin for Syrup  
Cefcapene Pivoxil Hydrochloride Fine Granules  
Cefditoren Pivoxil Fine Granules  
Cefdinir Fine Granules  
Ceferam Pivoxil Fine Granules  
Cefroxadine for Syrup  
Droxicidopa Fine Granules  
Troxipide Fine Granules  
Nicergoline Powder  
Hydralazine Hydrochloride Powder  
Famotidine Powder  
Faropenem Sodium for Syrup  
Phenytoin Powder  
10% Phenobarbital Powder  
Betamethazone Valerate and Gentamicin Sulfate Cream  
10% dl-Methylephedrine Hydrochloride Powder  
Riboflavin Powder  

24. The monographs which ‘Particle size’ was deleted according to the revision of General Rules for Preparations were as follows:

Azelastine Hydrochloride Granules  
Ursodeoxycholic Acid Granules  
Ecabet Sodium Granules  
Chlorpheniramine Maleate Powder  
Cefaclor Compound Granules  
Nicergoline Powder  
Calcium Paraaminosalicylate Granules  
10% Phenobarbital Powder  

25. The monographs which ‘Method of preparation’ was revised according to the revision of the monograph ‘Water’ were as follows:

Adrenaline Solution  
Opium Tincture  
Opium Alkaloids Hydrochloride Injection  
Opium Alkaloids and Atropine Injection  
Opium Alkaloids and Scopolamine Injection  
Weak Opium Alkaloids and Scopolamine Injection  
Meglumine Sodium Amidotrizoate Injection  
L-Arginine Hydrochloride Injection  
Sulfur and Camphor Lotion  
Sodium Iotalamate Injection  
Meglumine Iotalamate Injection  
Ethanol for Disinfection  
10% Sodium Chloride Injection  
Hydrochloric Acid Lemonade  
Compound Oxycodone Injection  
Compound Oxycodone and Atropine Injection  
Potash Soap  
Diagnostic Sodium Citrate Solution  
Sodium Citrate Injection for Transfusion  
Glycerin and Potash Solution  
Absorptive Cream  
Hydropilic Cream  
Cresol Solution  
Saponated Cresol Solution  
Compound Salicylic Acid Spirit  
Silver Nitrate Opthalmic Solution  
Isotonic Sodium Chloride Solution  
Simple Syrup  
Deslanoside Injection  
Naphazoline and Chlorpheniramine Solution  
Phenolated Water for Disinfection  
Phenolated Water  
Phenol and Zinc Oxide Liniment  
Phenolsulfonphthalein Injection  
Benzalkonium Chloride Solution  
Benzethonium Chloride Solution  
Formalin Water  
Mercurochrome Solution  
Alum Solution  
Morphine and Atropine Injection  
Meglumine Sodium Iodamide Injection  
Iodine Tincture  
Dilute Iodine Tincture  
Dental Iodine Glycerin  
Compound Iodine Glycerin  
Iodine, Salicylic Acid and Phenol Spirit  
Zinc Sulfate Ophthalmic Solution
Magnesium Sulfate Mixture
Ringer's Solution
Foeniculated Ammonia Spirit
Uva Ursi Fluidextract
Glycyrrhiza Extract
Crude Glycyrrhiza Extract
Platycodon Fluidextract
Apricot Kernel Water
Bitter Tincture
Condurango Fluidextract
Sodium Bicarbonate and Bitter Tincture Mixture
Senega Syrup
Orange Peel Syrup
Orange Peel Tincture
Ipecac Syrup
Mentha Water
Belladonna Extract
Nux Vomica Extract
Nux Vomica Extract Powder
Nux Vomica Tincture
Scopolia Extract
Scopolia Extract Powder

26. Monographs in which the test “Content determination” was changed to “Assay” were as follows:
Aloe
Powdered Aloe
Turmeric
Powdered Turmeric
Uva Ursi
Uva Ursi Fluidextract
Corydalis Tuber
Powdered Corydalis Tuber
Apricot Kernel
Magnolia Bark
Powdered Magnolia Bark
Bupleurum Root
Gardenia Fruit
Powdered Gardenia Fruit
Cornus Fruit
Toad Venom
Perilla Herb
Uncaria Hook
Capsicum
Powdered Capsicum
Capsicum Tincture
Peach Kernel
Powdered Peach Kernel
Ipecac
Powdered Ipecac
Ipecac Syrup
Moutan Bark
Powdered Moutan Bark

Royal Jelly
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Yoshikazu Tashiro
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Kazuichi Umemoto
Masaaki Wada
Eiji Watanabe
Haruo Watanabe
Takehiko Yajima
Toshiyasu Yamada
Teruhide Yamaguchi
Tetsuji Yamaguchi
Keiichi Yamamoto
Keiji Yamamoto
Tosuke Yamamoto
Chikamasa Yamashita
Takeshi Yamazaki
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Shiho Yasuo
Hikaru Yoden
Hitoo Yoshida
Kumi Yoshida
Sumie Yoshioka
Chikako Yomota
Etsuo Yonemochi

*: Chairman, the Committee on JP
**: Acting Chairman, the Committee on JP
1. The official name of this pharmacopoeia is 第十六改正日本薬局方, and may be abbreviated as 日局十六, 日局16, JP XVI or JP 16.
2. The English name of this pharmacopoeia is The Japanese Pharmacopoeia, Sixteenth Edition.
3. Among drugs, the Japanese Pharmacopoeia Drugs (the JP Drugs) are those specified in the monographs. The title names and the commonly used names adopted in the monographs should be used as official names. In the drug monographs, in addition to English name, chemical names or Latin names can be mentioned in the titles, as appropriate.
4. “Crude Drugs and related drugs” are placed together in the posterior part of the Official Monographs. These include: Crude Drugs being applied the requirements of the General Rules for Crude Drugs, or Powders, Extracts, Tinctures, Syrups, Spirits, Fluidextracts or Suppositories containing Crude Drugs as the active ingredient, combination preparations containing Crude Drugs as the principal active ingredient.
5. Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. However, the items of “Description” and “Storage” under Containers and storage in the monographs on preparations are given for information, and should not be taken as indicating standards for conformity.
6. In principle, unless otherwise specified, animals used for preparing the JP Drugs or their source materials must be healthy.
7. In this English version, the JP Drugs described in the monographs begin with a capital letter.
8. The molecular formulas or constitution formulas in parentheses ( ) after the name of drugs or chemicals designate chemically pure substances. Atomic masses adopted in the Japanese Pharmacopoeia conform to the table of “Standard Atomic Weights 2010”. Molecular masses are indicated to two decimal places rounded from three decimals.
9. The following abbreviations are used for the principal units.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>meter</td>
<td>m</td>
</tr>
<tr>
<td>centimeter</td>
<td>cm</td>
</tr>
<tr>
<td>millimeter</td>
<td>mm</td>
</tr>
<tr>
<td>micrometer</td>
<td>μm</td>
</tr>
<tr>
<td>nanometer</td>
<td>nm</td>
</tr>
<tr>
<td>kilogram</td>
<td>kg</td>
</tr>
<tr>
<td>gram</td>
<td>g</td>
</tr>
<tr>
<td>milligram</td>
<td>mg</td>
</tr>
<tr>
<td>microgram</td>
<td>μg</td>
</tr>
<tr>
<td>nanogram</td>
<td>ng</td>
</tr>
<tr>
<td>picogram</td>
<td>pg</td>
</tr>
<tr>
<td>Celsius degree</td>
<td>°C</td>
</tr>
<tr>
<td>mole</td>
<td>mol</td>
</tr>
<tr>
<td>millimole</td>
<td>mmol</td>
</tr>
<tr>
<td>square centimeter</td>
<td>cm²</td>
</tr>
<tr>
<td>liter</td>
<td>L</td>
</tr>
<tr>
<td>milliliter</td>
<td>mL</td>
</tr>
<tr>
<td>microliter</td>
<td>μL</td>
</tr>
<tr>
<td>megahertz</td>
<td>MHz</td>
</tr>
<tr>
<td>per centimeter</td>
<td>cm⁻¹</td>
</tr>
<tr>
<td>newton</td>
<td>N</td>
</tr>
<tr>
<td>kilopascal</td>
<td>kPa</td>
</tr>
<tr>
<td>pascal</td>
<td>Pa</td>
</tr>
<tr>
<td>pascal second</td>
<td>Pa·s</td>
</tr>
<tr>
<td>millipascal second</td>
<td>mPa·s</td>
</tr>
<tr>
<td>square millimeter per second</td>
<td>mm²/s</td>
</tr>
<tr>
<td>lux</td>
<td>lx</td>
</tr>
<tr>
<td>mole per liter</td>
<td>mol/L</td>
</tr>
<tr>
<td>millimole per liter</td>
<td>mmol/L</td>
</tr>
<tr>
<td>mass per cent</td>
<td>%</td>
</tr>
<tr>
<td>mass parts per million</td>
<td>ppm</td>
</tr>
<tr>
<td>mass parts per billion</td>
<td>ppb</td>
</tr>
<tr>
<td>volume per cent</td>
<td>vol%</td>
</tr>
<tr>
<td>volume parts per million</td>
<td>vol ppm</td>
</tr>
<tr>
<td>mass per volume per cent</td>
<td>w/v%</td>
</tr>
<tr>
<td>microsimens per centimeter</td>
<td>μS·cm⁻¹</td>
</tr>
<tr>
<td>endotoxin unit</td>
<td>EU</td>
</tr>
<tr>
<td>colony forming unit</td>
<td>CFU</td>
</tr>
</tbody>
</table>

Note: “ppm” used in the Nuclear Magnetic Resonance Spectroscopy indicates the chemical shift, and “w/v%” is used in the formula or composition of preparations.
10. The unit used for expressing the potency of drug is recognized as the quantity of drug. Usually it is expressed by a definite quantity of a definite standard substance which shows a definite biological activity, and differs according to each drug. The units are determined, in principle, by comparison with each reference standard by means of biological methods. The term “Unit” used for the JP articles indicates the unit
defined in the Japanese Pharmacopoeia.

11. The statement "Being specified separately." in the monographs means that the tests are to be specified when the drugs are granted approval based on the Pharmaceutical Affairs Law.

12. When an assurance that a product is of the JP Drug quality is obtained consistently from data derived from the manufacturing process validation studies, and from the records of appropriate manufacturing process control and of the test results of the quality control, some of the test items in the monograph being performed for the release of a product may be omitted as occasion demands.

13. The test methods specified in the Japanese Pharmacopoeia can be replaced by alternative methods which give better accuracy and precision. However, where a difference in test results is suspected, only the result obtained by the procedure given in the Pharmacopoeia is effective for the final judgment.

14. The details of the biological test methods may be changed insofar as they do not affect the essential qualities of the test.

15. The temperature for the tests or storage is described, in principle, in specific figures. However, the following expressions may be used instead.

Standard temperature, ordinary temperature, room temperature, and lukewarm are defined as 20°C, 15 – 25°C, 1 – 30°C, and 30 – 40°C, respectively. A cold place, unless otherwise specified, shall be a place having a temperature of 1 – 15°C.

The temperature of cold water, lukewarm water, warm water, and hot water are defined as not exceeding 10°C, 30 – 40°C, 60 – 70°C, and about 100°C, respectively.

The term “heated solvent” or “hot solvent” means a solvent heated almost to the boiling point of the solvent, and the term “warmed solvent” or “warm solvent” usually means a solvent heated to a temperature between 60°C and 70°C. The term “heat on or in a water bath” indicates, unless otherwise specified, heating with a boiling water bath or a steam bath at about 100°C.

Cold extraction and warm extraction are usually performed at temperatures of 15 – 25°C and 35 – 45°C, respectively.

16. To measure the number of drops, a dropping device which delivers 20 drops of water weighing 0.90 – 1.10 g at 20°C shall be used.

17. The term “in vacuum” indicates, unless otherwise specified, a pressure not exceeding 2.0 kPa.

18. The acidity or alkalinity of a solution, unless otherwise specified, is determined by blue or red litmus papers. To indicate these properties more precisely, pH values are used.

19. The terms in Table 1 are used to express the degree of cutting of Crude Drugs or fineness of powder Drugs.

<table>
<thead>
<tr>
<th>Sieve No.</th>
<th>4</th>
<th>6.5</th>
<th>8.6</th>
<th>18</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal Designation of sieve</td>
<td>4750 μm</td>
<td>2800 μm</td>
<td>2000 μm</td>
<td>850 μm</td>
<td>500 μm</td>
<td>150 μm</td>
<td>75 μm</td>
</tr>
<tr>
<td>Names of the drugs which pass through the respective sieves</td>
<td>Coarse cutting</td>
<td>Moderate finely cutting</td>
<td>Fine cutting</td>
<td>Coarse powder</td>
<td>Moderate finely powder</td>
<td>Fine powder</td>
<td>Very fine powder</td>
</tr>
</tbody>
</table>

20. The water to be used in the tests of drugs shall be the water suitable for performing the relevant test, such as the water not containing any substance that would interfere with the test.

21. As for wording “solution of a solute”, where the name of the solvent is not stated, the term “solution” indicates a solution in water.

22. For solution an expression such as “(1 in 3)”, “(1 in 10)”, or “(1 in 100)” means that 1 g of a solid is dissolved in, or 1 mL of a liquid is diluted with the solvent to make the total volume of 3 mL, 10 mL or 100 mL, respectively. For the liquid mixture an expression such as “(10:1)” or “(5:3:1)” means that the respective numbers of parts, by volume, of the designated liquids are to be mixed.

23. The term “weigh accurately” means to weigh down to the degree of 0.1 mg, 0.01 mg or 0.001 mg by taking into account the purpose of the test and using a relevant weighing device. The term “weigh exactly” means to weigh to the given decimal places.

24. A value of “n” figures in a test of a JP Drug shall be obtained by rounding off a value of “n + 1” figures.

25. Unless otherwise specified, all tests of the drugs shall be performed at the ordinary temperature and observations of the results shall follow immediately after the operations. However, the judgment for a test which is affected by temperature should be based on the conditions at the standard temperature.

26. The terms “immediately”/“at once” used in the test of a JP Drug mean that the procedure is to be performed within 30 seconds after the preceding procedure.

27. In the section under the heading Description, the term “white” is used to indicate white or practically white, and “colorless” is colorless or practically colorless. Unless otherwise specified, the test of color is carried out by placing 1 g of a solid drug on a sheet of
white paper or in a watch glass placed on white paper. A liquid drug is put into a colorless test tube of 15-mm internal diameter and is observed in front of a white background through a layer of 30 mm. For the test of clarity of liquid drugs the same procedure is applied with either a black or white background. For the observation of fluorescence of a liquid drug, only a black background shall be used.

28. In the section under the heading Description, the term “odorless” is used to indicate odorless or practically odorless. Unless otherwise specified, the test of odor shall be carried out by placing 1 g of a solid drug or 1 mL of a liquid drug in a beaker.

29. In the section under the heading Description, solubilities are expressed by the terms in Table 2. Unless otherwise specified, solubility means the degree of dissolution of a JP Drug, previously powdered in the case of a solid drug, within 30 minutes in a solvent at 20 ± 5°C, by vigorous shaking for 30 seconds each time at 5-minute intervals.

Table 2

<table>
<thead>
<tr>
<th>Descriptive term</th>
<th>Volume of solvent required for dissolving 1 g or 1 mL of solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very soluble</td>
<td>Less than 1 mL</td>
</tr>
<tr>
<td>Freely soluble</td>
<td>From 1 mL to less than 10 mL</td>
</tr>
<tr>
<td>Soluble</td>
<td>From 10 mL to less than 30 mL</td>
</tr>
<tr>
<td>Sparingly soluble</td>
<td>From 30 mL to less than 100 mL</td>
</tr>
<tr>
<td>Slightly soluble</td>
<td>From 100 mL to less than 1000 mL</td>
</tr>
<tr>
<td>Very slightly soluble</td>
<td>From 1000 mL to less than 10000 mL</td>
</tr>
<tr>
<td>Practically insoluble, or insoluble</td>
<td>10000 mL and over</td>
</tr>
</tbody>
</table>

30. In the test of a drug, the term “dissolve” or “miscible” indicates that it dissolves in, or mixes in arbitrary proportion with the solvent to form a clear solution or mixture. Insoluble materials other than the drug including fibers should not be detected or practically invisible, if any.

31. Identification is the test to identify the active ingredient(s) of the drug based upon its specific property.

32. Purity is the test to detect impurities/contaminants in drugs, and it, as well as other requirements in each monograph, specifies the purity of the drug usually by limiting the kind/nature and quantity of the impurities/contaminants. The impurities/contaminants subject to the purity test are those supposed to generate/contaminate during the manufacturing process or storage, including hazardous agents such as heavy metals, arsenic, etc. If any foreign substances are used or supposed to be added, it is necessary to perform tests to detect or limit the presence of such substances.

33. The term “constant mass” in drying or ignition, unless otherwise specified, means that the mass difference after an additional 1 hour of drying or ignition is not more than 0.10% of the preceding mass of the dried substance or ignited residue. For crude drugs, the difference is not more than 0.25%. However, when the difference does not exceed 0.5 mg in a chemical balance, 0.05 mg in a semi-microbalance, or 0.005 mg in a microbalance, the constant mass has been attained.

34. Assay is the test to determine the composition, the content of the active ingredients, and the potency unit of medicine by physical, chemical or biological procedures.

35. In stating the appropriate quantities to be taken for assay, the use of the word “about” indicates a quantity within 10% of the specified mass. The word “dry” in respect of the sample indicates drying under the same conditions, as described in Loss on drying in the monograph.

36. For the content of an ingredient determined by Assay in the monographs, if it is expressed simply as “not less than a certain percentage” without indicating its upper limit, 101.0% is understood as the upper limit.

37. The container is the device which holds drugs. The stopper or cap, etc., is considered as part of the container. The containers have no physical and chemical reactivity affecting the specified description and quality of the contents.

38. A well-closed container protects the contents from extraneous solids and from loss of the drug under ordinary or customary conditions of handling, shipment, and storage.

Where a well-closed container is specified, it may be replaced by a tight container.

39. A tight container protects the contents from extraneous solids or liquids, from loss of the contents, and from efflorescence, deliquescence, or evaporation under ordinary or customary conditions of handling, shipment, and storage.

Where a tight container is specified, it may be replaced by a hermetic container.

40. A hermetic container is impervious to air or any other gas under ordinary or customary conditions of handling, shipment, and storage.

41. The term “light-resistant” means that it can prevent transmittance of light affecting in the specified properties and quality of the contents and protect the
contained medicament from the light under ordinary
or customary conditions of handling, shipment, and
storage.

42. For the JP Drugs, the contents or potency in
terms of units of the active ingredient(s), or the speci-
fied expiration date in the monographs have to be
shown on the immediate container or wrapping of
them.

43. The origin, numerical value or physical proper-
ties of the JP Drugs, being stipulated by the special
labeling requirements in the monographs, have to be
shown on the immediate container or wrapping of
them.

44. The harmonized General Tests and Monographs
among the Japanese Pharmacopoeia, the European
Pharmacopoeia and the United States Pharmacopeia
are preceded by the statement as such.

The parts of the text, being not harmonized, are
surrounded by the symbols (• •).

—Abbreviations—
CS: Colorimetric Stock Solution
RS: Reference Standard
TS: Test Solution
GENERAL RULES FOR CRUDE DRUGS

1. Crude drugs in the monographs include medicinal parts obtained from plants or animals, cell inclusions and secretes separated from the origins, their extracts, and minerals. General Rules for Crude Drugs and Crude Drugs Test are applicable to the following:


2. Crude drugs are usually used in the forms of whole crude drugs, cut crude drugs or powdered crude drugs.

Whole crude drugs are the medicinal parts or their ingredients prepared by drying and/or simple processes, as specified in the monographs.
Cut crude drugs are small pieces or small blocks prepared by cutting or crushing of the whole crude drugs, and also coarse, medium or fine cutting of the crude drugs in whole, and, unless otherwise specified, are required to conform to the specifications of the whole crude drugs used as original materials.

Powdered crude drugs are coarse, medium, fine or very fine powder prepared from the whole crude drugs or the cut crude drugs; usually powdered crude drugs as fine powder are specified in the monographs.

3. Unless otherwise specified, crude drugs are used in dried form. The drying is usually carried out at a temperature not exceeding 60°C.

4. The origin of crude drugs is to serve as the criteria. Such statements as 'other species of the same genus' and 'allied plants' or 'allied animals' appearing in the origin of crude drugs usually indicate plants or animals which may be used as materials for crude drugs containing the same effective constituents.

5. Description in each monograph for crude drugs usually covers the crude drug derived from its typical original plant or animal and includes statements of characteristic properties of the crude drug. As for the color, odor and solubility, apply correspondingly to the prescription of the General Notices, except the odor which is to serve as the criteria. The taste and aspects obtained by microscopic observation are to serve as the criteria.

6. Powdered crude drugs, otherwise specified, may be mixed with diluents so as to attain proper content and potency.

7. Powdered crude drugs do not contain fragments of tissues, cells, cell inclusions or other foreign matter alien to the original crude drugs or cut crude drugs.

8. Crude drugs are as free as possible from contaminants and other impurities due to molds, insects and other animals and from other foreign matters, and are required to be kept in a clean and hygienic state.

9. Crude drugs are preserved under protection from moisture and insect damage, unless otherwise specified. In order to avoid insect damage, suitable fumigants may be used to preserve crude drugs, provided that the fumigants are so readily volatilized as to be harmless at the usual dosage of the crude drugs, and such fumigants that may affect the therapeutic efficacy of the crude drugs or interfere with the testing are precluded.

10. Crude drugs are preserved in well-closed containers unless otherwise specified.
GENERAL RULES
FOR PREPARATIONS

[1] General Notices for Preparations

(1) General Notices for Preparations present general rules for pharmaceutical dosage forms.

(2) In Monographs for Preparations, dosage forms are classified mainly by administration routes and application sites, and furthermore are subdivided according to their forms, functions and characteristics.

Those preparations containing mainly crude drugs as active raw materials are described under Monographs for Preparations Related to Crude Drugs.

(3) In Monographs for Preparations and Monographs for Preparations Related to Crude Drugs, dosage forms, which are generally or widely used, are described. However, any other appropriate dosage forms may be used where appropriate. For example, a dosage form suitable for a particular application may be designated by combining an administration route and a name of a dosage form listed in these chapters.

(4) In these monographs, preparation characteristics are specified for the dosage forms. The preparation characteristics are confirmed by appropriate tests.

(5) In the case of preparations, functions that control the release rate of active substance(s) may be added for the purpose of controlling the onset and duration of therapeutic effects and/or decreasing adverse or side effects. The preparations modified in release rate must have an appropriate function of controlled release for the intended use. The added functional modification must generally be displayed on the pack insert and on the direct container or packaging of these preparations.

(6) Pharmaceutical excipients are substances other than active substances contained in preparations, and they are used to increase the utility of the active substance(s) and preparation, to make formulation process easier, to keep the product quality, to improve the usability, and so forth. Suitable excipients may be added for these purposes. The excipients to be used, however, must be pharmacologically inactive and harmless in the administered amount and must not interfere with the therapeutic efficacy of the preparations.

(7) Purified water to be used for preparations is Purified Water or Purified Water in Containers, and water for injection is Water for Injection or Water for Injection in Containers.

Vegetable oils to be used for preparations are usually edible oils listed in the Pharmacopoeia. When starch is called for, unless otherwise specified, any kind of starch listed in the Pharmacopoeia may be used.

In addition, ethanol specified in vol% is prepared by adding Purified Water or Water for Injection to ethanol at the specified vol%.

(8) Even non-sterile preparations should be prepared with precautions to prevent contamination and growth of microorganisms, and they are applied to the test of Microbial Limit Test <4.05>, if necessary.

(9) The test for Content Uniformity under the Uniformity of Dosage Units <6.02> and the Dissolution Test <6.10> are not intended to apply to the crude drug component of preparations which are prepared using crude drugs or preparations related to crude drugs as raw materials.

(10) Containers and packaging for preparations must be suitable for their proper use and for ensuring safe application, as well as for maintaining the quality of the preparations. To protect preparations that may be susceptible to oxygen in the air, deoxidants or containers made of low-gas-permeability material may be used. For preparations susceptible to degradation by moisture, packages with desiccants or moisture-proof packaging using low-moisture-permeability materials for containers may be used. For preparations susceptible to degradation by evaporation of water, containers of low-moisture-permeability material may be used.

Preparations for single-dose use are referred to as “preparations in single-dose packages”.

(11) Unless otherwise specified, preserve preparations at room temperature. Store them in light-resistant containers or packaging, if light affects the quality of the preparation.

(1) In the Monographs for Preparations, the definitions of dosage forms, manufacturing methods, test methods, containers and packaging, and storage are described.

(2) The descriptions of the test methods and the containers and packaging in these monographs are fundamental requirements, and the manufacturing methods represent commonly used methods.

1. Preparations for Oral Administration

(1) Immediate-release dosage forms are preparations showing a release pattern of active substance(s) that is not intentionally modified and is generally dependent on the intrinsic solubility of the active substance.

(2) Modified-release dosage forms are preparations showing a release pattern of active substance(s) that is suitably modified for the desired purpose by means of a specific formulation design and/or manufacturing method. Modified-release dosage forms include enteric-coated and extended-release preparations.

(i) Enteric-coated (delayed-release) preparations

Enteric-coated preparations are designed to release the bulk of the active substance(s) not in stomach but mainly in small intestine, in order to prevent degradation or decomposition of the active substance(s) in stomach or to decrease the irritation of the active substance(s) on stomach. Enteric-coated preparations are generally coated with an acid-insoluble enteric film.

(ii) Extended-release preparations

Extended-release preparations are designed to control the release rate and release period of active substance(s) and to restrict the release to appropriate sites in the gastrointestinal tracts in order to decrease the dosing frequency and/or to reduce adverse or side effects. Extended-release preparations are generally prepared by using suitable agents that prolong the release of the active substance(s).

(3) Oral dosage forms such as capsules, granules and tablets can be coated with appropriate coating agents, such as sugars, sugar alcohols, or polymers, for the purpose of enabling the ingestion easy or of preventing degradation of the active substance(s).

1-1. Tablets

(1) Tablets are solid preparations having a desired shape and size, intended for oral administration. Orally Disintegrating Tablets, Chewable Tablets, Effervescent Tablets, Dispersible Tablets and Soluble Tablets are included in this category.

(2) Tablets are usually prepared by the following procedures. Enteric-coated or extended-release tablets can be prepared by appropriate methods.

(i) Mix homogeneously active substance(s) and excipients such as diluents, binders and disintegrators, granulate with water or a binder solution by a suitable method, mix with a lubricant, and then compress into a desired shape and size.

(ii) Mix homogeneously active substance(s) and excipients such as diluents, binders, and disintegrators, and then directly compress with a lubricant, or compress after adding active substance(s) and a lubricant to granules previously prepared from excipients and then mixing homogeneously.

(iii) Mix homogeneously active substance(s) and excipients such as diluents and binders, moisten with a solvent, form into a certain shape and size or mold the mixed mass into a certain shape and size, and then dry by a suitable method.

(iv) Plain Tablets are usually prepared according to (i), (ii) or (iii).

(v) Film-coated Tablets can be prepared, usually, by coating Plain Tablets with thin films using suitable film coating agents such as polymers.

(vi) Sugar-coated Tablets can be prepared, usually, by coating Plain Tablets using suitable coating agents including sugars or sugar alcohols.

(vii) Multiple-layer Tablets can be prepared by compressing granules of different compositions to form layered tablets by a suitable method.

(viii) Pressure-coated Tablets can be prepared by compressing granules to cover inner core tablets with different compositions.

(3) Unless otherwise specified, Tablets meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Unless otherwise specified, Tablets meet the requirements of Dissolution Test <6.10> or Disintegration Test <6.09>. For Effervescent tablets from which active substance(s) are dissolved before use and Soluble tablets, these tests are not required.

(5) Well-closed containers are usually used for the preparations. For preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

1-1-1. Orally Disintegrating Tablets/Orodispersible Tablets

(1) Orally Disintegrating Tablets are tablets which are quickly dissolved or disintegrated in the oral cavity.

(2) Orally Disintegrating Tablets shows an appropriate disintegration.
1-1-2. Chewable Tablets
(1) Chewable Tablets are tablets which are administered by chewing.
(2) Chewable Tablets must be in shape and size avoiding danger of suffocation.

1-1-3. Effervescent Tablets
(1) Effervescent Tablets are tablets which are quickly dissolved or dispersed with bubbles in water.
(2) Effervescent tablets are usually prepared using suitable acidic substances and carbonates or hydrogen carbonates.

1-1-4. Dispersible Tablets
(1) Dispersible Tablets are tablets which are administered after having been dispersed in water.

1-1-5. Soluble Tablets
(1) Soluble Tablets are tablets which are administered after having been dissolved in water.

1-2. Capsules
(1) Capsules are preparations enclosed in capsules or wrapped with capsule bases, intended for oral administration. Capsules are classified into Hard Capsules and Soft Capsules.
(2) Capsules are usually prepared by the following methods. Enteric-coated or extended-release capsules can be prepared by a suitable method. Coloring agents, preservatives, etc. may be added to the capsule bases.
   (i) Hard Capsules: A homogeneous mixture of active substance(s) with diluents and other suitable excipients, or granules or formed masses prepared by a suitable method, are filled into capsule shells as they are or after slight compression.
   (ii) Soft Capsules: Active substance(s) and suitable excipients (including solvents) are mixed, enclosed by a suitable capsule base such as gelatin plasticized by addition of glycerin, D-sorbitol, etc. and molded in a suitable shape and size.
(3) Unless otherwise specified, Capsules meet the requirements of Uniformity of Dosage Units <6.02>.
(4) Unless otherwise specified, Capsules meet the requirements of Dissolution Test <6.10> or Disintegration Test <6.09>.
(5) Well-closed containers are usually used for Capsules. For Capsules susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

1-3. Granules
(1) Granules are preparations prepared by granulation, intended for oral administration. Effervescent Granules are included in this category.
(2) Granules are usually prepared by the following methods. Granules can be coated using suitable coating agents if necessary. Extended-release or entericoated granules can also be prepared by a suitable method.
   (i) To powdery active substance(s) add diluents, binders, disintegrators, or other suitable excipients, mix to homogenize, and granulate by a suitable method.
   (ii) To previously granulated active substance(s) add excipients such as diluents, and mix to homogenize.
   (iii) To previously granulated active substance(s) add excipients such as diluents, and granulate by a suitable method.
(3) Among Granules, the preparations may be referred to as “Fine Granules” if, when Particle Size Distribution Test for Preparations <6.03> is performed, all granules pass through a No. 18 (850 μm) sieve, and not more than 10% of which remain on a No. 30 (500 μm) sieve.
(4) Unless otherwise specified, the Granules in single-dose packages meet the requirements of Uniformity of Dosage Units <6.02>.
(5) Unless otherwise specified, Granules comply with Dissolution Test <6.10> or Disintegration Test <6.09>. However, this provision is not to be applied to Effervescent granules, which are dissolved before use, and Disintegration Test <6.09> is not required for the Granules not more than 10% of which remain on a No. 30 (500 μm) sieve when the test is performed as directed under Particle Size Distribution Test for Preparations <6.03>.
(6) Among Granules, the particulate preparations may be referred to as “Powders” if, when the Particle Size Distribution Test for Preparations <6.03> is performed, all granules pass through a No. 18 (850 μm) sieve, and not more than 5% remain on a No. 30 (500 μm) sieve.
(7) Well-closed containers are usually used for Granules. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

1-3-1. Effervescent Granules
(1) Effervescent granules are granules which are quickly dissolved or dispersed with bubbles in water.
(2) Effervescent granules are usually prepared using suitable acidic substances and carbonates or hydrogen carbonates.

1-4. Powders
(1) Powders are preparations in powder form, intended for oral administration.
(2) Powders are usually prepared by homogene-
ously mixing active substance(s) with diluents or other suitable excipients.

3) Unless otherwise specified, the Powders in single-dose packages meet the requirements of Uniformity of Dosage Units <6.02>.

4) Unless otherwise specified, Powders meet the requirements of Dissolution Test <6.10>.

5) Well-closed containers are usually used for Powders. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

1-5. Liquids and Solutions for Oral Administration

1) Liquids and Solutions for Oral Administration are preparations in liquid form or flowable and viscous gelatinous state, intended for oral administration. Elixirs, Suspensions, Emulsions and Lemonades are included in this category.

2) Liquids and Solutions for Oral Administration are usually prepared by dissolving, emulsifying or suspending active substance(s) in Purified Water together with excipients, and by filtering if necessary.

3) For Liquids and Solutions for Oral Administration which are apt to deteriorate, prepare before use.

4) Unless otherwise specified, the preparations in single-dose packages meet the requirement of Uniformity of Dosage Units <6.02>.

5) Tight containers are usually used for Liquids and Solutions for Oral Administration. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

1-5-1. Elixirs

1) Elixirs are clear, sweetened and aromatic liquid preparations, containing ethanol, intended for oral administration.

2) Elixirs are usually prepared by dissolving solid active substance(s) or their extractives in ethanol and Purified Water, adding aromatic agents and sucrose, other sugars or sweetening agents, and clarifying by filtration or other procedure.

1-5-2. Suspensions

1) Suspensions are liquid preparations of active substance(s) suspended finely and homogeneously in a vehicle, intended for oral administration.

2) Suspensions are usually prepared by adding suspending agent or other suitable excipients and Purified Water or oil to solid active substance(s), and suspending homogeneously as the whole by a suitable method.

3) Mix homogeneously before use, if necessary.

4) Unless otherwise specified, Suspensions meet the requirements of Dissolution Test <6.10>.

1-5-3. Emulsions

1) Emulsions are liquid preparations of active substance(s) emulsified finely and homogeneously in a liquid vehicle, intended for oral administration.

2) Emulsions are usually prepared by adding emulsifying agents and Purified Water to liquid active substance(s), and emulsifying finely and homogeneously by a suitable method.

3) Mix homogeneously before use, where necessary.

1-5-4. Lemonades

1) Lemonades are sweet and sour, clear liquid preparations, intended for oral administration.

1-6. Syrups

1) Syrups are viscous liquid or solid preparations containing sugars or sweetening agents, intended for oral administration. Preparations for Syrups are included in this category.

2) Syrups are usually prepared by dissolving, mixing, suspending or emulsifying active substance(s) in a solution of sucrose, other sugars or sweetening agents, or in Simple Syrup. Where necessary, the mixture is boiled, and filtered while hot.

3) For Syrups which are apt to deteriorate, prepare before use.

4) Unless otherwise specified, Syrups in single-dose packages meet the requirements of Uniformity of Dosage Units <6.02>.

5) Unless otherwise specified, Syrups in which active substance(s) is suspended meet the requirements of Dissolution Test <6.10>.

6) Tight containers are usually used for Syrups. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

1-6-1. Preparations for Syrups

1) Preparations for Syrups are preparations in form of granules or powders, which become syrups by adding water. They may be termed "Dry Syrups".

2) Preparations for Syrup are usually prepared with sugars or sweetening agents as directed under 1-3. Granules or 1-4. Powders.

3) Preparations for Syrups are usually to be used after having been dissolved or suspended in water.

4) Unless otherwise specified, the Preparations for Syrups other than preparations which are to be used after having been dissolved meet the requirements of Dissolution Test <6.10> or Disintegration Test <6.09>. However, Disintegration Test <6.09> is not required for the Preparations, if, when the Particle Size Distribution Test for Preparations <6.03> is
performed, not more than 10% of the total amount remains on a No. 30 (500 mm) sieve.

(5) Well-closed containers are usually used for Preparations for Syrups. For the Preparations for Syrups susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

1-7. Jellies for Oral Administration

(1) Jellies for Oral Administration are non-flowable gelatinous preparations having a certain shape and size, intended for oral administration.

(2) Jellies for oral application are usually prepared by mixing active substance(s) with suitable excipients and polymer gel base, gelatinizing and forming into a certain shape and size by a suitable method.

(3) Unless otherwise specified, Jellies for Oral Administration meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Unless otherwise specified, Jellies for Oral Administration meet the requirements of Dissolution Test <6.10> or show an appropriate disintegration.

(5) Tight containers are usually used for Jellies for Oral Administration. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

2. Preparations for Oro-mucosal Application

2-1. Tablets for Oro-mucosal Application

(1) Tablets for Oro-mucosal Application are solid preparations having a certain form, intended for oral cavity application.

Troches/Lozenges, Sublingual Tablets, Buccal Tablets, Mucoadhesive Tablets and Medicated Chewing Gums are included in this category.

(2) Tablets for Oro-mucosal Application are prepared as directed under 1-1. Tablets.

(3) Unless otherwise specified, Tablets for Oro-mucosal Application meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Tablets for Oro-mucosal Application have an appropriate dissolution or disintegration.

(5) Well-closed containers are usually used for Tablets for Oro-mucosal Application. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

2-1-1. Troches/Lozenges

(1) Troches/Lozenges are tablets for oro-mucosal application, which are gradually dissolved or disintegrated in the mouth, and are intended for application locally to the oral cavity or the throat.

(2) Troches/Lozenges must be in shape and size avoiding danger of suffocation.

2-1-2. Sublingual Tablets

(1) Sublingual Tablets are tablets for oro-mucosal application, from which active substance(s) are quickly dissolved sublingually and absorbed via the oral mucosa.

2-1-3. Buccal Tablets

(1) Buccal Tablets are tablets for oro-mucosal application, from which the active substance(s) are dissolved gradually between the cheek and teeth, and absorbed via the oral mucosa.

2-1-4. Mucoadhesive Tablets

(1) Mucoadhesive Tablets are tablets for oro-mucosal application that are applied by adhesion to the oral mucosa.

(2) Mucoadhesive Tablets are usually prepared by using hydrophilic polymers to form hydrogel.

2-1-5. Medicated Chewing Gums

(1) Medicated Chewing Gums are tablets for oro-mucosal application, releasing active substance(s) by chewing.

(2) Medicated Chewing Gums are usually prepared using suitable gum bases such as vegetable resin, thermoplastic resin and elastomer.

2-2. Sprays for Oro-mucosal Application

(1) Sprays for Oro-mucosal Application are preparations that are applied active substance(s) by spraying into the oral cavity in mist, powder, foam or paste forms.

(2) Sprays for Oro-mucosal Application are usually prepared by the following methods:

(i) Dissolve or suspend active substance(s) and suitable excipients in a solvent, filter, where necessary, and fill into a container together with liquefied or compressed gas.

(ii) Dissolve or suspend active substance(s) and suitable excipients in a solvent, fill into a container, and fit with a pump for spraying.

(3) Unless otherwise specified, metered-dose types among Sprays for Oro-mucosal Application have an appropriate uniformity of delivered dose.

(4) Tight containers or pressure-resistant containers are usually used for Sprays for Oro-mucosal Application.

2-3. Semi-solid Preparations for Oro-mucosal Application

(1) Semi-solid Preparations for Oro-mucosal Application are preparations in cream, gel or ointment forms, intended for application to the oral mucosa.

(2) Semi-solid Preparations for Oro-mucosal Application are usually prepared by emulsifying active substance(s) together with excipients using “Purified
Water” and oil component such as petrolatum, or by homogenizing active substance(s) together with suitable excipients using polymer gel or oil and fats as the base.

(i) Creams for oro-mucosal application are prepared as directed under 11-5. Creams.

(ii) Gels for oro-mucosal application are prepared as directed under 11-6. Gels.

(iii) Ointments for oro-mucosal application are prepared as directed under 11-4. Ointments.

For Semi-solid Preparations for Oro-mucosal Application which are apt to deteriorate, prepare before use.

(3) Sufficient amounts of suitable preservatives to prevent the growth of microorganisms may be added for Semi-solid Preparations for Oro-mucosal Application filled in multiple-dose containers.

(4) Semi-solid Preparations for Oro-mucosal Application have a suitable viscosity to apply to the oral mucosa.

(5) Tight containers are usually used for Semi-solid Preparations for Oro-mucosal Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

2-4. Preparations for Gargle

(1) Preparations for Gargle are liquid preparations intended to apply locally to the oral and throat cavities. Solid type preparations to be dissolved in water before use are also included in this category.

(2) Preparations for Gargle are usually prepared by dissolving active substance(s) in a solvent together with suitable excipients, and filtering where necessary. The solid preparations are prepared as directed under 1-1. Tablets or 1-3. Granules.

(3) Unless otherwise specified, Preparations for Gargle in single-dose packages meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Tight containers are usually used for Preparations for Gargle. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

3. Preparations for Injection

3-1. Injections

(1) Injections are sterile preparations to be administered directly into the body through skin, muscle or blood vessel, usually in form of a solution, a suspension or an emulsion of active substance(s), or of a solid that contains active substance(s) to be dissolved or suspended before use.

Parenteral Infusions, Implants/Pellets and Prolonged-Release Injections are included in this category.

(2) Injections in solution, suspension or emulsion form are usually prepared by the following methods.

(i) Dissolve, suspend or emulsify active substance(s) with or without excipients in Water for Injection or an aqueous or nonaqueous vehicle homogeneously, fill into containers for injection, seal, and sterilize.

(ii) Dissolve, suspend or emulsify active substance(s) with or without excipients in Water for Injection or an aqueous or nonaqueous vehicle, and filtrate aseptically, or prepare aseptically a homogeneous liquid, fill into containers for injection, and seal.

Every care should be taken to prevent contamination with microorganisms. The overall processes of preparing injections, from the preparation of active solution to the sterilization, should be completed as rapidly as possible, taking into consideration the composition of the injection and the storage conditions.

The concentration of active substance(s) expressed in % represents w/v%.

Injections that are to be dissolved or suspended before use and are designated in the name as “for injection” may be accompanied by a suitable vehicle to dissolve or suspend the supplied preparation (hereinafter referred to as “vehicle attached to preparation”).

(3) Injections may be prepared as Freeze-dried Injections or Powders for Injections to prevent degradation or deactivation of the active substance(s) in solution.

(i) Freeze-dried Injections

Freeze-dried Injections are usually prepared by dissolving active substance(s) with or without excipients such as diluents in Water for Injection, sterilizing the solution by aseptic filtration, filling the filtrate directly into individual containers for injection and being freeze-dried, or dividing the filtrate in special containers, being freeze-dried and transferred into individual containers for injection.

(ii) Powders for Injections

Powders for injections are usually prepared by filtrating aseptically a solution of active substance(s), obtaining powders by crystallization from the solution or mixing additionally the powders with sterilized excipients, and filling the powders into individual containers for injections.

(4) To prevent errors in the preparation with vehicles attached or administration of injections, or bacterial or foreign matter contamination, or for the purpose of urgent use, prefilled syringes or cartridges may be prepared.

(i) Prefilled Syringes for Injections
Prefilled Syringes for injections are usually prepared by dissolving, suspending or emulsifying active substance(s) with or without excipients in a vehicle, and filling into syringes.

(ii) Cartridges for Injections

Cartridges for Injections are usually prepared by dissolving, suspending or emulsifying active substance(s) with or without excipients in a vehicle, and filling into cartridges.

The cartridges are used by fixing in an injection device for exclusive use.

(5) Vehicles used in Injections or attached to preparations must be harmless in the amounts usually administered and must not interfere with the therapeutic efficacy of the active substance(s).

The vehicles are classified into the following two groups. They should meet each requirement.

(i) Aqueous vehicles: As the vehicle of aqueous injections, Water for Injection is usually used. Isotonic Sodium Chloride Solution, Ringer’s Solution, or other suitable aqueous solutions may be used instead.

Unless otherwise specified, these aqueous vehicles, other than those exclusively for intracutaneous, subcutaneous or intramuscular administration, meet the requirements of Bacterial Endotoxins Test <4.01>.

When the Bacterial Endotoxins Test <4.01> is not applicable to aqueous vehicles, the Pyrogen Test <4.04> may be applied instead.

(ii) Non-aqueous vehicles: Vegetable oils are usually used as vehicles for non-aqueous injections. These oils, unless otherwise specified, are clear at 10°C, the acid value is not more than 0.56, the saponification value is between 185 and 200, and the iodine value falls between 79 and 137. They meet the requirements of Mineral Oil Test <1.05>.

Several suitable organic solvents other than vegetable oils may be used as non-aqueous vehicles.

(6) Unless otherwise specified, any coloring agent must not be added solely for the purpose of coloring the preparations.

(7) Sodium chloride or other excipients may be added to aqueous injections to adjust them isotonic to blood or other body fluids. Acids or alkalis may be added to adjust the pH.

(8) Injections supplied in multiple-dose containers may be added sufficient amounts of suitable preservatives to prevent the growth of microorganisms.

(9) Unless otherwise specified, Injections and vehicles attached to preparations other than those used exclusively for intracutaneous, subcutaneous or intramuscular administration meet the requirements of Bacterial Endotoxins Test <4.01>. In the case where the Bacterial Endotoxins Test <4.01> is not applicable, Pyrogen Test <4.04> may be applied instead.

(10) Unless otherwise specified, Injections and vehicles attached to preparations meet the requirements of Sterility Test <4.06>.

(11) Containers of Injections are colorless and meet the requirements of Test for Glass Containers for Injections <7.01>. Where specified in individual monographs, these containers may be replaced by colored containers meeting the requirements of Test for Glass Containers for Injections <7.01> or by plastic containers for aqueous injections meeting the requirements of Test Methods for Plastic Containers <7.02>.

(12) Unless otherwise specified, rubber stoppers used for glass containers of 100 mL or more of aqueous infusions meet the requirements of Test for Rubber Closure for Aqueous Infusions <7.03>.

(13) Unless otherwise specified, Injections and vehicles attached to preparations meet the requirements of Foreign Insoluble Matter Test for Injections <6.06>.

(14) Unless otherwise specified, Injections and vehicles attached to preparations meet the requirements of Insoluble Particulate Matter Test for Injections <6.07>.

(15) Unless otherwise specified, the actual volume of Injections meets the requirements of Test for Extractable Volume of Parenteral Preparations <6.05>.

(16) Unless otherwise specified, Injections to be dissolved or suspended before use meet the requirements of Uniformity of Dosage Units <6.02>.

(17) Suspensions for injection are usually not to be injected into the blood vessels or spinal cord, and emulsions for injection are not to be injected into the spinal cord.

(18) The maximum size of particles observed in suspensions for injection is usually not larger than 150 μm, and that of particles in emulsions for injection is usually not larger than 7 μm.

(19) The following information, unless otherwise specified, must be written on the package leaflet, or the container or wrapper.

(i) In cases where the vehicle is not specified, the name of the employed vehicle, with the exception of Water for Injection, sodium chloride solution not exceeding 0.9 w/v% and those vehicles in which acids or alkalis are used in order to adjust the pH.

(ii) In case of vehicle attached to preparation, the name of the vehicle, content volume, ingredients and quantities or ratios, and a statement of the presence of the vehicle on the outer container or outer wrapper.

(iii) Name and quantity of stabilizers, preserva-
Prolonged Release Injections are injections to be used for intramuscular administration, for the purpose of releasing active substance(s) for a long period of time.

(2) Prolonged Release Injections are usually prepared by dissolving or suspending active substance(s) in a non-aqueous vehicle such as vegetable oil, or by suspending microspheres prepared with biodegradable polymers.

(3) Prolonged Release Injections have an appropriate function of controlled release.

4. Preparations for Dialysis
4-1. Dialysis Agents
(1) Dialysis Agents are preparations in liquid, or in solid which are to be dissolved before use, intended for peritoneal dialysis or hemodialysis.

They are classified into Peritoneal dialysis agents and Hemodialysis agents.

(2) Unless otherwise specified, Dialysis Agents meet the requirements of Bacterial Endotoxins Test \(<4.01\>.

(3) The solid preparations which are to be dissolved before use among Dialysis agents have an appropriate uniformity of dosage units.

4-1-1. Peritoneal Dialysis Agents
(1) Peritoneal Dialysis Agents are sterile dialysis agents, intended to be used for peritoneal dialysis.

(2) Peritoneal Dialysis Agents are usually prepared by dissolving active substance(s) with suitable excipients in a vehicle to make a certain volume, or by filling active substance(s) combined with suitable excipients in a container, and sealing it. Sterilize if necessary. Every care should be taken to prevent microbial contamination. The overall processes from preparation to sterilization for preparing the agents should be completed as rapidly as possible, taking into consideration the composition of the agents and the storage conditions. The concentration of Peritoneal dialysis agents expressed in % represents w/v%. In the case of solid preparations which are dissolved before use, prepare as directed under 1-1. Tablets or 1-3. Granules.

(3) If necessary, pH adjusting agents, isotonic agents or other excipients may be added.

(4) Unless otherwise specified, the vehicle used for Peritoneal dialysis agents is Water for Injection.

(5) Unless otherwise specified, Peritoneal Dialysis Agents meet the requirements of Sterility Test \(<4.06\>.

(6) Unless otherwise specified, Peritoneal Dialysis Agents meet the requirements of (4) Parenteral infusions under Test for Extractable Volume of Parenteral Preparations \(<6.05\>.

(7) Unless otherwise specified, Peritoneal Dialysis Agents meet the requirements of Foreign Insoluble Matter Test for Injections \(<6.06\>.

3-1-1. Parenteral Infusions
(1) Parenteral Infusions are usually injections of not less than 100 mL, intended for intravenous administration.

(2) Parenteral Infusions are mainly administered for the purpose of water supply, correction of electrolyte abnormality and nutritional support, and they are also used by mixing with other injections for treatments by continual infusion.

3-1-2. Implants/Pellets
(1) Implants/Pellets are solid or gel-like form injections, intended for subcutaneous or intramuscular administration by means of an implant device or operative treatment, for the purpose of releasing active substance(s) for a long period of time.

(2) Implants/Pellets are usually prepared in a form of pellet, microsphere or gel using biodegradable polymers.

(3) Unless otherwise specified, Implants/Pellets meet the requirements of Uniformity of Dosage Units \(<6.02\>.

(4) Implants/Pellets have an appropriate function of controlled release.

(5) Implants/Pellets are not required the requirements of Foreign Insoluble Matter Test for Injections, Insoluble Particulate Matter for Injections and Test for Extractable Volume of Parenteral Preparations.

3-1-3. Prolonged Release Injections
(1) Prolonged Release Injections are injections to be used for intramuscular administration, for the purpose of releasing active substance(s) for a long period
(8) Unless otherwise specified, Peritoneal Dialysis Agents meet the requirements of Insoluble Particulate Matter Test for Injections <6.07>.

(9) Colorless containers meeting the requirements of Test for Glass Containers for Injections <7.01> are used for Peritoneal Dialysis Agents. Where specified otherwise, the colored containers meeting the requirements of Test for Glass Containers for Injections <7.01> or the plastic containers for aqueous injections meeting the requirements of Test Methods for Plastic Containers <7.02> may be used.

(10) Unless otherwise specified, the rubber closures of the containers meet the requirements of Test for Rubber Closure for Aqueous Infusions <7.03>.

(11) Hermetic containers, or tight containers which are able to prevent microbial contamination are usually used for Peritoneal Dialysis Agents. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

4-1-2. Hemodialysis Agents

(1) Hemodialysis agents are dialysis agents to be used for hemodialysis.

(2) Hemodialysis Agents are usually prepared by dissolving active substance(s) with excipients in a vehicle to make a certain volume, or by filling active substance(s) with excipient(s) in a container. In the case of the solid preparations to be dissolved before use, prepare as directed under 1-1. Tablets or 1-3. Granules.

(3) If necessary, pH adjusting agents, isotonic agents or other excipients may be added.

(4) Unless otherwise specified, the vehicle used for Hemodialysis agents is Water for Injection or water suitable for dialysis.

(5) Tight containers which are able to prevent microbial contamination are usually used for Hemodialysis Agents. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

5. Preparations for Inhalation

5-1. Inhalations

(1) Inhalations are preparations intended for administration as aerosols to the bronchial tubes or lung. Inhalations are classified to Dry Powder Inhalers, Inhalation Liquid Preparations and Metered-dose Inhalers.

(2) For administration of Inhalations, suitable devices or apparatus are used, or they are placed in containers which have a appropriate function of inhalation device.

5-1-1. Dry Powder Inhalers

(1) Dry Powder Inhalers are preparations which deliver a constant respiratory intake, intended for administration as solid particle aerosols.

(2) Dry Powder Inhalers are usually prepared by pulverizing active substance(s) into fine particles. Where necessary, lactose or other suitable excipients are added to make homogenous mixture.

(3) Metered-dose types among Dry Powder Inhalers have an appropriate uniformity of delivered dose of the active substance(s).

(4) The particles of active substance(s) in Dry Powder Inhalers have an aerodynamically appropriate size.

(5) Well-closed containers are usually used for Dry Powder Inhalers. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

5-1-2. Inhalation Liquid Preparations

(1) Inhalation Liquid Preparations are liquid inhalations which are administered by an inhalation device such as operating nebulizers.

(2) Inhalation Liquid Preparations are usually prepared by mixing active substance(s) with a vehicle and suitable isotonic agents and/or pH adjusting agents to make a solution or suspension, and by filtering where necessary.

(3) Sufficient amounts of suitable preservatives may be added to Inhalation Liquid Preparations to prevent the growth of microorganisms.

(4) Tight containers are usually used for Inhalation Liquid Preparations. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

5-1-3. Metered-dose Inhalers

(1) Metered-dose Inhalers are preparations which deliver a constant dose of active substance(s) from the container together with propellant filled in.

(2) Metered-dose Inhalers are usually prepared by dissolving active substance(s) with a suitable dispersing agents and stabilizers in a vehicle to make a solution or suspension, and by filling in pressure-resistant containers together with liquid propellant, and setting metering valves.

(3) Metered-dose Inhalers have an appropriate uniformity of delivered dose of active substance(s).

(4) Particles of active substance(s) in Metered-dose Inhalers have an aerodynamically appropriate size.

(5) Pressure-resistant and hermetic containers are usually used for Metered-dose Inhalers.

6. Preparations for Ophthalmic Application

6-1. Ophthalmic Liquids and Solutions
(1) Ophthalmic Liquids and Solutions are sterile preparations of liquid, or solid to be dissolved or suspended before use, intended for application to the conjunctival sac or other ocular tissues.

(2) Ophthalmic Liquids and Solutions are usually prepared by dissolving, suspending active substance(s) in a vehicle after adding excipients to make a constant volume, or mixing active substance(s) and excipients, and filling into containers. The overall processes, from preparation to sterilization, should be completed with sufficient care to prevent microbial contamination as rapidly as possible, taking into consideration the composition of the preparations and the storage conditions. The concentration of active substance expressed in % represents w/v%.

Ophthalmic Liquids and Solutions to be dissolved or suspended before use and designated in the name as “for ophthalmic application” may be accompanied by a vehicle for dissolving or suspending the preparation (hereinafter referred to as “vehicle attached to preparation”).

(3) Vessels to prepare Ophthalmic Liquids and Solutions or vehicle attached to the preparations must be harmless in the amounts usually administered and must not interfere with the therapeutic efficacy of the active substance(s).

Vehicles for Ophthalmic Liquids and Solutions are classified into the following two groups.

(i) Aqueous vehicles: As the vehicles for the aqueous preparations Purified Water or suitable aqueous vehicles are used. For vehicles attached to the preparations sterilized Purified Water or sterilized aqueous vehicles are used.

(ii) Non-aqueous vehicles: As the vehicles for the non-aqueous preparations vegetable oils are usually used. Suitable organic solvents may be also used as the non-aqueous vehicles.

(4) Unless otherwise specified, any coloring agents must not be added solely for the purpose of coloring Ophthalmic Liquids and Solutions or vehicles attached to the preparations.

(5) Sodium chloride or other excipients may be added to Ophthalmic Liquids and Solutions to adjust them isotonic to lacrimal fluid. Acids or alkalis may be also added to adjust the pH.

(6) Unless otherwise specified, Ophthalmic Liquids and Solutions and vehicles attached to the preparations meet the requirements of Sterility Test <4.06>.

(7) Sufficient amounts of appropriate preservatives to prevent the growth of microorganisms may be added to the preparations filled in multiple dose containers.

(8) Unless otherwise specified, Ophthalmic Liquids and Solutions prepared in aqueous solutions or the vehicles attached to the preparations meet the requirements of Foreign Insoluble Matter Test for Ophthalmic Solutions <6.11>.

(9) Unless otherwise specified, Ophthalmic Liquids and Solutions and the vehicles attached to the preparations meet the requirements of Insoluble Particulate Matter Test for Ophthalmic Solutions <6.08>.

(10) The maximum particle size observed in Ophthalmic suspensions is usually not larger than 75 μm.

(11) Transparent tight containers, which do not disturb the test of Foreign Insoluble Matter Test for Ophthalmic Solutions <6.11>, are usually used for Ophthalmic Liquids and Solutions. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

6-2. Ophthalmic Ointments

(1) Ophthalmic Ointments are sterile preparations of semi-solid, intended for application to the conjunctival sac or other ocular tissues.

(2) Ophthalmic Ointments are usually prepared by mixing homogeneously solution of or finely powdered active substance(s) with petrolatum or other bases, and filling into containers. The overall processes, from preparation to sterilization, should be completed with sufficient care to prevent microbial contamination as rapidly as possible, taking into consideration the composition of the preparations and the storage conditions.

(3) Sufficient amounts of suitable preservatives may be added to Ophthalmic Ointments filled in multiple dose containers to prevent the growth of microorganisms.

(4) Unless otherwise specified, Ophthalmic Ointments meet the requirements of Sterility Test <4.06>, and unless otherwise specified, the test is carried out by the Membrane filtration method.

(5) Unless otherwise specified, Ophthalmic Ointments meet the requirements of Test for Metal Particles in Ophthalmic Ointments <6.01>.

(6) The maximum particle size of active substance(s) in Ophthalmic Ointments is usually not larger than 75 μm.

(7) Ophthalmic Ointments have a suitable viscosity for applying to the ocular tissues.

(8) Tight containers which are able to prevent microbial contamination are usually used for Ophthalmic Ointments. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.
7. Preparations for Otic Application

7-1. Ear Preparations

(1) Ear Preparations are liquid, semi-solid, or solid preparations which are to be dissolved or suspended before use, intended for application to the external or internal ear.

(2) Ear Preparations are usually prepared by filling in containers with liquids in which active substance(s) and excipients are dissolved or suspended in a vehicle to make a constant volume, or with powders in which active substance(s) and excipients are mixed. The overall processes, from preparation to sterilization, should be completed with sufficient care to prevent microbial contamination as rapidly as possible, taking into consideration the composition of the preparations and the storage conditions. The concentration of active substance of Ear Preparations expressed in % represents w/v%.

In the case where the sterile preparations are prepared, proceed as directed under 6-1. Ophthalmic Liquids and Solutions.

Ear Preparations which are to be dissolved or suspended before use and designated in the name as “for otic preparation” may be accompanied by a vehicle to dissolve or suspend (hereinafter referred to as “vehicle attached to preparation”).

(3) Vehicles used for Ear Preparations or the vehicle attached to the preparation are classified into the following two groups.

(i) Aqueous vehicles: As the vehicles for the aqueous preparations or the vehicles attached to the preparations, Purified Water or suitable aqueous vehicles are used. For the sterile preparations, Sterilized Purified Water or suitable sterilized aqueous vehicles are used as the vehicle attached to the preparations.

(ii) Non-aqueous vehicles: As the vehicles for the non-aqueous preparations vegetable oils are usually used. Suitable organic solvents may be also used as non-aqueous vehicles.

(4) Unless otherwise specified, any coloring agents must not be added solely for the purpose of coloring Ear Preparations or vehicle attached to the preparations.

(5) Sufficient amounts of suitable preservatives to prevent the growth of microorganisms may be added to the preparations filled in multiple dose containers.

(6) Unless otherwise specified, sterile Ear preparations and the vehicles attached to the sterile preparations meet the requirements of Sterility Test <4.06>.

(7) Tight containers are usually used for Ear Preparations. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

8. Preparations for Nasal Application

8-1. Nasal Preparations

(1) Nasal Preparations are preparations intended for application to the nasal cavities or nasal mucous membrane.

Nasal preparations are classified into Nasal dry powder inhalers and Nasal Liquid Preparations.

(2) Where necessary, Nasal Preparations are sprayed for inhalation by using a suitable atomizing device such as spray-pump.

(3) Unless otherwise specified, metered-dose type preparations among Nasal Preparations show the appropriate uniformity of delivered dose.

8-1-1. Nasal Dry Powder Inhalers

(1) Nasal Dry Powder Inhalers are fine powdered preparations, intended for application to the nasal cavities.

(2) Nasal Dry Powder Inhalers are usually prepared by pulverizing active substance(s) into moderately fine particles, or by mixing homogeneously with excipients where necessary.

(3) Well-closed containers are usually used for Nasal Dry Powder Inhalers. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

8-1-2. Nasal Liquids and Solutions

(1) Nasal Liquids and Solutions are liquid preparations, or solid preparations to be dissolved or suspended before use, intended for application to the nasal cavities.

(2) Nasal Liquids and Solutions are usually prepared by dissolving or suspending active substance(s) in a vehicle together with excipients, and filtering where necessary. Isotonic agents and/or pH adjusting agents may be used.

(3) Nasal Liquids and Solutions, which are to be dissolved or suspended before use and designated in the name as “for nasal application”, may be accompanied by a vehicle to dissolve or suspend.

(4) Sufficient amounts of suitable preservatives to prevent the growth of microorganisms may be added to the preparations filled in multiple dose containers.

(5) Tight containers are usually used for Nasal Liquids and Solutions. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

9. Preparations for Rectal Application

9-1. Suppositories for Rectal Application

(1) Suppositories for Rectal Application are semi-
solid preparations of a desired shape and size, intended for intrarectal application, which release active substance(s) by melting at body temperature or dissolving or dispersing gradually in the secretions.

(2) Suppositories for Rectal Application are usually prepared by mixing homogeneously active substance(s) and excipients such as dispersing agents and emulsifying agents, dissolving or suspending uniformly in a base which is liquefied by warming, filling a constant volume of the resultant material into containers, and molding it into a shape and size. Lipophilic bases or hydrophilic bases are usually used.

(3) Suppositories for Rectal Application are usually conical- or spindle-shaped.

(4) Unless otherwise specified, Suppositories for Rectal Application meet the requirements of Uniformity of Dosage Units \(<6.02\)\).

(5) Suppositories for Rectal Application show an appropriate release.

(6) Well-closed containers are usually used for Suppositories for Rectal Application. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

9-2. Semi-solid Preparations for Rectal Application

(1) Semi-solid Preparations for Rectal Application are preparations which are in a form of cream, gel or ointment intended for application to around or inside of the anus.

(2) Semi-solid Preparations for Rectal Application are usually prepared by emulsifying active substance(s) with excipients in Purified Water and oil component such as vaseline, or by homogenously mixing active substance(s) and excipients in a base of polymer gel or grease.

   (i) Creams for rectal application: Prepare as directed under 11-5. Creams.
   (ii) Gels for rectal application: Prepare as directed under 11-6. Gels.
   (iii) Ointments for rectal application: Prepare as directed under 11-4. Ointments.

For the preparations which are apt to deteriorate, prepare before use.

(3) Sufficient amounts of suitable preservatives to prevent the growth of microorganisms may be added to the Preparations filled in multiple dose containers.

(4) Semi-solid Preparations for Rectal Application have a suitable viscosity for applying to the rectum.

(5) Tight containers are usually used for Semi-solid Preparations for Rectal Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

9-3. Enemas for Rectal Application

(1) Enemas for Rectal Application are preparations in liquid form or viscous and gelatinous state, intended for application via the anus.

(2) Enemas for Rectal Application are usually prepared by dissolving or suspending active substance(s) in Purified Water or a suitable aqueous vehicle to make a given volume, and filling in containers. Dispersing agents, stabilizers and/or pH adjusting agents may be used.

(3) Tight containers are usually used for Enemas for Rectal Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

10. Preparations for Vaginal Application

10-1. Tablets for Vaginal Use

(1) Tablets for Vaginal Use are solid preparations of a desired shape and size, intended for application to the vagina, which release active substance(s) by dissolving or dispersing gradually in the secretions.

(2) Tablets for Vaginal Use are usually prepared as directed under 1-1. Tablets.

(3) Unless otherwise specified, Tablets for Vaginal Use meet the requirements of Uniformity of Dosage Units \(<6.02\)\).

(4) Tablets for Vaginal Use show an appropriate release.

(5) Well-closed containers are usually used for Tablets for Vaginal Use. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

10-2. Suppositories for Vaginal Use

(1) Suppositories for Vaginal Use are semi-solid preparations of a desired shape and size, intended for application to the vagina, which release active substance(s) by melting at body temperature or by dissolving or dispersing gradually in the secretions.

(2) Suppositories for Vaginal Use are prepared according to 9-1. Suppositories for Rectal Application.

(3) Suppositories for Vaginal Use are usually spherical or ovoid shaped.

(4) Unless otherwise specified, Suppositories for Vaginal Use meet the requirements of Uniformity of Dosage Units \(<6.02\)\).

(5) Suppositories for Vaginal Use show an appropriate release.

(6) Well-closed containers are usually used for Suppositories for Vaginal Use. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.
11. Preparations for Cutaneous Application

11-1. Solid Preparations for Cutaneous Application

1. Solid Preparations for Cutaneous Application are solid preparations intended for application to the skin (including scalp) or nails. Powders for Cutaneous Application are included in this category.

2. Unless otherwise specified, Solid Preparations for Cutaneous Application in single-dose packages meet the requirements of Uniformity of Dosage Units \(<6.02\>.

3. Well-closed containers are usually used for Solid Preparations for Cutaneous Application. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

11-1-1. Powders for Cutaneous Application

1. Powders for Cutaneous Application are powdery solid preparations intended for external application.

2. Powders for Cutaneous Application are usually prepared by mixing homogeneously active substance(s) and excipients such as diluents and pulverizing the mixture.

11-2. Liquids and Solutions for Cutaneous Application

1. Liquids and Solutions for Cutaneous Application are liquid preparations intended for application to the skin (including scalp) or nails. Liniments and Lotions are included in this category.

2. Liquids and Solutions for Cutaneous Application are usually prepared by mixing active substance(s) with excipients in a vehicle, and filtering if necessary.

For the preparations which are apt to deteriorate, prepare before use.

3. Unless otherwise specified, Liquids and Solutions for Cutaneous Application in single-dose packages meet the requirements of Uniformity of Dosage Units \(<6.02\>\), except for emulsified or suspended preparations.

4. Tight containers are usually used for Liquids and Solutions for Cutaneous Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

11-2-1. Liniments

1. Liniments are liquid or muddy preparations intended for external application to the skin by rubbing.

11-2-2. Lotions

1. Lotions are external liquids in which active substance(s) are dissolved, emulsified or finely dispersed in an aqueous vehicle.

2. Lotions are usually prepared by dissolving, suspending or emulsifying active substance(s) in Purified Water with excipients and making homogeneous as a whole.

3. Lotions in which the components have separated out during storage may be used after mixing to re-homogenize them, provided that the active substance(s) has not deteriorated.

11-3. Sprays for Cutaneous Application

1. Sprays for Cutaneous Application are preparations intended for spraying active substance(s) onto the skin in mists, powders, foams or paste state.

Sprays for Cutaneous Application are classified into Aerosols for Cutaneous Application and Pump Sprays for Cutaneous Application.

2. Sprays for Cutaneous Application are usually prepared by dissolving or suspending active substance(s) in a vehicle, filtering where necessary, and filling in containers.

3. Unless otherwise specified, metered-dose type sprays show an appropriate uniformity of delivered dose.

11-3-1. Aerosols for Cutaneous Application

1. Aerosols for Cutaneous Application are sprays which atomize active substance(s) together with liquefied or compressed gas filled in containers.

2. Aerosols for Cutaneous Application are usually prepared by dissolving or suspending active substance(s) in a vehicle, filling with liquefied propellants in pressure-resistant containers, and setting a continuous spray valve. If necessary, dispersing agents and stabilizers may be used.

3. Pressure-resistant containers are usually used for Aerosols for Cutaneous Application.

11-3-2. Pump Sprays for Cutaneous Application

1. Pump Sprays for Cutaneous Application are sprays which atomize active substance(s) in containers by pumping.

2. Pump Sprays for Cutaneous Application are usually prepared by dissolving or suspending active substance(s) with excipients in a vehicle, filling in containers and setting pumps to the containers.

3. Tight containers are usually used for Pump Sprays for Cutaneous Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.
11-4. Ointments

(1) Ointments are semi-solid preparations to be applied to the skin, which dissolve or disperse active substance(s) in a base. There are two types, hydrophobic ointments and hydrophilic ointments.

(2) Hydrophobic ointments are usually prepared by warming to melt hydrophobic bases such as fatty oils, waxes or paraffin, adding and mixing active substance(s) in the bases to be dissolved or dispersed, and kneading the whole to make homogeneous.

Hydrophilic ointments are usually prepared by warming to melt hydrophilic bases such as macrogol, adding and mixing active substance(s) in the bases, and kneading the whole to make homogeneous.

For Ointments which are apt to deteriorate, prepare before use.

(3) Ointments have a suitable viscosity for application to the skin.

(4) Tight containers are usually used for Ointments. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

11-5. Creams

(1) Creams are semi-solid preparations to be applied to the skin, which are in the form of oil-in-water or water-in-oil emulsions. Hydrophobic preparations in the form of water-in-oil emulsions may be termed “Oily creams”.

(2) Creams are usually prepared by mixing homogenously and emulsifying an oil-phase component and a water-phase component, both warmed, of which either one contains the active substance(s). These components have the following constituents.

Oil-phase component: Vaseline, fatty alcohols, etc., with or without emulsifying agent(s) or other suitable excipients.

Water-phase component: Purified Water with or without emulsifying agent(s) or other suitable excipients.

For Creams which are apt to deteriorate, prepare before use.

(3) Creams have a suitable viscosity for applying to the skin.

(4) Tight containers are usually used for Creams. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

11-6. Gels

(1) Gels are gelatinous preparations intended for application to the skin.

There are Aqueous Gels and Oily Gels.

(2) Gels are usually prepared by the following methods.

(i) Aqueous Gels: To active substance(s) add polymers, other excipients and Purified Water, dissolve or suspend, and gelatinize by warming and cooling or by adding a gelatinizing agents.

(ii) Oily Gels: To active substance(s) add liquid oily bases such as glycols, fatty alcohols and other excipients, and mix.

(3) Gels have a suitable viscosity for application to the skin.

(4) Tight containers are usually used for Gels. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

11-7. Patches

(1) Patches are preparations intended to be attached on the skin.

Patches are classified into Tapes/Plasters and Cataplasms/Gel Patches.

(2) Patches are usually prepared by mixing active substance(s) homogeneously with a base such as a polymer or a mixture of polymers, spreading on a backing layer or liner, and cutting into a given size. Percutaneous absorption type preparations may be prepared by using a release rate-controlling membrane. Where necessary, adhesive agents or penetration enhancers may be used.

(3) Unless otherwise specified, Patches of Transdermal Systems meet the requirements of Uniformity of Dosage Units.<6.02>.

(4) Patches have a suitable adhesion for application to the skin.

(5) Patches which are regulated the release rate have an appropriate function of controlled release.

11-7-1. Tapes/Plasters

(1) Tapes/Plasters are patches which are prepared with bases of practically no water contain.

Plasters are included in this category.

(2) Tapes/Plasters are usually prepared by mixing homogeneously active substance(s) with or without excipients and a base of non water-soluble natural or synthetic polymers such as resins, plastics or rubber, and spreading on a cloth or spreading and sealing on a cloth or plastic film, cutting into a given size. The preparations may be also prepared by filling a mixture of active substance(s) and a base with or without other excipients in releasers composed with a release-controlling film, supporter and liner.

(3) Well-closed containers are usually used for Tapes/Plasters. For the preparations susceptible to degradation by moisture, a moisture-proof container
or packaging may be used.

11-7-2. Cataplasms/Gel Patches

(1) Cataplasms/Gel Patches are patches using water containing bases.

(2) Cataplasms/Gel patches are usually prepared by mixing active substance(s), Purified Water, and Glycerin or other liquid materials, or by mixing and kneading natural or synthetic polymers, which are soluble in water or absorbent of water, with Purified Water, adding active substance(s), mixing the whole homogeneously, spreading on a cloth or film, and cutting into a given size.

(3) Tight containers are usually used for Cataplasms/Gel Patches. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

[3] Monographs for Preparations Related to Crude Drugs

Preparations Related to Crude Drugs

(1) Preparations related to crude drugs are preparations mainly derived from crude drugs. Extracts, Pills, Spirits, Infusions and Decoctions, Teabags, Tinctures, Aromatic Waters, and Fluidextracts are included in this category.

Definitions, methods of preparations, test methods, containers and packaging, and storage of these preparations are described in this chapter.

(2) The descriptions of the test methods and the containers and packaging in this chapter are fundamental requirements, and the preparation methods represent commonly used methods.

1. Extracts

(1) Extracts are preparations, prepared by concentrating extractives of crude drugs. There are following two kinds of extracts.

(i) Viscous extracts

(ii) Dry extracts

(2) Unless otherwise specified, Extracts are usually prepared as follows.

(i) Crude drugs, pulverized to suitable sizes, are extracted for a certain period of time with suitable solvents by means of cold extraction or warm extraction, or by percolation as directed in (ii) of (2) under 6. Tinctures. The extractive is filtered, and the filtrate is concentrated or dried by a suitable method to make a millet jelly-like consistency for the viscous extracts, or to make crushable solid masses, granules or powder for the dry extracts.

Extracts, which are specified the content of active substance(s), are prepared by assaying active substance(s) in a portion of sample and adjusting, if necessary, to specified strength with suitable diluents.

(ii) Weigh crude drugs, pulverized to suitable sizes, according to the prescription and heat for a certain period of time after adding 10 – 20 times amount of water. After separating the solid and liquid by centrifugation, the extractive is concentrated or dried by a suitable method to make a millet jelly-like consistency for the viscous extracts, or to make crushable solid masses, granules or powder for the dry extracts.

(3) Extracts have order and taste derived from the crude drugs used.

(4) Unless otherwise specified, Extracts meet the requirements of Heavy Metals Limit Test <1.07> when the test solution and the control solution are prepared as follows.

Test solution: Ignite 0.30 g of Extracts to ash, add 3 mL of dilute hydrochloric acid, warm, and filter. Wash the residue with two 5-mL portions of water. Neutralize the combined filtrate and washings (indicator: a drop of phenolphthalein TS) by adding ammonia TS until the color of the solution changes to pale red, filter where necessary, and add 2 mL of dilute acetic acid and water to make 50 mL.

Control solution: Proceed with 3 mL of dilute hydrochloric acid in the same manner as directed in the preparation of the test solution, and add 3.0 mL of Standard Lead Solution and water to make 50 mL.

(5) Tight containers are used for these preparations.

2. Pills

(1) Pills are spherical preparations, intended for oral administration.

(2) Pills are usually prepared by mixing drug substance(s) uniformly with diluents, binders, disintegrators or other suitable excipient(s) and rolling into spherical form by a suitable method. They may be coated with a coating agent by a suitable method.

(3) Unless otherwise specified, Pills comply with Disintegration Test <6.09>.

(4) Well-closed or tight containers are usually used for these preparations.

3. Spirits

(1) Spirits are fluid preparations, usually prepared by dissolving volatile drug substance(s) in ethanol or in a mixture of ethanol and water.

(2) Spirits should be stored remote from fire.

(3) Tight containers are used for these preparations.
4. Infusions and Decoctions

(1) Infusions and Decoctions are fluid preparations, usually obtained by macerating crude drugs in water.

(2) Infusions and Decoctions are usually prepared by the following method.

Cut crude drugs into a size as directed below, and transfer suitable amounts to an infusion or decoction apparatus.

Leaves, flowers and whole plants: Coarse cutting
Woods, stems, barks, roots and rhizomes: Medium cutting
Seeds and fruits: Fine cutting

(i) Infusions: Usually, damp 50 g of crude drugs with 50 mL of water for about 15 minutes, pour 900 mL of hot water to them, and heat for 5 minutes with several stirrings. Filter through a cloth after cooling.

(ii) Decoctions: Usually, heat one-day dose of crude drugs with 400 – 600 mL of water until to lose about a half amount of added water spending more than 30 minutes, and filter through a cloth while warm.

Prepare Infusions or Decoctions when used.

(3) These preparations have odor and taste derived from the crude drugs used.

(4) Tight containers are usually used for these preparations.

5. Teabags

(1) Teabags are preparations, usually packed one-day dose or one dose of crude drugs cut into a size of between coarse powder and coarse cutting in paper or cloth bags.

(2) Teabags are usually used according to the preparation method as directed under 4. Infusions and Decoctions.

(3) Well-closed or tight containers are usually used for these preparations.

6. Tinctures

(1) Tinctures are liquid preparations, usually prepared by extracting crude drugs with ethanol or with a mixture of ethanol and purified water.

(2) Unless otherwise specified, Tinctures are usually prepared from coarse powder or fine cuttings of crude drugs by means of either maceration or percolation as described below.

(i) Maceration: Place crude drugs in a suitable container, and add an amount of a solvent, equivalent to the same volume or about three-fourths of the volume of the crude drugs. Stopper container, and allow the container to stand for about 5 days or until the soluble constituents have satisfactorily dissolved at room temperature with occasional stirring. Separate the solid and liquid by centrifugation or other suitable methods. In the case where about three-fourths volume of the solvent is added, wash the residue with a suitable amount of the solvent, and squeeze the residue, if necessary. Combine the extract and washings, and add sufficient solvent to make up the volume. In the case where the total volume of the solvent is added, sufficient amounts of the solvent may be added to make up for reduced amount, if necessary. Allow the mixture to stand for about 2 days, and obtain a clear liquid by decantation or filtration.

(ii) Percolation: Pour solvent in small portions to crude drugs placed in a container, and mix well to moisten the crude drugs. Stopper container, and allow it to stand for about 2 hours at room temperature. Pack the contents as tightly as possible in an appropriate percolator, open the lower opening, and slowly pour sufficient solvent to cover the crude drugs. When the percolate begins to drip, close the opening, and allow the mixture to stand for 2 to 3 days at room temperature. Then, open the opening, and allow the percolate to drip at a rate of 1 to 3 mL per minute. Add an appropriate quantity of the solvent to the percolator, and continue to percolate until the desired volume has passed. Mix thoroughly, allow standing for 2 days, and obtain a clear liquid by decantation or filtration. The time of standing and the flow rate may be varied depending on the kind and amount of crude drugs to be percolated.

Tinctures, prepared by either of the above methods and specified the content of marker constituent or ethanol, are prepared by assaying the content using a portion of the sample and adjusting the content with a sufficient amount of the percolate or solvent as required on the basis of the result of the assay.

(3) Tinctures should be stored remote from fire.

(4) Tight containers are used for these preparations.

7. Aromatic Waters

(1) Aromatic Waters are clear liquid preparations, saturated essential oils or other volatile substances in water.

(2) Unless otherwise specified, Aromatic Waters are usually prepared by the following process.

Shake thoroughly for 15 minutes 2 mL of an essential oil or 2 g of a volatile substance with 1000 mL of lukewarm purified water, set the mixture aside for 12 hours or longer, filter through moistened filter paper, and add purified water to make 1000 mL. Alterna-
tively, incorporate thoroughly 2 mL of an essential oil or 2 g of a volatile substance with sufficient talc, refined siliceous earth or pulped filter-paper, add 1000 mL of purified water, agitate thoroughly for 10 minutes, and then filter the mixture. To obtain a clear filtrate repeat the filtration if necessary, and add sufficient purified water passed through the filter paper to make 1000 mL.

3) Aromatic Waters have odor and taste derived from the essential oils or volatile substances used.

4) Tight containers are used for these preparations.

8. Fluidextracts

(1) Fluidextracts are liquid percolates of crude drugs, usually prepared so that each mL contains soluble constituents from 1 g of the crude drugs. Where the content is specified, it takes precedence.

(2) Unless otherwise specified, Fluidextracts are usually prepared from coarse powder or fine cutting of crude drugs by either of following maceration or percolation.

(i) Maceration: Place a certain amounts of crude drugs in a suitable vessel, add a solvent to cover the crude drugs, close the vessel, and allow the vessel to stand at room temperature with occasional stirring for about 5 days or until the soluble constituents have satisfactorily dissolved. Separate the solid and liquid by centrifugation or other suitable method. Usually, reserve a volume of the liquid equivalent to about three-fourths of the total volume, and use it as the first liquid. Wash the residue with appropriate amount of the solvent, combine the washings and the remaining of the first liquid, concentrate if necessary, mix with the first liquid, and use it as solution (A). To the solution (A) add the solvent, if necessary, to make equal amount of the mass of the crude drugs. Allow the mixture to stand for about 2 days, and collect a clear liquid by decantation or filtration.

(ii) Percolation: Mix well 1000 g of the crude drugs with the first solvent to moisten them, close the container, and allow it to stand for about 2 hours at room temperature. Transfer the content to a suitable percolator, stuff it as tightly as possible, open the lower opening of the percolator, and slowly pour the second solvent to cover the crude drugs. Close the lower opening when the solvent begins to drop, and allow the mixture to stand for 2 to 3 days at room temperature. Open the lower opening, and allow the percolate to run out at the rate of 0.5 to 1.0 mL per minute.

Set aside the first 850 mL of the percolate as the first percolate. Add the second solvent to the percolator, then drip the percolate, and use it as the second percolate.

The period of standing and the flow rate during percolation may be varied depending on the kind and the amount of crude drugs used. The flow rate is usually regulated as follows, depending on the using amount of crude drugs.

<table>
<thead>
<tr>
<th>Mass of crude drug</th>
<th>Volume of solution running per minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not more than 1000 g</td>
<td>0.5 – 1.0 mL</td>
</tr>
<tr>
<td>Not more than 3000 g</td>
<td>1.0 – 2.0 mL</td>
</tr>
<tr>
<td>Not more than 10000 g</td>
<td>2.0 – 4.0 mL</td>
</tr>
</tbody>
</table>

Concentrate the second percolate, taking care not to lose the volatile substances of the crude drug, mix with the first percolate, and use it as solution (A). To the solution (A) add the second solvent to make 1000 mL, and allow the mixture to stand for about 2 days. Decant the supernatant liquid or filter the liquid to obtain a clear solution.

Fluidextracts for which the content of marker constituent or ethanol is specified are obtained by adjusting the content with a sufficient amount of the second solvent as required on the basis of the result of the assay made with a portion of the solution (A).

(3) Fluidextracts have odor and taste derived from the crude drugs used.

(4) Unless otherwise specified, Fluidextracts meet the requirements of Heavy Metals Limit Test <1.07> when the test solution and the control solution are prepared as follows.

Test solution: Ignite 1.0 g of Fluidextracts to ash, add 3 mL of dilute hydrochloric acid, warm, and filter. Wash the residue with two 5-mL portions of water. Neutralize the combined filtrate and washings (indicator: a drop of phenolphthalein TS) by adding ammonia TS until the color of the solution changes to pale red, filter if necessary, and add 2 mL of the dilute acetic acid and water to make 50 mL.

Control solution: Proceed with 3 mL of dilute hydrochloric acid in the same manner as directed in the preparation of the test solution, and add 3.0 mL of Standard Lead Solution and water to make 50 mL.

(5) Tight containers are used for these preparations.
GENERAL TESTS, PROCESSES AND APPARATUS

General Tests, Processes and Apparatus includes common methods for tests, useful test methods for quality recognition of drugs and other articles related to them. Unless otherwise specified, acid-neutralizing capacity determination of gastrointestinal medicines, alcohol number determination, amino acid analysis of proteins, ammonium determination, arsenic determination, atomic absorption spectrophotometry, boiling point determination, chloride determination, conductivity measurement, congealing point determination, determination of bulk and tapped densities, digestion test, disintegration test, dissolution test, distilling range determination, endpoint determination in titrimetry, flame coloration, fluorometry, foreign insoluble matter test for injections, foreign soluble matter test for ophthalmic solutions, gas chromatography, heavy metal determination, infrared spectrophotometry, insoluble particulate matter test for injections, insusolate particulate matter test for ophthalmic solutions, iron determination, liquid chromatography, loss on drying determination, loss on ignition determination, melting point determination, methanol determination, microbial assay for antibiotics, mineral oil determination, nitrogen determination, nuclear magnetic resonance spectroscopy, optical rotation determination, osmolality determination, oxygen flask combustion method, particle size determination, particle size distribution test for preparations, pH determination, powder particle density determination, qualitative test, refractive index determination, residual solvents test, residue on ignition determination, specific gravity and density determination, specific surface area determination, sulfate determination, test for bacterial endotoxins, test for glass containers for injections, test for metal particles in ophthalmic ointments, test for microbial limit, test for microbial limit for crude drugs, test for plastic containers, test for pyrogen, test for readily carbonizable substances, test for rubber closure for aqueous infusions, test for stability, test for total organic carbon, test of extractable volume for injection, thermal analysis, thin-layer chromatography, ultraviolet-visible spectrophotometry, uniformity of dosage units (test for content uniformity, mass variation test), viscosity determination, vitamin A assay, water determination, and X-ray powder diffraction are performed as directed in the corresponding articles under the General Tests, Processes and Apparatus. The tests for melting point of fats, congealing point of fatty acids, specific gravity, acidity, saponification value, ester value, hydroxyl value, unsaponifiable matter and iodine value of fats and fatty oils are performed as directed in the corresponding items under Fats and Fatty Oils Test, and sampling, preparation of sample for analysis, microscopic examination, purity test, loss on drying, total ash, acid-insoluble ash, extract content, and essential oil content of crude drugs are performed as directed in the corresponding items under Crude Drugs Test.

The number of each test method is a category number given individually. The number in brackets (<>) appeared in monograph indicates the number corresponding to the general test method.

1. Chemical Methods

1.01 Alcohol Number Determination

Alcohol Number Determination represents the number of milliliters of ethanol at 15°C obtained from 10 mL of tincture or other preparations containing ethanol by the following procedures.

1. Method 1 Distilling method

This is a method to determine the Alcohol Number by reading the number of milliliters of ethanol distillate at 15°C obtained from 10 mL of a sample measured at 15°C by the following procedures.

1.1. Apparatus

Use hard glass apparatus as illustrated in Fig. 1.01-1. Ground glass may be used for the joints.

1.2. Reagent

Alkaline phenolphthalein solution: To 1 g of phenolphthalein add 7 mL of sodium hydroxide TS and water to make 100 mL.

1.3. Procedure

Transfer 10 mL of the sample preparation, accurately measured at 15 ± 2°C, to the distilling flask A, add 5 mL of water and boiling chips. Distil ethanol carefully into the glass-stoppered, volumetric cylinder D.

By reference to Table 1.01-1, a suitable volume of distillate (mL) should be collected, according to the content of ethanol in the sample preparation.

Prevent bumping during distillation by rendering the sample strongly acidic with phosphoric acid or sulfuric acid, or by adding a small amount of paraffin, beeswax or silicone resin before starting the distillation.

Table 1.01-1

<table>
<thead>
<tr>
<th>Ethanol content in the sample (vol%)</th>
<th>Distillate to be collected (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>more than 80</td>
<td>13</td>
</tr>
<tr>
<td>80 – 70</td>
<td>12</td>
</tr>
<tr>
<td>70 – 60</td>
<td>11</td>
</tr>
<tr>
<td>60 – 50</td>
<td>10</td>
</tr>
<tr>
<td>50 – 40</td>
<td>9</td>
</tr>
<tr>
<td>40 – 30</td>
<td>8</td>
</tr>
<tr>
<td>less than 30</td>
<td>7</td>
</tr>
</tbody>
</table>
When the samples contain the following substances, carry out pretreatment as follows before distillation.

(i) Glycerin: Add sufficient water to the sample so that the residue in the distilling flask, after distillation, contains at least 50% of water.

(ii) Iodine: Decolorize the sample with zinc powder.

(iii) Volatile substances: Preparations containing appreciable proportions of essential oil, chloroform, diethyl ether or camphor require treatment as follows. Mix 10 mL of the sample, accurately measured, with 10 mL of saturated sodium chloride solution in a separator, add 10 mL of petroleum benzin, and shake. Collect the separated aqueous layer. The petroleum benzin layer was extracted with two 5 mL portions of saturated sodium chloride solution. Combine the aqueous layers, and distill. According to the ethanol content in the sample, collect a volume of distillate 2 to 3 mL more than that shown in the above Table.

(iv) Other substances: Render preparations containing free ammonia slightly acidic with dilute sulfuric acid. If volatile acids are present, render the preparation slightly alkaline with sodium hydroxide TS, and if the preparations contain soap along with volatile substances, decompose the soap with an excess of dilute sulfuric acid before the extraction with petroleum benzin in the treatment described in (iii).

To the distillate add 4 to 6 g of potassium carbonate and 1 to 2 drops of alkaline phenolphthalein solution, and shake vigorously. If the aqueous layer shows no white turbidity, agitate the distillate with additional potassium carbonate. After allowing to stand in water at 15 ± 2°C for 30 minutes, read the volume of the upper reddish ethanol layer in mL, and regard it as the Alcohol Number. If there is no clear boundary surface between these two layers, shake vigorously after addition of a few drops of water, then observe in the same manner.

2. Method 2 Gas chromatography

This is a method to determine the alcohol number by determining ethanol (C₂H₅OH) content (vol%) from a sample measured at 15°C by the following procedures.

2.1. Reagent

Ethanol for alcohol number: Ethanol (99.5) with determined ethanol (C₂H₅OH) content. The relation between specific gravity \( d_{15}^{15} \) of ethanol and content of ethanol (C₂H₅OH) is 0.797:99.46 vol%, 0.796:99.66 vol%, and 0.795:99.86 vol%.

2.2. Preparation of sample solution and standard solution

Sample solution: Measure accurately a volume of sample at 15 ± 2°C equivalent to about 5 mL of ethanol (C₂H₅OH), and add water to make exactly 50 mL. Measure accurately 25 mL of this solution, add exactly 10 mL of the internal standard solution, and add water to make 100 mL.

Standard solution: Measure accurately 5 mL of ethanol for alcohol number at the same temperature as the sample, and add water to make exactly 50 mL. Measure accurately 25 mL of this solution, add exactly 10 mL of the internal standard solution, and add water to make 100 mL.

2.3. Procedure

Place 25 mL each of the sample solution and the standard solution in a 100-mL, narrow-mouthed, cylindrical glass bottle sealed tightly with a rubber closure and aluminum band, immerse the bottle up to the neck in water, allowed to stand at room temperature for more than 1 hour in a room with little change in temperature, shake gently so as not to splash the solution on the closure, and allow to stand for 30 minutes. Perform the test with 1 mL each of the gas in the bottle with a syringe according to the Gas Chromatography <2.02> under the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak height of ethanol to that of the internal standard.

\[
\text{Alcohol number} = \frac{Q_T}{Q_S} \times \frac{5 \text{ (mL)}}{\text{a volume (mL) of sample}} \times \frac{\text{ethanol (C}_2\text{H}_5\text{OH) content (vol\%) of ethanol for alcohol number}}{9.406}
\]

*Internal standard solution*—A solution of acetonitrile (3 in 50).

*Operating conditions*—

Detector: A hydrogen flame-ionization detector.

Column: A glass tube about 3 mm in inside diameter and about 1.5 m in length, packed with 150- to 180-μm porous ethylvinylbenzene-divinylbenzene copolymer (mean pore
1.02 Ammonium Limit Test

Ammonium Limit Test is a limit test for ammonium contained in drugs.

In each monograph, the permissible limit for ammonium (as NH$_4^+$) is described in terms of percentage (%) in parentheses.

1. Apparatus

Use a distilling apparatus for ammonium limit test as illustrated in Fig. 1.02-1. For the distillation under reduced pressure, use the apparatus shown in Fig. 1.02-2. Either apparatus are composed of hard glass, and ground-glass joints may be used. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

2. Procedure

2.1. Preparation of test solution and control solution

Unless otherwise specified, test solutions and control solution are prepared as directed in the following.

Place an amount of the sample, directed in the monograph, in the distilling flask A. Add 140 mL of water and 2 g of magnesium oxide, and connect the distillation apparatus. To the receiver (measuring cylinder) F add 20 mL of boric acid solution (1 in 200) as an absorbing solution, and immerse the lower end of the condenser. Adjust the heating to give a rate of 5 to 7 mL per minute of distillate, and distill until the distillate measures 60 mL. Remove the receiver from the lower end of the condenser, rinsing the end part with a small quantity of water, add sufficient water to make 100 mL and designate it as the test solution.

For the distillation under reduced pressure, take the amount of sample specified in the monograph to the vacuum distillation flask L, add 70 mL of water and 1 g of magnesium oxide, and connect to the apparatus (Fig. 1.02-2). To the receiver M add 20 mL of a solution of boric acid (1 in 200) as absorbing liquid, put the end of the branch tube of the distillation flask L in the absorbing liquid, and keep at 60°C using a water bath or alternative equipment. Adjust the reduced pressure to get the distillate at a rate of 1 to 2 mL per minute, and continue the distillation until to get 30 mL of the distillate. Cool the receiver M with running water during the distillation. Get off the end of the branch tube from surface of the absorbing liquid, rinse in the end with a small amount of water, then add water to the liquid to make 100 mL, and perform the test using this solution as the test solution.

Place a volume of Standard Ammonium Solution, directed in the monograph, in the distilling flask A or the vacuum distillation flask L, proceed as for the preparation.
1.03 Chloride Limit Test

Chloride Limit Test is a limit test for chloride contained in drugs.

In each monograph, the permissible limit for chloride (as Cl) is described in terms of percentage (%) in parentheses.

1. Procedure

Unless otherwise specified, transfer the quantity of the sample, directed in the monograph, to a Nessler tube, and dissolve it in a proper volume of water to make 40 mL. Add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the test solution. Transfer the volume of 0.01 mol/L hydrochloric acid VS, directed in the monograph, to another Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution.

When the test solution is not clear, filter both solutions by using the same procedure.

Add 1 mL of silver nitrate TS to the test solution and to the control solution, mix well, and allow to stand for 5 minutes protecting from light. Compare the opalescence developed in both solutions against a white background by viewing downward or transversely: the color developed in the test solution is not more intense than that of the control solution.

1.04 Flame Coloration Test

Flame Coloration Test is a method to detect an element, by means of the property that the element changes the colorless flame of a Bunsen burner to its characteristic color.

(1) Salt of metal—The platinum wire used for this test is about 0.8 mm in diameter, and the end part of it is straight. In the case of a solid sample, make the sample into a gruel by adding a small quantity of hydrochloric acid, apply a little of the gruel to the 5-mm end of the platinum wire, and test by putting the end part in a colorless flame, keeping the platinum wire horizontal. In the case of a liquid sample, immerse the end of the platinum wire into the sample to about 5 mm in length, remove from the sample gently, and perform the test in the same manner as for the solid sample.

(2) Halide—Cut a copper net, 0.25 mm in opening and 0.174 mm in wire diameter, into a strip 1.5 cm in width and 5 cm in length, and wind in round one end of a copper wire. Heat the copper net strongly in the colorless flame of Bunsen burner until the flame no longer shows a green or blue color, and then cool it. Repeat this procedure several times, and coat the net completely with cupric oxide. After cooling, unless otherwise specified, apply about 1 mg of the sample to the copper net, ignite, and burn it. Repeat this procedure three times, and then test by putting the copper net in the colorless flame.

The description, “Flame coloration persists”, in a monograph, indicates that the reaction persists for 4 seconds.

1.05 Mineral Oil Test

Mineral Oil Test is a method to test mineral oil in nonaqueous solvents for injections and for eye drops.

1. Procedure

Pour 10 mL of the sample into a 100-mL flask, and add 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of ethanol (95). Put a short-stemmed, small funnel on the neck of the flask, and heat on a water bath to make clear, with frequent shaking. Then transfer the solution to a shallow porcelain dish, evaporate the ethanol on a water bath, add 100 mL of water to the residue, and heat on a water bath: no turbidity is produced in the solution.

1.06 Oxygen Flask Combustion Method

Oxygen Flask Combustion Method is a method for the identification or the determination of halogens or sulfur produced by combusting organic compounds, which contain chlorine, bromine, iodine, fluorine or sulfur, in a flask filled with oxygen.

1. Apparatus

Use the apparatus shown in Fig. 1.06-1.

2. Preparation of test solution and blank solution

Unless otherwise specified, prepare them by the following method.

2.1. Preparation of sample

(i) For solid samples: Place the quantity of the sample specified in the monograph on the center of the filter illustrated in the figure, weigh accurately, wrap the sample carefully along the dotted line without scattering, and place the parcel in a platinum basket or cylinder B, leaving its fusetrip on the outside.

(ii) For liquid samples: Roll a suitable amount of absorbent cotton with filter paper, 50 mm in length and 5 mm in width, so that the end part of the paper is left to a length of about 20 mm as a fuse-strip, and place the parcel in a platinum basket or cylinder B. Place the sample in a suitable glass tube, weigh accurately, and moisten the cotton with the quantity of the sample specified in the monograph, bringing the edge of the sample in contact with the cotton.

2.2. Method of combustion

Place the absorbing liquid specified in the monograph in flask A, fill it with oxygen, moisten the ground part of the stopper C with water, then ignite the fuse-strip, immediately transfer it to the flask, and keep the flask airtight until the combustion is completed. Shake the flask occasionally until
3. Procedure of determination

3.1. Chlorine and bromine

Apply a small amount of water to the upper part of A, pull out C carefully, and transfer the test solution to a beaker. Wash C, B and the inner side of A with 15 mL of 2-propanol, and combine the washings with the test solution. To this solution add 1 drop of bromophenol blue TS, then add dilute nitric acid dropwise until a yellow color develops, and titrate \(<2.50\) with 0.005 mol/L silver nitrate VS according to the potentiometric titration. Perform the test with the blank solution in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L silver nitrate VS = 0.6345 mg of Cl

Each mL of 0.005 mol/L silver nitrate VS = 0.3995 mg of Br

3.2. Iodine

Apply a small amount of water to the upper part of A, pull out C carefully, add 2 drops of hydrazine hydrate to the test solution, put C on A, and decolorize the solution by vigorous shaking. Transfer the content of A to a beaker, wash C, B and the inner side of A with 25 mL of 2-propanol, and transfer the washings to the above beaker. To this solution add 1 drop of bromophenol blue TS, then add dilute nitric acid dropwise until a yellow color develops, and titrate \(<2.50\) with 0.005 mol/L silver nitrate VS according to the potentiometric titration. Perform the test with the blank solution in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L silver nitrate VS

3.3. Fluorine

Apply a small amount of water to the upper part of A, pull out C carefully, transfer the test solution and the blank solution to 50 mL volumetric flasks separately, wash C, B and the inner side of A with water, add the washings and water to make 50 mL, and use these solutions as the test solution and the correction solution. Pipet the test solution \((V\ mL)\) equivalent to about 30 \(\mu\)g of fluorine, add 25 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, and allow to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>, using a blank prepared with 5 mL of water in the same manner. Determine the absorbances, \(A_T\), \(A_C\) and \(A_S\) of the subsequent solutions of the test solution, the correction solution and the standard solution at 600 nm.

\[
\text{Amount (mg) of fluorine (F) in the test solution} = \frac{\text{amount (mg) of fluorine in 5 mL of the standard solution} \times (A_T - A_C) \times 50}{V}
\]

Standard Fluorine Solution: Dry sodium fluoride (standard reagent) in a platinum crucible between 500°C and 550°C for 1 hour, cool it in a desiccator (silica gel), weigh accurately about 66.3 mg of it, and dissolve in water to make exactly 500 mL. Pipet 10 mL of this solution, and dilute with sufficient water to make exactly 100 mL.

3.4. Sulfur

Apply a small amount of water to the upper part of A, pull out C carefully, and wash C, B and the inner side of A with 15 mL of methanol. To this solution add 40 mL of methanol, then add exactly 25 mL of 0.005 mol/L barium perchlorate VS, allow to stand for 10 minutes, add 0.15 mL of arsenazo III TS with a measuring pipet, and titrate \(<2.50\) with 0.005 mol/L sulfuric acid VS. Perform the test with the blank solution in the same manner.

Each mL of 0.005 mol/L barium perchlorate VS

1.07 Heavy Metals Limit Test

Heavy Metals Limit Test is a limit test of the quantity of heavy metals contained as impurities in drugs. The heavy metals are the metallic inclusions that are darkened with so-
dium sulfide TS in acidic solution, as their quantity is expressed in terms of the quantity of lead (Pb).

In each monograph, the permissible limit for heavy metals (as Pb) is described in terms of ppm in parentheses.

1. Preparation of test solutions and control solutions

Unless otherwise specified, test solutions and control solutions are prepared as directed in the following:

1.1. Method 1

Place an amount of the sample, directed in the monograph, in a Nessler tube. Dissolve in water to make 40 mL. Add 2 mL of dilute acetic acid and water to make 50 mL, and designate it as the test solution.

The control solution is prepared by placing the volume of Standard Lead Solution directed in the monograph in a Nessler tube, and adding 2 mL of dilute acetic acid and water to make 50 mL.

1.2. Method 2

Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle ignition. After cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat cautiously until white fumes are no longer evolved, and incinerate by ignition between 500°C and 600°C. Cool, add 2 mL of hydrochloric acid, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Then add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate a mixture of 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid on a water bath, further evaporate to dryness on a sand bath, and moisten the residue with 3 drops of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

1.3. Method 3

Place an amount of the sample, directed in the monograph, in a platinum or porcelain crucible, mix with 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and carbonize by gradual heating. Cool, add 1 mL of sulfuric acid, heat carefully, and incinerate by ignition between 500°C and 600°C. If a carbonized substance remains, moisten with a small amount of sulfuric acid, and incinerate by ignition. Cool, dissolve the residue in 3 mL of hydrochloric acid, evaporate on a water bath to dryness, wet the residue with 3 drops of hydrochloric acid, add 10 mL of water, and dissolve by warming. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until a pale red color develops, then add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and the washing to a Nessler tube, add water to make 50 mL, and use this solution as the test solution.

The control solution is prepared as follows: Take 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. Cool, add 1 mL of sulfuric acid, heat carefully, and ignite between 500°C and 600°C. Cool, and add 3 mL of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

2. Procedure

Add 1 drop of sodium sulfide TS to each of the test solution and the control solution, mix thoroughly, and allow to stand for 5 minutes. Then compare the colors of both solutions by viewing the tubes downward or transversely against a white background. The test solution has no more color than the control solution.

1.08 Nitrogen Determination (Semimicro-Kjeldahl Method)

Nitrogen Determination is a method to determine nitrogen in an organic substance in which the nitrogen is converted into ammonia nitrogen by thermal decomposition of the organic substance with sulfuric acid, and the ammonia liberated by alkali and trapped by distillation with steam is determined by titration.

1. Apparatus

Use the apparatus illustrated in Fig. 1.08-1. It is thoroughly constructed of hard glass, and ground glass surfaces may be used for joints. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

Alternatively, apparatus can be used in which some of the procedures, such as digestion of organic substances, distillation of the liberated ammonia, and endpoint detection methods in titrimetry (e.g., potentiometric titration or titration by colorimeter) are automated.

2. System suitability

If an automated apparatus is used, it is necessary to confirm periodically the suitability of the apparatus according to the following method:

Weigh accurately about 1.7 g of amidosulfuric acid (standard reagent), previously dried in a desicator (in vacuum, silica gel) for about 48 hours, dissolve in water to make exactly 200 mL. Pipet 2 mL of this solution, and transfer to a
digestion flask. When the test is performed as directed in the
instrumental manual the nitrogen content (%) in amidosulfuric acid should be determined between 14.2% and 14.6%.

3. Reagents, Test Solutions
Decomposition accelerator: Unless otherwise specified, use 1 g of a powdered mixture of 10 g of potassium sulfate and 1 g of copper (II) sulfate pentahydrate. The composition and amount of the digestion accelerator may be modified if it is confirmed that the modified one give almost the same results using the sample as those obtained from the conventional catalyst.

4. Procedure
Unless otherwise specified, proceed by the following method. Weigh accurately or pipet a quantity of the sample corresponding to 2 to 3 mg of nitrogen (N:14.01), and place in the Kjeldahl flask A. Add the decomposition accelerator and wash down any adhering sample from the neck of the flask with a small quantity of water. Add 7 mL of sulfuric acid, allowing it to flow down the inside wall of the flask.

Then, while shaking the flask, add cautiously 1 mL of hydrogen peroxide (30) drop by drop along the inside wall of the flask. Heat the flask gradually, then heat so strong that

the vapor of sulfuric acid is condensed at the neck of the flask, until the solution changes through a blue and clear to a vivid green and clear, and the inside wall of the flask is free from a carbonaceous material. If necessary, add a small quantity of hydrogen peroxide (30) after cooling, and heat again. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus (Fig. 1.08.1) washed beforehand by passing stream through it. To the absorption flask K add 15 mL of boric acid solution (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube J. Add 30 mL of sodium hydroxide solution (2 in 5) through the funnel F, rinse cautiously the funnel with 10 mL of water, lose the clamp attached to the rubber tubing G, then begin the distillation with stream, and continue until the distillate measures 80 to 100 mL. Remove the absorption flask from the lower end of the condenser tube J, rinsing the end part with a small quantity of water, and titrate with 0.005 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L sulfuric acid VS

= 0.1401 mg of N

If an automated apparatus is used, proceed as directed in the instrumental procedure.

1.09 Qualitative Tests

Qualitative Tests are applied to the identification of drugs and are done generally with quantities of 2 to 5 mL of the test solution.

Acetate
(1) When warmed with diluted sulfuric acid (1 in 2), acetates evolve the odor of acetic acid.
(2) When an acetate is warmed with sulfuric acid and a small quantity of ethanol (95), the odor of ethyl acetate is evolved.
(3) Neutral solutions of acetates produce a red-brown color with iron (III) chloride TS, and a red-brown precipitate when boiled. The precipitate dissolves and the color of the solution changes to yellow upon addition of hydrochloric acid.

Aluminum salt
(1) Solutions of aluminum salts, when treated with ammonium chloride TS and ammonia TS, yield a gelatinous, white precipitate which does not dissolve in an excess of ammonia TS.
(2) Solutions of aluminum salts, when treated with sodium hydroxide TS, yield a gelatinous, white precipitate which dissolves in an excess of the reagent.
(3) Solutions of aluminum salts, when treated with sodium sulfide TS, yield a gelatinous, white precipitate which dissolves in an excess of the reagent.
(4) Add ammonia TS to solutions of aluminum salts until a gelatinous, white precipitate is produced. The color of the precipitate changes to red upon addition of 5 drops of alizarin red S TS.
Ammonium salt
When heated with an excess of sodium hydroxide TS, ammonium salts evolve the odor of ammonia. This gas changes moistened red litmus paper to blue.

Antimony salt, primary
1. When primary antimony salts are dissolved in a slight excess of hydrochloric acid for the test and then diluted with water, a white turbidity is produced. The mixture produces an orange precipitate upon addition of 1 to 2 drops of sodium sulfide TS. When the precipitate is separated, and sodium sulfide TS is added to one portion of the precipitate and sodium hydroxide TS is added to another portion, it dissolves in either of these reagents.
2. Add water to acidic solutions of primary antimony salts in hydrochloric acid until a small quantity of precipitate is produced, and then add sodium thiosulfate TS: the precipitate dissolves. A red precipitate is reproduced when the solution is heated.

Aromatic amines, primary
Acidic solutions of primary aromatic amines, when cooled in ice, mixed with 3 drops of sodium nitrite TS under agitation, allowed to stand for 2 minutes, mixed well with 1 mL of ammonium amidosulfate TS, allowed to stand for 1 minute, and then mixed with 1 mL of N,N-diethyl-N'-1-naphthylethylenediamine oxalate TS, exhibit a red-purple color.

Arsenate
1. Neutral solutions of arsenates produce no precipitate with 1 to 2 drops of sodium sulfide TS, but produce a yellow precipitate with hydrochloric acid subsequently added. The separated precipitate dissolves in ammonium carbonate TS.
2. Neutral solutions of arsenates produce a dark red-brown precipitate with silver nitrate TS. When dilute nitric acid is added to one portion of the suspension, and ammonium TS is added to another portion, the precipitate dissolves in either of these reagents.
3. Neutral or ammonia alkaline solutions of arsenates produce with magnesia TS a white, crystalline precipitate, which dissolves by addition of dilute hydrochloric acid.

Arsenite
1. Acidic solutions of arsenites in hydrochloric acid produce a yellow precipitate with 1 to 2 drops of sodium sulfide TS. When hydrochloric acid is added to one portion of the separated precipitate, it does not dissolve. When ammonium carbonate TS is added to another portion, the precipitate dissolves.
2. Slightly alkaline solutions of arsenites produce a yellowish white precipitate with silver nitrate TS. When ammonium TS is added to one portion of the suspension, and dilute nitric acid is added to another portion, the precipitate dissolves in either of these reagents.
3. Slightly alkaline solutions of arsenites produce a green precipitate with copper (II) sulfate TS. When the separated precipitate is boiled with sodium hydroxide TS, it changes to red-brown.

Barium salt
1. When the Flame Coloration Test (1) C1.04 is applied to barium salts, a persistent yellow-green color develops.
2. Solutions of barium salts produce with dilute sulfuric acid a white precipitate, which does not dissolve upon addition of dilute nitric acid.
3. Acidic solutions of barium salts in acetic acid produce a yellow precipitate with potassium chromate TS. The precipitate dissolves by addition of dilute nitric acid.

Bicarbonate
1. Bicarbonates effervesce upon addition of dilute hydrochloric acid, generating a gas, which produces a white precipitate immediately, when passed into calcium hydroxide TS (common with carbonates).
2. Solutions of bicarbonates produce no precipitate with magnesium sulfate TS, but produce a white precipitate when boiled subsequently.
3. A cold solution of bicarbonates remains unchanged or exhibits only a slightly red color upon addition of 1 drop of phenolphthalein TS (discrimination from carbonates).

Bismuth salt
1. Bismuth salts, dissolved in a slight excess of hydrochloric acid, yield a white turbidity upon dilution with water. A dark brown precipitate is produced with 1 to 2 drops of sodium sulfide TS subsequently added.
2. Acidic solutions of bismuth salts in hydrochloric acid exhibit a yellow color upon addition of thiourea TS.
3. Solution of bismuth salts in dilute nitric acid or in dilute sulfuric acid yield with potassium iodide TS a black precipitate, which dissolves in an excess of the reagent to give an orange-colored solution.

Borate
1. When ignite a mixture of a borate with sulfuric acid and methanol, it burns with a green flame.
2. Turmeric paper, when moistened with acidic solutions of borates in hydrochloric acid and dried by warming, exhibits a red color, which changes to blue with ammonia TS added dropwise.

Bromate
1. Acidic solutions of bromates in nitric acid yield with 2 to 3 drops of silver nitrate TS a white, crystalline precipitate, which dissolves upon heating. When 1 drop of sodium nitrite TS is added to this solution, a pale yellow precipitate is produced.
2. Acidic solutions of bromates in nitric acid exhibit a yellow to red-brown color upon addition of 5 to 6 drops of sodium nitrite TS. When 1 mL of chloroform is added to the mixture and shaken, the chloroform layer exhibits a yellow to red-brown color.

Bromide
1. Solutions of bromides yield a pale yellow precipitate with silver nitrate TS. Upon addition of dilute nitric acid to a portion of the separated precipitate, it does not dissolve. When ammonia solution (28) is added to another portion and shaken, the separated solution yields a white turbidity upon acidifying with dilute nitric acid.
(2) Solutions of bromides exhibit a yellow-brown color with chlorine TS. The mixture is separated into 2 portions. When one portion is shaken with chloroform, the chloroform layer exhibits a yellow-brown to red-brown color. When phenol is added to the other portion, a white precipitate is produced.

**Calcium salt**

(1) When the Flame Coloration Test (1) is applied to calcium salts, a yellow-red color develops.
(2) Solutions of calcium salts yield a white precipitate with ammonium carbonate TS.
(3) Solutions of calcium salts yield a white precipitate with ammonium oxalate TS. The separated precipitate does not dissolve in dilute acetic acid, but dissolves in dilute hydrochloric acid.
(4) Neutral solutions of calcium salts produce no precipitate, when mixed with 10 drops of potassium chromate TS and heated (discrimination from strontium salts).

**Carbonate**

(1) Carbonates effervesce upon addition of dilute hydrochloric acid, generating a gas, which produces a white precipitate immediately, when passed into calcium hydroxide TS (common with biconarbonates).
(2) Solutions of carbonates yield with magnesium sulfate TS a white precipitate, which dissolves by addition of dilute acetic acid.
(3) Cold solutions of carbonates exhibit a red color with 1 drop of phenolphthalein TS (discrimination from bicarbonates).

**Ceric salt**

(1) When a cerous salt is mixed with 2.5 times its mass of lead (IV) oxide, nitric acid is added and the solution is boiled, it exhibits a yellow color.
(2) Solutions of cerous salts yield a yellow to red-brown precipitate upon addition of hydrogen peroxide TS and ammonia TS.

**Chlorate**

(1) Solutions of chlorates yield no precipitate with silver nitrate TS. When 2 to 3 drops of sodium nitrite TS and dilute nitric acid are added to the mixture, a white precipitate is produced gradually, which dissolves by addition of ammonia TS.
(2) When indigocarmine TS is added dropwise to neutral solutions of chlorates until a pale blue color appears, and the mixture is acidified with dilute sulfuric acid, the blue color vanishes promptly upon subsequent dropwise addition of sodium hydrosulfite TS.

**Chloride**

(1) Solution of chlorides evolve an odor of chlorine, when mixed with sulfuric acid and potassium permanganate, and heated. The gas evolved turns moistened potassium iodide starch paper blue.
(2) Solutions of chlorides yield a white precipitate with silver nitrate TS. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When an excess of ammonia TS is added to another portion, the precipitate dissolves.

**Chromate**

(1) Solutions of chromates exhibit a yellow color.
(2) Solutions of chromates produce a yellow precipitate with lead (II) acetate TS. When acetic acid (31) is added to a portion of the suspension, the precipitate does not dissolve. When dilute nitric acid is added to another portion, the precipitate dissolves.
(3) When acidic solutions of chromates in sulfuric acid are mixed with an equal volume of ethyl acetate and 1 to 2 drops of hydrogen peroxide TS, shaken immediately and allowed to stand, the ethyl acetate layer exhibits a blue color.

**Citrate**

(1) When 20 mL of a mixture of pyridine and acetic anhydride (3:1) is added to 1 or 2 drops of a solution of citrate, and the solution is allowed to stand for 2 to 3 minutes, a red-brown color develops.
(2) Neutral solutions of citrates, when mixed with an equal volume of dilute sulfuric acid and two-thirds volume of potassium permanganate TS, heated until the color of permanganate is discharged, and then treated dropwise with bromine TS to one-tenth of total volume, yield a white precipitate.
(3) Neutral solutions of citrates, when boiled with an excess of calcium chloride TS, yield a white crystalline precipitate. When sodium hydroxide TS is added to a portion of the separated precipitate, it does not dissolve. When dilute hydrochloric acid is added to another portion, the precipitate dissolves.

**Cupric salt**

(1) When a well polished iron plate is immersed in acidic solutions of cupric salts in hydrochloric acid, a red metallic film appears on its surface.
(2) Solutions of cupric salts produce a pale blue precipitate with a small quantity of ammonia TS. The precipitate dissolves in an excess of the reagent, yielding a deep blue-colored solution.
(3) Solutions of cupric salts yield a red-brown precipitate with potassium hexacyanoferrate (II) TS. When dilute nitric acid is added to a portion of the suspension, the precipitate does not dissolve. When ammonia TS is added to another portion, the precipitate dissolves, yielding a deep blue-colored solution.
(4) Solutions of cupric salts produce a black precipitate with sodium sulfide TS. When dilute hydrochloric acid, dilute sulfuric acid or sodium hydroxide TS is added to a portion of the separated precipitate, it does not dissolve. When hot dilute nitric acid is added to another portion, the precipitate dissolves.

**Cyanide**

(1) Solutions of cyanides yield a white precipitate with an excess of silver nitrate TS. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When ammonia TS is added to another portion, the precipitate dissolves.
(2) Solutions of cyanides yield a blue precipitate, when mixed by shaking with 2 to 3 drops of iron (II) sulfate TS, 2 to 3 drops of dilute iron (III) chloride TS and 1 mL of sodium hydroxide TS, and then acidified with dilute sulfuric acid.

**Dichromate**

(1) Solutions of dichromates exhibit a yellow-red color.
(2) Solutions of dichromates produce a yellow precipitate with lead (II) acetate TS. When acetic acid (31) is added to one portion of the suspension, the precipitate does not
Ferricyanide

(1) Slightly acidic solutions of ferric salts yield with potassium hexacyanoferrate (II) TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferric salts yield with sodium hydroxide TS a gelatinous, red-brown precipitate, which changes to black upon addition of sodium sulfide TS. The separated precipitate dissolves in dilute hydrochloric acid, yielding a white turbidity.

(3) Slightly acidic solutions of ferric salts exhibit a purple color with 5-sulfosalicylic acid TS.

Ferricyanide

(1) Solutions of ferricyanides exhibit a yellow color.

(2) Solutions of ferricyanides yield with iron (II) sulfate TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

Ferrocyanide

(1) Solutions of ferrocyanides yield with iron (III) chloride TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferrocyanides yield with copper (II) sulfate TS a red-brown precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

Ferrous salt

(1) Slightly acidic solutions of ferrous salts yield with potassium hexacyanoferrate (III) TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferrous salts yield with sodium hydroxide TS a greenish gray, gelatinous precipitate, which changes to black with sodium sulfide TS. The separated precipitate dissolves in dilute hydrochloric acid.

(3) Neutral or slightly acidic solutions of ferrous salts exhibit an intense red color upon dropwise addition of a solution of 1,10-phenanthroline monohydrate in ethanol (95) (1 in 50).

Fluoride

(1) When solutions of fluorides are heated with chromic acid-sulfuric acid TS, the inside of the test tube is not moistened uniformly.

(2) Neutral or slightly acidic solutions of fluorides exhibit a blue-purple color after standing with 1.5 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS (1:1:1).

Glycerophosphate

(1) Solutions of glycerophosphates remain unaffected by addition of calcium chloride TS, but yield a precipitate when boiled.

(2) Solutions of glycerophosphates yield no precipitate with hexaammonium heptamolybdate TS in the cold, but yield a yellow precipitate when boiled for a long time.

(3) When glycerophosphates are mixed with an equal mass of powdered potassium hydrogen sulfate and heated gently over a free flame, the pungent odor of acrolein is evolved.

Iodide

(1) Solutions of iodides yield a yellow precipitate with silver nitrate TS. When dilute nitric acid is added to one portion of the suspension, and ammonia solution (28) to another portion, the precipitates do not dissolve in either of these reagents.

(2) Acidic solutions of iodides exhibit a yellow-brown color with 1 to 2 drops of sodium nitrite TS and then yield a black-purple precipitate. The solutions exhibit a deep blue color with starch TS subsequently added.

Lactate

Acidic solutions of lactates in sulfuric acid, when heated with potassium permanganate TS, evolve the odor of acetaldehyde.

Lead salt

(1) Solutions of lead salts yield a white precipitate with dilute sulfuric acid. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When sodium hydroxide TS is added to another portion and warmed, or when ammonium acetate TS is added to another portion, the precipitate dissolves.

(2) Solutions of lead salts yield with sodium hydroxide TS a white precipitate, which dissolves in an excess of sodium hydroxide TS, and yields a black precipitate upon subsequent addition of sodium sulfide TS.

(3) Acidic solutions of lead salts in dilute acetic acid yield with potassium chromate TS a yellow precipitate, which does not dissolve in ammonia TS but dissolves in sodium hydroxide TS subsequently added.

Lithium salt

(1) When the Flame Coloration Test (1) \( <1.04\) is applied to lithium salts, a persistent red color develops.

(2) Solutions of lithium salts yield with disodium hydrogenphosphate TS a white precipitate, which dissolves upon subsequent addition of dilute hydrochloric acid.

(3) Solutions of lithium salts yield no precipitate with dilute sulfuric acid (discrimination from strontium salts).

Magnesium salt

(1) Solutions of magnesium salts yield upon warming with ammonium carbonate TS a white precipitate, which dissolves in ammonium chloride TS. A white, crystalline precipitate is reproduced by subsequent addition of disodium hydrogenphosphate TS.

(2) Solutions of magnesium salts yield with sodium hydroxide TS a white, gelatinous precipitate. When iodine TS is added to one portion of the suspension, the precipitate develops a dark-brown color. When excess sodium hydroxide TS is added to another portion, the precipitate does not dissolve.

Manganese salt

(1) Solutions of manganese salts yield a white precipitate with ammonia TS. When silver nitrate TS is added to a portion of the suspension, the precipitate changes to black. When another portion is allowed to stand, the upper part of the precipitate exhibits a brownish color.

(2) Acidic solutions of manganese salts in dilute nitric...
acid exhibit a purple-red color with a small quantity of powdered bismuth sodium trioxide.

Mercuric salt
(1) A copper plate is immersed in solutions of mercuric salts, allowed to stand, taken out, and then washed with water. The plate becomes bright and silvery white in appearance, when rubbed with paper or cloth (common with mercuric salts).
(2) Solutions of mercuric salts yield with a small quantity of sodium sulfide TS a black precipitate, which dissolves in an excess of the reagent. The black precipitate is reproduced by subsequent addition of ammonium chloride TS.
(3) When potassium iodide TS is added dropwise to neutral solutions of mercuric salts, a red precipitate is produced. The precipitate dissolves in an excess of the reagent.
(4) Acidic solutions of mercuric salts in hydrochloric acid yield with a small quantity of tin (II) chloride TS a white precipitate, which changes to grayish black upon addition of an excess of the reagent.

Mercurous salt
(1) A copper plate is immersed in solutions of mercurous salts, allowed to stand, taken out, and then washed with water. The plate becomes bright and silvery white in appearance, when rubbed with paper or cloth (common with mercurous salts).
(2) Mercurous salts or their solutions exhibit a black color with sodium hydroxide TS.
(3) Solutions of mercurous salts yield a white precipitate with dilute hydrochloric acid. The separated precipitate changes to black upon addition of ammonium TS.
(4) Solutions of mercurous salts yield with potassium iodide TS a yellow precipitate, which changes to green, then to black upon subsequent addition of an excess of the reagent.

Mesilate
(1) To mesilates add twice its mass of sodium hydroxide, heat gently to melt, and continue heating for 20 to 30 seconds. After cooling, add a little amount of water, then add dilute hydrochloric acid, and warm: the gas evolved changes moistened potassium iodate-starch paper to blue.
(2) To mesilates add threefold its mass of sodium nitrate and anhydrous sodium carbonate, mix, and heat gradually. After cooling, dissolve the residue in diluted hydrochloric acid (1 in 5), and filter if necessary. The filtrate yields a white precipitate upon addition of barium chloride TS.

Nitrate
(1) When a solution of nitrates is mixed with an equal volume of sulfuric acid, the mixture is cooled, and iron (II) sulfate TS is superimposed, a dark brown ring is produced at the junction of the two liquids.
(2) Solutions of nitrates exhibit a blue color with diphenylamine TS.
(3) When potassium permanganate TS is added to acidic solutions of nitrates in sulfuric acid, the red-purple color of the reagent does not fade (discrimination from nitrites).

Nitrite
(1) Solutions of nitrates, when acidified with dilute sulfuric acid, evolve a yellow-brown gas with a characteristic odor. The solutions exhibit a dark brown color upon addition of a small quantity of iron (II) sulfate crystals.
(2) Solutions of nitrates, when 2 to 3 drops of potassium iodide TS and dilute sulfuric acid are added dropwise, exhibit a yellow-brown color, and then yield a black-purple precipitate. When the mixture is shaken with 2 mL of chloroform, the chloroform layer exhibits a purple color.
(3) Solutions of nitrates, when mixed with thiourea TS and acidified with dilute sulfuric acid, and iron (III) chloride TS is added dropwise, exhibit a dark red color. When the mixture is shaken with 2 mL of diethyl ether, the diethyl ether layer exhibits a red color.

Oxalate
(1) When potassium permanganate TS is added dropwise to warm acidic solutions of oxalates in sulfuric acid, the reagent is decolorized.
(2) Solutions of oxalates yield a white precipitate with calcium chloride TS. The separated precipitate does not dissolve in dilute acetic acid but dissolves upon subsequent addition of dilute hydrochloric acid.

Permanganate
(1) Solutions of permanganates exhibit a red-purple color.
(2) When an excess of hydrogen peroxide TS is added to acidic solutions of permanganates in sulfuric acid, the solutions effervescence and decolorize permanganates.
(3) Acidic solutions of permanganates in sulfuric acid are decolorized, when an excess of oxalic acid TS is added and heated.

Peroxide
(1) Solutions of peroxides are mixed with an equal volume of ethyl acetate and 1 to 2 drops of potassium dichromate TS, and then acidified with dilute sulfuric acid. When the mixture is shaken immediately and allowed to stand, the ethyl acetate layer exhibits a blue color.
(2) Acidic solutions of peroxides in sulfuric acid decolorize dropwise added potassium permanganate TS, and effervesce to evolve a gas.

Phosphate (Orthophosphate)
(1) Neutral solutions of phosphates yield with silver nitrate TS a yellow precipitate, which dissolves upon addition of dilute nitric acid or ammonia TS.
(2) Acidic solutions in dilute nitric acid of phosphates yield a yellow precipitate with hexammonium heptamolybdate TS on warming. The precipitate dissolves upon subsequent addition of sodium hydroxide TS or ammonia TS.
(3) Neutral or ammonia-alkaline solutions of phosphates yield with magnesia TS a white, crystalline precipitate, which dissolves upon subsequent addition of dilute hydrochloric acid.

Potassium salt
(1) When the Flame Coloration Test (1) applied to potassium salts, a pale purple color develops. When it gives a yellow color, a red-purple color can be seen through cobalt glass.
(2) Neutral solutions of potassium salts yield a white, crystalline precipitate with sodium hydrogen tartrate TS. The formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod. The separated precipitate dissolves upon addition of any of ammonia TS, sodium hydroxide TS or sodium carbonate TS.
(3) Acidic solutions of potassium salts in acetic acid (31)...
yield a yellow precipitate with sodium hexanitrocobaltate (III) TS.

(4) Potassium salts do not evolve the odor of ammonia, when an excess of hydroxido TS is added and warmed (discrimination from ammonium salts).

Saliicylate

(1) Salicylates evolve the odor of phenol, when an excess of soda-lime is added and heated.

(2) Concentrated solutions of salicylates yield a white, crystalline precipitate with dilute hydrochloric acid. The separated precipitate, washed well with cold water and dried, melts <1.04° at about 159°C.

(3) Neutral solutions of salicylates exhibit with 5 to 6 drops of dilute iron (III) chloride TS a red color, which changes to purple and then fades when dilute hydrochloric acid is added dropwise.

Silver salt

(1) Solutions of silver salts yield a white precipitate with dilute hydrochloric acid. When dilute nitric acid is added subsequently to a portion of the suspension, the precipitate does not dissolve. When an excess of ammonia TS is added to another portion, the precipitate dissolves.

(2) Solutions of silver salts yield with potassium chromate TS a red precipitate, which dissolves upon addition of dilute nitric acid.

(3) Solutions of silver salts yield a brownish gray precipitate with ammonia TS added dropwise. When ammonia TS is added dropwise until the precipitate dissolves, then 1 to 2 drops of formaldehyde solution are added and warmed, a mirror of metallic silver is deposited on the inside wall of the container.

Sodium salt

(1) When the Flame Coloration Test (1) <1.04° is applied to sodium salts, a yellow color develops.

(2) Concentrated, neutral or slightly alkaline solutions of sodium salts yield a white, crystalline precipitate with potassium hexahydroxoantimonate (V) TS. The formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod.

Stannic salt

(1) When the outside bottom of a test tube containing water is moistened with acidic solutions of stannic salts in hydrochloric acid and is placed in a nonluminous flame of a Bunsen burner, a blue flame mantle is seen around the bottom of the test tube (common with stannous salts).

(2) When granular zinc is immersed in acidic solutions of stannic salts in hydrochloric acid, a spongy, gray substance is deposited on the surface of the granules (common with stannous salts).

(3) Add iron powder to acidic solutions of stannic salts in hydrochloric acid, allow to stand, and then filter. When iodine-starch TS is added dropwise to the filtrate, the color of the test solution disappears.

(4) Acidic solutions of stannic salts in hydrochloric acid, to which ammonia TS is added dropwise until a small quantity of precipitate is produced, yield a pale yellow precipitate with 2 to 3 drops of sodium sulfide TS. The separated precipitate dissolves upon addition of sodium sulfide TS and pale yellow precipitate is reproduced by subsequent addition of hydrochloric acid.

Stannous salt

(1) When the outside bottom of a test tube containing water is moistened with acidic solutions of stannous salts in hydrochloric acid and is placed in a nonluminous flame of a Bunsen burner, a blue flame mantle is seen around the bottom of the test tube (common with stannic salts).

(2) When a solution, prepared by mixing 2 to 3 drops of a solution of resorcitol (1 in 50) and 2 to 3 drops of a solution of potassium bromide (1 in 10) with 5 mL of sulfuric acid, is added to 2 to 3 drops of solutions of tartrates, and then heated for 5 to 10 minutes on a water bath, a deep blue
color is produced. The solution exhibits a red to red-orange color when poured to 3 mL of water after cooling.

Thiocyanate

(1) Solutions of thiocyanates yield a white precipitate with an excess of silver nitrate TS. When dilute nitric acid is added to a portion of the suspension, the precipitate does not dissolve. When ammonia solution (28) is added to another portion, the precipitate dissolves.

(2) Solutions of thiocyanates produce with iron (III) chloride TS a red color, which is not decolored by addition of hydrochloric acid.

Thiosulfate

(1) When iodine TS is added dropwise to acidic solutions of thiosulfates in acetic acid (31), the color of the reagent fades.

(2) When an equal volume of dilute hydrochloric acid is added, solutions of thiosulfates evolve the odor of sulfur dioxide, and yield gradually a white turbidity, which changes to yellow on standing.

(3) Solutions of thiosulfates yield with an excess of silver nitrate TS a white precipitate, which changes to black on standing.

Zinc salt

(1) Neutral to alkaline solutions of zinc salts yield a whitish precipitate with ammonium sulfide TS or sodium sulfide TS. The separated precipitate does not dissolve in dilute acetic acid but dissolves upon subsequent addition of dilute hydrochloric acid.

(2) Solutions of zinc salts yield a white precipitate with potassium hexacyanoferrate (II) TS. When dilute hydrochloric acid is added to a portion of the suspension, the precipitate does not dissolve. When sodium hydroxide TS is added to another portion, the precipitate dissolves.

(3) Neutral to weakly acidic solutions of zinc salts yield a white precipitate, when 1 or 2 drops of pyridine and 1 mL of potassium thiocyanate TS are added.

1.10 Iron Limit Test

Iron Limit Test is a limit test for iron contained in drugs. The limit is expressed in terms of ppm in parentheses.

In each monograph, the permissible limit for iron (as Fe) is described in terms of ppm in parentheses.

1. Preparation of test solutions and control solutions

Unless otherwise specified, test solutions and control solutions are prepared as follows:

1.1. Method 1

Weigh the amount of sample specified in individual monograph, add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, dissolve by warming if necessary, and designate this solution as the test solution.

Prepare the control solution as follows: To the amount of Standard Iron Solution specified in individual monograph add 20 mL of dilute hydrochloric acid, and proceed as directed for the test solution.

1.2. Method 2

Weigh the amount of sample specified in individual monograph, add 10 mL of dilute hydrochloric acid, and dissolve by warming if necessary. Dissolve 0.5 g of L-tartaric acid, and add one drop of phenolphthalein TS. Add ammonia TS dropwise until the solution develops a pale red color. Add 20 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, and designate this solution as the test solution.

Prepare the control solution as follows: To the amount of Standard Iron Solution specified in individual monograph add 10 mL of dilute hydrochloric acid, and proceed as directed for the test solution.

1.3. Method 3

Place the amount of sample specified in individual monograph in a crucible, moisten with a small amount of sulfuric acid, heat cautiously and gently at first, and then incinerate by ignition. After cooling, add 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and to the residue add 0.5 mL of diluted hydrochloric acid (2 in 3) and 10 mL of water. After dissolving by warming, add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, and designate this solution as the test solution.

Prepare the control solution as follows: Transfer the amount of Standard Iron Solution specified in individual monograph to a crucible, and add 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and proceed as directed for the test solution.

In this procedure, use a quartz or porcelain crucible, which is immersed in boiling dilute hydrochloric acid for 1 hour and washed throughly with water and dried.

2. Procedure

Unless otherwise specified, proceed as follows:

2.1. Method A

Transfer the test solution and the control solution to separate Nessler tubes, to each add 2 mL of a solution of L-ascorbic acid (1 in 100), mix well, and allow to stand for 30 minutes. Add 1 mL of a solution of α, α′-dipyridyl in ethanol (95) (1 in 200), add water to make 50 mL, and allow to stand for 30 minutes. Then compare the colors developed in both solutions against a white background. The test solution has no more color than the control solution.

2.2. Method B

Dissolve 0.2 g of L-ascorbic acid in the test solution and the control solution, and allow to stand for 30 minutes. Add 1 mL of a solution of α, α′-dipyridyl in ethanol (95) (1 in 200), and allow to stand for 30 minutes. Then add 2 mL of a solution of 2,4,6-trinitrophenol (3 in 1000) and 20 mL of 1,2-dichloroethane, shake vigorously, collect the 1,2-dichloroethane layer, and filter through a pledge of absorbent cotton in a funnel on which 5 g of anhydrous sodium sulfate is placed if necessary. Then compare the colors developed in both solutions against a white background. The test solution has no more color than the control solution.

1.11 Arsenic Limit Test

Arsenic Limit Test is a limit test for arsenic contained in drugs. The limit is expressed in terms of arsenic (III) trioxide (As2O3).

In each monograph, the permissible limit for arsenic (as As2O3) is described in terms of ppm in parentheses.
1. Apparatus
   Use the apparatus illustrated in Fig. 1.11-1.
   Place glass wool F in the exit tube B up to about 30 mm in height, moisten the glass wool uniformly with a mixture of an equal volume of lead (II) acetate TS and water, and apply gentle suction to the lower end to remove the excess of the mixture. Insert the tube vertically into the center of the rubber stopper H, and attach the tube to the generator bottle A so that the small perforation E in the lower end of B extends slightly below. At the upper end of B, attach the rubber stopper J to hold the tube C vertically. Make the lower end to the exit tube of C level with that of the rubber stopper J.

2. Preparation of the test solution
   Unless otherwise specified, proceed as directed in the following.
   2.1. Method 1
   Weigh the amount of the sample directed in the monograph, add 5 mL of water, dissolve by heating if necessary, and designate the solution as the test solution.
   2.2. Method 2
   Weigh the amount of the sample directed in the monograph, add 5 mL of water, and add 1 mL of sulfuric acid except in the cases that the samples are inorganic acids. Add 10 mL of sulfurous acid solution, transfer to a small beaker, and evaporate the mixture on a water bath until it is free from sulfurous acid and is reduced to about 2 mL in volume. Dilute with water to make 5 mL, and designate it as the test solution.
   2.3. Method 3
   Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), ignite the ethanol, and heat gradually to incinerate. If carbonized material still remains by this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate. After cooling, add 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and designate it as the test solution.
   2.4. Method 4
   Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, heat gradually, and ignite to incinerate. If carbonized material still remains by this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate in the same manner. After cooling, add 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and designate it as the test solution.
   2.5. Method 5
   Weigh the amount of the sample directed in the monograph, add 10 mL of N,N-dimethylformamide, dissolve by heating if necessary, and designate the solution as the test solution.

3. Test solutions
   Absorbing solution for hydrogen arsenide: Dissolve 0.50 g of silver N,N-diethyldithiocarbamate in pyridine to make 100 mL. Preserve this solution in a glass-stoppered bottle protected from light, in a cold place.
   Standard Arsenic Stock Solution: Weigh accurately 0.100 g of finely powdered arsenic (III) trioxide dried at 105°C for 4 hours, and add 5 mL of sodium hydroxide solu-
tion (1 in 5) to dissolve. Add dilute sulfuric acid to neutralize, add further 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL.

Standard Arsenic Solution: Pipet 10 mL of Standard Arsenic Stock Solution, add 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL. Each mL of the solution contains 1 µg of arsenic (III) trioxide (As₂O₃). Prepare Standard Arsenic Solution just before use and preserve in a glass-stoppered bottle.

4. Procedure

Unless otherwise specified, proceed using apparatus shown in Fig. 1.11-1. Carry out the preparation of the standard color at the same time.

Place the test solution in the generator bottle A and, if necessary, wash down the solution in a bottle with a small quantity of water. Add 1 drop of methyl orange TS, and after neutralizing with ammonia TS, ammonia solution (28%) or dilute hydrochloric acid, add 5 mL of diluted hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acidic tin (II) chloride TS, and allow to stand for 10 minutes. Then add water to make 40 mL, add 2 g of zinc for arsenic analysis, and immediately connect the rubber stopper H fitted with B and C with the generator bottle A. Transfer 5 mL of the absorbing solution for hydrogen arsenide to the absorber tube D, insert the tip of C to the bottom of the absorber tube D, then immerse the generator bottle A up to the shoulder in water maintained at 25°C, and allow to stand for 1 hour. Disconnect the absorber tube, add pyridine to make 5 mL, if necessary, and observe the color of the absorbing solution: the color produced corresponds to 2 µg of arsenic (III) trioxide (As₂O₃) and is used as the standard.

5. Note

Apparatus, reagents and test solutions used in the test should contain little or no arsenic. If necessary, perform a blank determination.

1.12 Methanol Test

Methanol Test is a method to determine methanol adhering in ethanol.

1. Reagents

(i) Standard Methanol Solution—To 1.0 g of methanol, accurately measured, add water to make exactly 1000 mL. To 5 mL of this solution, exactly measured, add 2.5 mL of methanol-free ethanol and water to make exactly 50 mL.

(ii) Solution A—To 75 mL of phosphoric acid add water to make 500 mL, then dissolve 15 g of potassium permanganate in this solution.

(iii) Solution B—Add sulfuric acid carefully to an equal volume of water, cool, and dissolve 25 g of oxalic acid hydrate in 500 mL of this dilute sulfuric acid.

2. Procedure

Pipet 1 mL of the sample, and add water to make exactly 20 mL. Use this solution as the sample solution. Transfer 5 mL each of the sample solution and the Standard Methanol Solution, accurately measured, to test tubes, add 2 mL of Solution A to each solution, and allow to stand for 15 minutes. Decolorize these solutions by adding 2 mL of Solution B, and mix with 5 mL of fuchsin-sulfurous acid TS. Allow to stand for 30 minutes at ordinary temperature. The sample solution has no more color than the Standard Methanol Solution.

1.13 Fats and Fatty Oils Test

Fats and Fatty Oils Test is a method applied to fats, fatty oils, waxes, fatty acids, higher alcohols, and related substances.

1. Preparation of test sample

For a solid sample, melt with care, and, if necessary, filter the melted sample with a dry filter paper by warming. For a turbid liquid sample, heat at about 50°C. If it is still turbid, filter it with a dry filter paper while warm. In either case, mix the sample to make it homogeneous.

2. Melting point

Proceed by the method described in Method 2 of Melting Point Determination (2.60).

3. Congealing point of fatty acids

3.1. Preparation of fatty acids

Dissolve 25 g of potassium hydroxide in 100 g of glycerin. Transfer 75 g of this solution into a 1-L beaker, and heat at 150°C. Add 50 g of the sample to this solution, and heat at a temperature not higher than 150°C for 15 minutes under frequent stirring to saponify completely. Cool the solution to 100°C, dissolve by addition of 500 mL of hot water, and add slowly 50 mL of diluted sulfuric acid (1 in 4). Heat the solution under frequent stirring until the clear layer of fatty acid is separated distinctly. Separate the fatty acid layer, and wash the fatty acid layer with hot water until the washing shows no acidity to methyl orange TS. Transfer the fatty acid layer to a small beaker, and heat on a water bath until the fatty acid becomes clear owing to the separation of water. Filter the warm solution, and complete the evaporation of water by carefully heating the filtered solution to 130°C.

3.2. Measurement of congealing point

Proceed by the method described in Congealing Point Determination (2.42).

4. Specific gravity

4.1. Liquid sample at ordinary temperature

Proceed by the method described in Determination of Specific Gravity and Density (2.56).

4.2. Solid sample at ordinary temperature

Unless otherwise specified, fill a pycnometer with water at 20°C. Weigh accurately the pycnometer, and, after discarding the water and drying, weigh accurately the empty pycnometer. Then, fill the pycnometer with the melted sample to about three-fourths of the depth, and allow to stand at a temperature a little higher than the melting temperature of the sample for 1 hour to drive off the air in the sample. After keeping at the specified temperature, weigh accurately the
5. **Acid value**

The acid value is the number of milligrams of potassium hydroxide (KOH) required to neutralize the free acids in 1 g of sample.

### 5.1. Procedure

Unless otherwise specified, weigh accurately the amount of sample shown in Table 1.13-1, according to the expected acid value of the sample, in a glass-stoppered, 250-mL flask, add 100 mL of a mixture of diethyl ether and ethanol (95:1:1 or 2:1) as the solvent, and dissolve the sample by warming, if necessary. Then, add a few drops of phenolphthalein TS as an indicator, and add 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red color which persists for 30 seconds. If the sample solutions is turbid at lower temperature, titration should be done while warm. To the solvent used add phenolphthalein TS as an indicator, and add 0.1 mol/L potassium hydroxide-ethanol VS before use, until the solvent remains light red for 30 seconds.

\[
d = \frac{M_1 - M}{(M_2 - M_1) - (M_3 - M_2)}
\]

- \(d\): mass (g) of the empty pycnometer
- \(M\): Mass (g) of the pycnometer filled with water
- \(M_1\): Mass (g) of the pycnometer filled with the sample
- \(M_2\): Mass (g) of the pycnometer filled with water
- \(M_3\): Mass (g) of the pycnometer filled with the sample and water

6. **Saponification value**

The saponification value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters in 1 g of sample.

### 6.1. Procedure

Unless otherwise specified, weigh accurately 1 to 2 g of the sample, transfer to a 200-mL flask, and add exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS. Attach a medium reflux condenser or an air condenser 750 mm in length and 6 mm in diameter to the neck of the flask, and heat gently in a water bath for 1 hour with frequent shaking. Cool the solution, add 1 mL of phenolphthalein TS, and titrate \(\leq 2.5\) with 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red color which persists for 30 seconds. If the sample solutions is turbid at lower temperature, titration should be done while warm. Perform a blank determination.

\[
\text{Acid value} = \frac{\text{consumed volume (mL) of 0.1 mol/L potassium hydroxide-ethanol VS} \times 5.611}{\text{amount (g) of sample}}
\]

### Table 1.13-1

<table>
<thead>
<tr>
<th>Acid value</th>
<th>Amount (g) of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 5</td>
<td>20</td>
</tr>
<tr>
<td>5 to 15</td>
<td>10</td>
</tr>
<tr>
<td>15 to 30</td>
<td>5</td>
</tr>
<tr>
<td>30 to 100</td>
<td>2.5</td>
</tr>
<tr>
<td>More than 100</td>
<td>1.0</td>
</tr>
</tbody>
</table>

7. **Ester value**

The ester value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters in 1 g of sample.

### 7.1. Procedure

Unless otherwise specified, designate the difference between the saponification value and the acid value determined as the ester value.

8. **Hydroxyl value**

The hydroxyl value is the number of milligrams of potassium hydroxide (KOH) required to neutralize acetic acid combined with hydroxyl groups, when 1 g of the sample is acetylated by the following procedure.

### 8.1. Procedure

Place about 1 g of the sample, weighed accurately, in a 200-mL round-bottom flask (shown in Fig. 1.13-1), and add exactly 5 mL of pyridine-acetic anhydride TS. Place a small funnel on the neck of the flask, and heat by immersing the flask up to 1 cm from the bottom in an oil bath between 95°C and 100°C. Put a thick, round paper with a round hole on the joint of the neck of the flask to protect the neck from the heat of the oil bath. After heating for 1 hour, take the flask from the oil bath, and cool by standing. Add 1 mL of water to the flask, and shake to decompose acetic anhydride. Heat the flask in the oil bath for 10 minutes again. After cooling, wash the funnel and neck with 5 mL of neutralized ethanol down into the flask, and titrate \(\leq 2.5\) with 0.5 mol/L potassium hydroxide-ethanol VS (indicator: 1 mL of phenolphthalein TS). Perform a blank determination.

\[
\text{Hydroxyl value} = \frac{(a - b) \times 28.05}{\text{amount (g) of sample}} + \text{acid value}
\]

- \(a\): Volume (mL) of 0.5 mol/L hydrochloric acid VS consumed in the blank determination
- \(b\): Volume (mL) of 0.5 mol/L hydrochloric acid VS consumed for titration of the sample

![Image](image-url)
9. Unsaponifiable matter

Unsaponifiable matter is calculated as the difference between the amount of materials, which are unsaponifiable by the procedure described below, soluble in diethyl ether and insoluble in water, and the amount of fatty acids expressed in terms of the amount of oleic acid. Its limit is expressed as a percentage in the monograph.

9.1. Procedure

Transfer about 5 g of the sample, accurately weighed, to a 250-mL flask. Add 50 mL of potassium hydroxide-ethanol TS, attach a reflux condenser to the flask, boil gently on a water bath for 1 hour with frequent shaking, and then transfer to the first separator. Wash the flask with 100 mL of warm water, and transfer the washing to the separator. Further, add 50 mL of water to the separator, and cool to room temperature. Wash the flask with 100 mL of diethyl ether, add the washing to the separator, extract by vigorous shaking for 1 minute, and allow to stand until both layers are separated clearly. Transfer the water layer to the second separator, add 50 mL of diethyl ether, shake, and allow to stand in the same manner. Transfer the water layer in the second separator to the third separator, add 50 mL of diethyl ether, and extract by shaking again in the same manner. Combine the diethyl ether extracts in the second and third separators into the first separator, wash each separator with 50 mL of diethyl ether, and extract by shaking again in the same manner. Combine the diethyl ether extracts in the second and third separators into the first separator, wash each separator with a small amount of diethyl ether, and combine the washings into the first separator. Wash the combined extracts in the first separator with 30 mL portions of water successively, until the washing does not develop a light red color with 2 drops of phenolphthalein TS. Add a small amount of anhydrous sodium sulfate to the diethyl ether extracts, and allow to stand for 1 hour. Filter the diethyl ether extracts with dry filter paper, and collect the filtrates into a tared flask. Wash the flask with 100 mL of diethyl ether, and extract by shaking again in the same manner. Combine the diethyl ether extracts in the second and third separators into the first separator, wash each separator with a small amount of diethyl ether, and add the washing to the flask through the above filter paper. After evaporation of the filtrate and washing almost to dryness on a water bath, add 3 mL of acetone, and evaporate again to dryness on a water bath. Complete the drying between 70°C and 80°C under reduced pressure (about 2.67 kPa) for 30 minutes, allow to stand for cooling in a desiccator (reduced pressure, silica gel) for 30 minutes, and then weigh. After weighing, add 2 mL of potassium chloride solution (1 in 10 and 100 mL of water, and shake. Then, titrate $2.50\text{mL}$ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

\[
\text{Iodine value} = \frac{(a - b) \times 1.269}{\text{amount (g) of sample}}
\]

\[
a: \text{Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination}
\]

\[
b: \text{Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed for titration of the sample}
\]

10. Iodine value

The iodine value, when measured under the following conditions, is the number of grams of iodine (I), representing the corresponding amount of halogen, which combines with 100 g of sample.

10.1. Procedure

Unless otherwise specified, weigh accurately the amount of sample shown in Table 1.13-2, according to the expected iodine value of the sample, in a small glass container. In a 500-mL glass-stoppered flask place the container containing the sample, add 20 mL of cyclohexane to dissolve the sample, then add exactly 25 mL of Wijs TS, and mix well. Stopper the flask, and allow to stand, protecting against light, between 20°C and 30°C for 30 minutes (when the expected iodine value is more than 100, for 1 hour) with occasional shaking. Add 20 mL of potassium iodoide solution (1 in 10) and 100 mL of water, and shake. Then, titrate $2.50\text{mL}$ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

\[
\text{Iodine value} = \frac{(a - b) \times 1.269}{\text{amount (g) of sample}}
\]

\[
a: \text{Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination}
\]

\[
b: \text{Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed for titration of the sample}
\]

1.14 Sulfate Limit Test

Sulfate Limit Test is a limit test for sulfate contained in drugs.

In each monograph, the permissible limit for sulfate (as SO₄) is described in terms of percentage (%) in parentheses.

1. Procedure

Unless otherwise specified, transfer the quantity of the sample, directed in the monograph, to a Nessler tube, dissolve it in sufficient water, and add water to make 40 mL. Add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the test solution. Transfer the volume of 0.005 mol/L sulfuric acid VS, directed in the monograph, to another Nessler tube, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions according to the same procedure. Add 2 mL of barium chloride TS to the test solution and to the control solution, mix well, and allow to stand for 10 minutes. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely.

The turbidity produced in the test solution is not thicker than that of the control solution.

1.15 Readily Carbonizable Substances Test

Readily Carbonizable Substances Test is a method to examine the minute impurities contained in drugs, which are readily colored by addition of sulfuric acid.
1. Procedure
Before use, wash the Nessler tubes thoroughly with sulfuric acid for readily carbonizable substances. Unless otherwise specified, proceed as follows. When the sample is solid, place 5 mL of sulfuric acid for readily carbonizable substances in a Nessler tube, to which add a quantity of the finely powdered sample, little by little, as directed in the monograph, and dissolve it completely by stirring with a glass rod. When the sample is liquid, transfer a volume of the sample, as directed in the monograph, to a Nessler tube, add 5 mL of sulfuric acid for readily carbonizable substances, and mix by shaking. If the temperature of the content of the tube rises, cool the content; maintain it at the standard temperature, if the reaction may be affected by the temperature. Allow to stand for 15 minutes, and compare the color of the liquid with that of the matching fluid in the Nessler tube specified in the monograph, by viewing transversely against a white background.

2. Physical Methods

Chromatography

2.01 Liquid Chromatography

Liquid Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a liquid as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a liquid or soluble sample, and is used for identification, purity test, and quantitative determination.

A mixture injected into the column is distributed between the mobile phase and the stationary phase with a characteristic ratio (k) for each component.

\[
k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}
\]

The ratio k represents the mass distribution ratio in liquid chromatography.

Since the relation given below exists among the ratio (k), the time for which the mobile phase is passed through the column \( t_0 \) (time measured from the time of injection of a compound with \( k = 0 \) to the time of elution at the peak maximum), and the retention time \( t_R \) (time measured from the time of injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

\[
t_R = (1 + k) \cdot t_0
\]

1. Apparatus

Basically, the apparatus required for the liquid chromatographic procedure consists of a pumping system for the mobile phase, a sample injection port, a column, a detector and a recorder. A mobile phase component regulator, a thermostat for the column, a pumping system for reaction reagents and a chemical reaction chamber are also used, if necessary. The pumping system serves to deliver the mobile phase and the reagents into the column and connecting tube at a constant flow rate. The sample injection port is used to deliver a quantity of the sample to the apparatus with high reproducibility. The column is a tube with a smooth interior, made of inert metal, etc., in which a packing material for liquid chromatography is uniformly packed. A column with a stationary phase chemically bound on the inside wall instead of the column packed with the packing material may be used. The detector is used to detect a property of the samples which is different from that of the mobile phase, and may be an ultraviolet or visible spectrophotometer, fluorometric detector, differential refractometer, electrochemical detector, chemiluminescence detector, electric conductivity detector, mass spectrophotometer, etc. The output signal is usually proportional to the concentration of samples at amounts of less than a few µg. The recorder is used to record the output signals of the detector. As required, a data processor may be used as the recorder to record or output the chromatogram, retention times or amounts of the components. The mobile phase component regulator is used to vary the ratio of the mobile phase components in a stepwise or gradient fashion.

2. Procedure

Fix the detector, column and mobile phase to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the sample solution or the standard solution specified in the individual monograph with the sample injector into the column through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram. If the components to be analyzed have no readily detectable physical properties such as absorbance or fluorescence, the detection is achieved by changing the components to suitable derivatives. Usually, the derivatization is performed as a pre- or post-column labeling.

3. Identification and purity test

When Liquid Chromatography is used for identification of a component of a sample, it is performed by confirming identity of the retention time of the component and that of an authentic specimen, or by confirming that the peak shape of the component is unchanged after mixing the sample with an authentic specimen. If a detector which is able to obtain chemical structural information of the component at the same time is used, highly specific identification can be achieved by confirming identity of the chemical structure of the component and that of an authentic specimen, in addition to the identity of their retention times.

When Liquid Chromatography is used for purity test, it is generally performed by comparing the peak area of target impurity from the sample solution with that of the main component from a standard solution, which is prepared by diluting the sample solution to a concentration corresponding to the specified limit of the impurity, or by calculating target impurity content using the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.
The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on its relative response factor to the principal component.

4. Assay

4.1. Internal standard method

In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the liquid chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound.

4.2. Absolute calibration curve method

Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve obtained, prepare a calibration curve by plotting the peak volume of these standard solutions. With the chromatogram of the authentic specimen, and inject accurately a fixed amount of the compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the liquid chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition.

5. Method for peak measuring

5.1. Peak height measuring method

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on both sides of the peak. (ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing system.

5.2. Peak area measuring method

(i) Width at half-height method: Multiply the peak width at the half-height by the peak height. (ii) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

6. System suitability

System suitability testing is an integral part of test methods using chromatography, and is used to ensure that the performance of the chromatographic systems used is as suitable for the analysis of the drug as was at the time when the verification of the test method was performed using the system. System suitability testing should be carried out at every series of drug analysis. The test procedures and acceptance criteria of system suitability testing must be prescribed in the test method of drugs. The results of drug analyses are not acceptable unless the requirements of system suitability have been met.

In system suitability testing of the chromatographic systems, the evaluation of “System performance” and “System repeatability” is usually required. For quantitative purity tests, the evaluation of “Test for required detectability” may also be required.

6.1. Test for required detectability

For purity tests, when it is confirmed that the target impurity is distinctly detected at the concentration of its specification limit, it is considered verified that the system used has adequate performance to achieve its intended use.

For quantitative purity tests, “Test for required detectability” is usually required, and in order to confirm, in some degree, the linearity of response near its specification limit, the range of expected response to the injection of a certain volume of target impurity solution at the concentration of its specification limit should be prescribed. For limit test, “Test for required detectability” is not required, if the test is performed by comparing the response from sample solution with that from standard solution at the concentration of its specification limit. “Test for required detectability” is also not required, if it is confirmed that the impurity can be detected at its specification limit by the evaluation of “System repeatability” or some other procedure.

6.2. System performance

When it is confirmed that the specificity for determining the test ingredient is ensured, it is considered verified that the system used has adequate performance to achieve its intended use.

In assay, “System performance” should be defined by the resolution between the test ingredient and a target substance to be separated (a closely eluting compound is preferable),
and when appropriate, by their order of elution. In purity tests, both the resolution and the order of elution between the test ingredient and a target substance to be separated (a closely eluting compound is preferable) should be prescribed. In addition, if necessary, the symmetry factor of the test ingredient should be prescribed together with them. However, if there is no suitable target substance to be separated, it is acceptable to define “System performance” using the number of theoretical plates and the symmetry factor of the test ingredient.

6.3. System repeatability

When it is confirmed that the degree of variation (precision) of the response of the test ingredient is at a level that meets the requirement of “System repeatability”, it is considered verified that the system used has adequate performance to achieve its intended use.

The allowable limit of “System repeatability” is normally defined as the relative standard deviation (RSD) of the response of the test ingredient in replicate injections of standard solution. It is acceptable to confirm the repeatability of the system not only by replicate injections of standard solution before sample injections, but also by divided injections of standard solution before and after sample injections, or by interspersed injections of standard solution among sample injections.

In principle, total number of replicate injections should be 6. However, in the case that a long time is necessary for one analysis, such as the analysis using the gradient method, or the analysis of samples containing late eluting components, it may be acceptable to decrease the number of replicate injections by adopting new allowable limit of “System repeatability” which can guarantee a level of “System repeatability” equivalent to that at 6 replicate injections.

The allowable limit of “System repeatability” should be set at an appropriate level based on the data when suitability of the method for the evaluation of quality of the drug was verified, and the precision necessary for the quality test.

7. Point to consider on changing the operating conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of the column, particle size of the packing material, column temperature, composition ratio of the mobile phase, pH of the mobile phase, concentration ratio of the mobile phase, composition of buffer solution before sample injections, but also by divided injections of standard solution before and after sample injections, or by interspersed injections of standard solution among sample injections.

In principle, total number of replicate injections should be 6. However, in the case that a long time is necessary for one analysis, such as the analysis using the gradient method, or the analysis of samples containing late eluting components, it may be acceptable to decrease the number of replicate injections by adopting new allowable limit of “System repeatability” which can guarantee a level of “System repeatability” equivalent to that at 6 replicate injections.

The allowable limit of “System repeatability” should be set at an appropriate level based on the data when suitability of the method for the evaluation of quality of the drug was verified, and the precision necessary for the quality test.

7. Point to consider on changing the operating conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of the column, particle size of the packing material, column temperature, composition ratio of the mobile phase, pH of the mobile phase, concentration of ion-pair forming agents in the mobile phase, ionic strength of the mobile phase, flow rate of the mobile phase, number and timing of mobile phase composition changes in gradient program, flow rate of mobile phase in gradient program, composition and flow rate of derivatizing reagents, and reaction time and chamber temperature in chemical reaction may be modified within the ranges in which the liquid chromatographic system used conforms to the requirements of system suitability.

8. Terminology

(i) SN ratio: It is defined by the following formula.

\[
S/N = \frac{2H}{h}
\]

\(H\): Peak height of the target ingredient peak from the baseline (the median value of background noise)

\(h\): Width of background noise of the chromatogram of sample solution or solvent blank around the peak of the target ingredient

The baseline and background noise are measured over a range 20 times of peak width at the center point of peak height of the target ingredient. When a solvent blank is used, measure over almost the same range as mentioned above around the point where the target ingredient elutes.

(ii) Symmetry factor: It shows the degree of symmetry of a peak in the chromatogram, and is defined as \(S\) in the following equation.

\[
S = \frac{W_{0.05h}}{2f}
\]

\(W_{0.05h}\): Width of the peak at one-twentieth of the peak height

\(f\): Distance between the perpendicular from the peak maximum and the leading edge of the peak at one-twentieth of the peak height

Where \(W_{0.05h}\) and \(f\) have the same unit.

(iii) Relative standard deviation: Generally, it is defined as RSD (%) in the following equation.

\[
\text{RSD} (\%) = \frac{100}{\bar{X}} \times \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{X})^2}{n - 1}}
\]

\(x_i\): Observed value

\(\bar{X}\): Mean of observed values

\(n\): Number of replicate measurements

(iv) Complete separation of peak: It means that the resolution between two peaks is not less than 1.5. It is also called as “baseline separation”.

(v) Peak-valley ratio: It indicates the degree of separation between 2 peaks on a chromatogram when baseline separation cannot be attained, and is defined as \(p/v\) by the following formula.

\[
p/v = \frac{H_p}{H_v}
\]

\(H_p\): peak height from the baseline of the minor peak

\(H_v\): height from the baseline of the lowest point (peak valley) of the curve between major and minor peaks

(vi) Separation factor: It shows the relation between the retention times of peaks in the chromatogram, and is defined as \(\alpha\) in the following equation.
Gas Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a gas (carrier gas) as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a gaseous or vaporizable sample, and is used for identification, purity test, and quantitative determination.

A mixture injected into the column is distributed between the mobile phase and the stationary phase with a characteristic ratio \( (k) \) for each component.

\[
k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}
\]

Since the relation given below exists among the ratio \( (k) \), the time for which the mobile phase is passed through the column \( t_0 \): time measured from the time of injection of a compound with \( k = 0 \) to the time of elution at the peak maximum, and the retention time \( t_R \): time measured from the time of injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

\[
t_R = (1 + k) t_0
\]

1. Apparatus

Basically, the apparatus required for the gas chromatographic procedure consists of a carrier gas-introducing port and flow regulator, a sample injection port, a column, a column oven, a detector and a recorder. Gas introducing port and flow regulator for a combustion gas, a burning supporting gas and an accessory gas and sample injection port for headspace are also used, if necessary. The carrier gas-introducing port and flow regulator serves to deliver the carrier gas into the column at a constant flow rate, and usually consist of a pressure regulation valve, a flow rate regulation valve and a pressure gauge. The sample injection port is used to deliver a quantity of the sample to the flow line of carrier gas with high reproducibility. There are sample injection ports for packed column and for capillary column. There are both divided injection mode and non-divided injection mode to sample injection port for capillary column. The columns are usually classified as packed column or capillary column. The packed column is a tube made of inert metal, glass or synthetic resin, in which a packing material for gas chromatography is uniformly packed. The packed column with not more than 1 mm in inside diameter is also called a packed capillary column (micro packed column). A capillary column is a tube made of inert metal, glass, quartz or synthetic resin, whose inside wall is bound chemically with stationary phase for gas chromatography. The column oven has the setting capacity for a column with required length and the temperature regulation system for keeping the constant column temperature. The detector is used to detect a component separated on the column, and may be an alkaline thermal ionization detector, a flame photometry detector, mass spectrometrometer, hydrogen flame-ionization detector, an electron capture detector, a thermal conductivity detector, etc. The recorder is used to record the output signals of the detector.

2. Procedure

Unless otherwise specified, proceed by the following method. Fix the detector, column and carrier gas to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions speci-
3. Identification and purity test

Identification of a component of a sample is performed by confirming identity of the retention time of the component and that of an authentic specimen, or by confirming that the peak shape of the component is unchanged after mixing the sample with an authentic specimen.

In general, the purity of the sample is determined by comparing the peak area of target impurity from the sample solution with that of the main component from a standard solution, which is prepared by diluting the sample solution to a concentration corresponding to the specified limit of the impurity, or by calculating target impurity content using the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on its response factor to the principal component.

4. Assay

In general, perform the assay by using the internal standard method. The absolute calibration curve method is used when a suitable internal standard is not available. Perform the assay by using the standard addition method when the effect of the component other than the compound to be assayed on the quantitative determination is not negligible against a result of the determination.

4.1. Internal standard method

In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the gas chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound.

4.2. Absolute calibration curve method

Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a sample solution according to the method specified in the individual monograph, perform the gas chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition.

4.3. Standard addition method

Pipet a fixed volume of more than 4 sample solutions, add exactly the standard solution so that stepwise increasing amounts of the object compound are contained in the solutions except 1 sample solution, diluted exactly each solution with and without standard solution to a definite volume, and use each solution as the sample solution. Based on the chromatogram obtained by exact injection of a fixed volume of individual sample solutions, measure the peak area or peak height of individual sample solutions. Calculate the concentration of standard objective compound added into each sample solution, plot the amounts (concentration) of added standard object compound on the abscissa and the peak area or peak height on the ordinate on the graph, extend the calibration curve obtained by linking the plots, and determine the amount of object compound to be assayed from the distance between the origin and the intersecting point of the calibration curve with the abscissa. This method is available only in the case that the calibration curve is a straight line, and passes through the origin when the absolute calibration curve method is employed. In this method, all procedures must be carried out under a strictly constant condition.

5. Method for peak measuring

Generally, the following methods are used.

5.1. Peak height measuring method

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on either side of the peak.

(ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing...
5.2. Peak area measuring method
   (i) Width at half-height method: Multiply the peak width at the half-height by the peak height.
   (ii) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

6. System suitability
   Refer to “System suitability” described under 2.01 Liquid Chromatography.

7. Point to consider in changing the operating conditions
   Among the operating conditions specified in the individual monograph, inside diameter and length of column, particle size of packing material, concentration or thickness of stationary phase, column temperature, temperature-rising rate, kind and flow rate of carrier gas, and split ratio may be modified within the ranges in which the gas chromatographic system used conforms to the requirements of system suitability. Headspace sample injection device and its operating conditions may be also modified, provided that they give equivalent or more accuracy and precision.

8. Terminology
   The definition of terms described under 2.01 Liquid Chromatography shall apply in 2.02 Gas Chromatography.

9. Note
   Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

2.03 Thin-layer Chromatography

Thin-layer Chromatography is a method to separate each ingredient by developing a mixture in a mobile phase, using a thin-layer made of a suitable stationary phase, and is applied for identification, purity test, etc. of substances.

1. Preparation of thin-layer plate
   Generally, proceed by the following method.
   A smooth and uniformly thick glass plate having a size of 50 mm × 200 mm or 200 mm × 200 mm is used for preparing a thin-layer plate. Using a suitable apparatus, apply a water suspension of powdered solid substance for the stationary phase, directed in the monograph, on one side of the glass plate to make a uniform layer of 0.2 to 0.3 mm in thickness. After air-drying, dry further by heating at a fixed temperature between 105°C and 120°C for 30 to 60 minutes. A suitable plastic plate may be used instead of the glass plate. Preserve the dried plate with protection from moisture.

2. Procedure
   Unless otherwise specified, proceed by the following method.
   Designate a line about 20 mm distant from the bottom of the thin-layer plate as the starting line, spot 2 to 6 mm in diameter the directed volumes of the sample solution or the standard solution in the monograph using micropipets at points on this line, separated by more than 10 mm, and air-dry. Unless otherwise specified, attach the filter paper along with the inside wall of the container, and wet the filter paper with the developing solvent. In the container, the developing solvent is placed up to about 10 mm in height from the bottom beforehand, seal the container closely, and allow it to stand for 1 hour at ordinary temperature. Place the plate in the container, avoiding contact with the inside wall, and seal the container. Develop it at ordinary temperature.
   When the solvent front has ascended to the distance directed in the monograph, remove the plate from the container. Immediately put a mark at the solvent front. After air-drying, observe the location, color, etc., of each spot by the method specified in the monograph. Calculate the Rf value by using the following equation:

\[ R_f = \frac{\text{distance from the starting line to the center of the spot}}{\text{distance from the starting line to the solvent front}} \]

2.04 Amino Acid Analysis of Proteins

Amino acid analysis of proteins refers to the methodology used to determine the amino acid composition or content of proteins, peptides, and other pharmaceutical preparations. Amino acid analysis can be used to quantify protein and peptides, to determine the identity of proteins or peptides based on their amino acid composition, to support protein and peptide structure analysis, to evaluate fragmentation strategies for peptide mapping, and to detect atypical amino acids that might be present in a protein or peptide. It is necessary to hydrolyze a protein/peptide to its individual amino acid constituents before amino acid analysis. Following protein/peptide hydrolysis, the amino acid analysis procedure can be the same as that practiced for free amino acids in other pharmaceutical preparations. The amino acid constituents of the test sample are typically derivatized for analysis.

1. Hydrolysis of Protein and Peptide
   Acid hydrolysis at 110°C for 24 hours using 6 mol/L hydrochloric acid containing phenol (Method 1) is the most common method for hydrolyzing protein and samples. The result should be analyzed carefully because several amino acids are chemically modified during the acid hydrolysis and thus not recovered quantitatively. Tryptophan is destroyed; serine and threonine are partially destroyed; methionine might undergo oxidation; and cysteine is typically recovered as cystine (but cystine recovery is usually poor because of partial destruction or reduction to cysteine). Peptide bonds involving isoleucine and valine are partially cleaved; and asparagine and glutamine are deamidated, resulting in aspartic acid and glutamic acid, respectively.
   The hydrolysis techniques, Methods 2 to 11, are used to address these concerns. Some of the hydrolysis techniques, Methods 4 to 11, may cause modifications of cysteine, methionine, asparagines and glutamine to other amino acids. Therefore, the benefits of using a given hydrolysis technique are weighed against the concerns with the technique and are tested adequately before employing a method other than acid hydrolysis by Method 1.

   (i) Method 1: Hydrolysis using hydrochloric acid containing phenol (liquid phase hydrolysis, vapor phase hy-
2.21 Nuclear Magnetic Resonance Spectroscopy / General Tests

A time-course study is often employed to analyze the starting concentration of amino acids that are partially destroyed or slow to cleave. An acceptable alternative to the time-course study is to subject an amino acid calibration standard to the same hydrolysis conditions as the test sample. This technique will allow the analyst to account for some residue destruction.

Microwave acid hydrolysis has been used and is rapid but requires special equipment as well as special precautions. Complete proteolysis, using a mixture of proteases, has been used but can be complicated, requires the proper controls, and is typically more applicable to peptides than proteins.

2. Methodologies of Amino Acid Analysis

The amino acid analysis techniques include the postcolumn derivatization for detection (Methods 1 to 2) after the separation of the free amino acids by ion-exchange chromatography and the precolumn derivatization of the free amino acids (Methods 2 to 7) followed by reversed-phase HPLC.

(ii) Method 2: Mercaptoethanesulfonic acid hydrolysis (vapor phase hydrolysis)

(iii) Method 3: Hydrolysis using hydrochloric acid containing thioglycolic acid (vapor phase hydrolysis)

(iv) Method 4: Hydrolysis by Method 1 or Method 2 after oxidation with performic acid

(v) Method 5: Hydrolysis using hydrochloric acid containing sodium azide (liquid phase hydrolysis)

(vi) Method 6: Hydrolysis using hydrochloric acid containing dimethylsulfoxide (vapor phase hydrolysis)

Cysteine-cystine oxidation

(vii) Method 7: Hydrolysis using hydrochloric acid after a vapor phase pyridylethylolation reaction

(viii) Method 8: Hydrochloric acid hydrolysis after a liquid phase pyridylethylolation reaction

(ix) Method 9: Hydrochloric acid hydrolysis after a liquid phase carboxymethylation reaction

Conversion of cysteine-cystine to mixed disulfide

(x) Method 10: Hydrochloric acid hydrolysis after a reaction with dithiodiglycolic acid or dithiodipropionic acid

Derivatization of asparagine and glutamine

(xi) Method 11: Hydrochloric acid hydrolysis after reaction with bis(1,1-trifluorooctoxy)iodobenzene

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(i) Method 1: Ninhydrin

(ii) Method 2: o-Phthalaldehyde (OPA)

(iii) Method 3: Phenylisothiocyanate (PITC)

(iv) Method 4: 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)

(v) Method 5: (Dimethylamino)azobenzenesulfonyl chloride (DABS-Cl)

(vi) Method 6: 9-Fluorenylmethyl chloroformate (FMOC-Cl)

(vii) Method 7: 7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F)

Among these methods, ion-exchange chromatography with postcolumn ninhydrin derivertization is one of the most common methods employed for quantitative amino acid analysis. The choice of any one technique often depends on the sensitivity required from the assay. Instruments and reagents for these procedures are available commercially. Furthermore, many modifications of these methodologies exist with different reagent preparations, reaction procedures, chromatographic systems, etc. Specific parameters may vary according to the exact equipment and procedure used.

Spectroscopic Methods

2.21 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy is based on the phenomenon that specific radio frequency radiation is absorbed by magnetic nuclei in a sample placed in a magnetic field; target nuclei are 1H, 13C, 15N, 19F, 31P, etc. These nuclei have intrinsic spin angular momentum, of which the magnitude is given by \( I (I + 1)/2 \), where \( I \) is the spin quantum number and is integral or half-integral (\( I = 1/2 \) for 1H and 13C). When the magnetic nuclei are placed in a magnetic field, they are oriented in \( 2I + 1 \) possible orientations corresponding to \( 2I + 1 \) equally spaced energy levels (two energy levels for 1H and 13C). The transition between two successive quantized energy levels corresponding to adjacent orientations can be induced by electromagnetic radiation with a suitable frequency. The precise relation between the field strength and the resonant frequency is given by

\[
v = \frac{v_R H_0}{2\pi} \]

where \( H_0 \) is the strength of the applied external magnetic field and \( v \) is the gyromagnetic ratio, a constant characterizing a particular isotope. The absorption of radiation (NMR signal) can occur only when the irradiating radio frequency satisfies the resonance condition. Since the absorption coefficient (the transition probability) does not depend on the environment in which the nuclei are located, the intensity is basically proportional to the number of nuclei. The excess spins shifted to the higher energy levels by the transition process return to the thermal equilibrium state at various rates determined by a characteristic time constant (known as the relaxation time).

A nucleus is shielded from the applied magnetic field by the electrons belonging to its own atom and to the molecule. Therefore nuclei in different environments are shielded to different extents and resonate at different frequencies. The difference in resonance frequencies is defined as chemical shift (\( \delta \)), which is independent of the strength of the magnetic field, and is given by

\[
\delta = \frac{v_o - v_R}{v_R} + \delta_R
\]

where,

- \( v_o \): The resonance frequency of the observed signal,
- \( v_R \): The resonance frequency of the reference signal,
- \( \delta_R \): The chemical shift of the reference signal (in the case of the value not being 0).

The chemical shifts are normally expressed in ppm, a
dimensionless unit, by assuming the chemical shift of the reference compound as 0 ppm. When the chemical shift of the reference compound is not assumed to be 0 ppm, chemical shifts of samples are corrected accordingly.

In addition to the shielding due to electrons, the nucleus is subjected to effects due to the spin orientations of other magnetic nuclei through chemical bonds, resulting in an additional splitting of the signal. The spacing between two adjacent components of the signal is known as the spin-spin coupling constant ($J$). Coupling constants are measured in hertz and are independent of the strength of the external magnetic field. The increased number of interacting nuclei will make the multiplet pattern more complex.

From the NMR spectrum the following four parameters can be obtained: chemical shift, spin-spin coupling constant, resonance intensity (intensities of $^1H$ are proportional to the number of nuclei and those of $^{13}C$ and others are susceptible to the nuclear Overhauser effect (NOE) and relaxation) and relaxation time. These parameters are useful for structural determination, identification and quantitative analysis of molecules. Spin decoupling, NOE, and two-dimensional NMR techniques are also available for structural analysis.

1. **Spectrometer**
   
   There are two types of spectrometers.

1.1. **Fourier transform NMR (FT-NMR) spectrometers** (Fig. 2.21-1)

   Target nuclei are simultaneously excited in all frequency range of the nuclei by means of an intense radio frequency pulse. The FID (free induction decay) after the pulse is detected, which is a time domain signal, is converted to a frequency domain spectrum by Fourier transformation. Number of data points suitable for the spectral range, flip angle, acquisition time, delay time and number of scans should be set appropriately.

   Recently FT-NMR is commonly used because of its high sensitivity and various advanced applications.

1.2. **Continuous wave NMR (CW-NMR) spectrometers** (Fig. 2.21-2)

   In the case of the CW method, a spectrum is obtained by sweeping the radio frequency or magnetic field continuously over the frequency range of the nuclei being observed.

2. **Measurement**

   Prior to measurements, the sensitivity and resolution of the instrument must be adjusted to the optimum levels using a standard sample (ethylbenzene, 1,2-dichlorobenzene or acetaldehyde) dissolved in an appropriate NMR solvent.

   The sample dissolved in a suitable solvent is transferred into an NMR tube. The reference compound can be added directly to the sample solution (internal reference), or a sealed capillary tube containing the reference compound can be inserted into the NMR tube (external reference). The sample solutions should be completely homogeneous. In particular, solid contaminants should be removed in order to obtain good spectra. Various deuterated NMR solvents are commonly used for NMR measurement and the following points should be considered in selecting an appropriate solvent: (i) The solvent signals do not overlap with the sample signals. (ii) The sample must be soluble in the solvent selected. (iii) The solvent does not react with the sample. Furthermore, it should be noted that chemical shifts can depend upon the solvent employed, sample concentration and deuterium ion concentration, and that viscous solutions usually give rather broad, poorly resolved spectra.

   For the reference standards use the reagents for nuclear magnetic resonance spectroscopy. For $^1H$ and $^{13}C$ spectra, tetramethylsilane (TMS) is usually used as the reference compound for samples dissolved in organic solvents. For samples dissolved in deuterium oxide, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) or sodium 3-(trimethylsilyl)propionate (TSP) is used. For other nuclei, nitromethane, trichlorofluoromethane and phosphoric acid are used as reference compounds for $^{15}N$, $^{19}F$ and $^{31}P$, respectively. Furthermore, chemical shifts of residual protons in deuterated solvents and $^{13}C$ in the solvent instead of a reference compound can be used for $^1H$ and $^{13}C$ NMR.

3. **Record of apparatus and measurement conditions**

   Type of instrument, frequency, solvent, temperature, sample concentration, reference compound, experimental technique, etc. should be recorded to allow appropriate comparison of spectra, because NMR spectra depend on the measurement conditions.

4. **Identification**

   The sample solution is prepared and tested by the method directed in each monograph. Usually in the case of $^1H$ NMR, the sample is identified by the following method.

4.1. **Identification by the use of chemical shift, multiplicity and relative intensity**

   When chemical shifts, multiplicities and relative intensities...
of signals are defined, the sample can be identified as being the same substance when all chemical shifts, multiplicities and relative intensities are the same as those prescribed.

4.2. Identification by the use of a Reference Standard

Measurement conditions should be the same as those used in the case of the Reference Standard. When the spectra of a sample and the Reference Standard exhibit the same multiplicities and relative intensities of signal at the same chemical shifts, the sample can be identified as being the same substance as the Reference Standard.

5. Experimental techniques of \( ^1H \) and \( ^{13}C \) NMR spectroscopy

NMR spectroscopy includes one-, two- and multi-dimensional techniques, which are used for various purposes.

Spin decoupling, and NOE are available in one-dimensional \( ^1H \) spectroscopy. Spin decoupling can assign coupling correlations. As NOE can observe correlations among spatially proximate protons, the configuration and the conformation can be analyzed.

Broadband decoupling, INEPT and DEPT are usually applied in one-dimensional \( ^{13}C \) spectroscopy. The broadband decoupling technique simplifies a spectrum and achieves enhancement of sensitivity. INEPT (insensitive nuclei enhanced by polarization transfer) and DEPT (distortionless enhancement of polarization transfer) enhance the sensitivity of \( ^{13}C \) by means of polarization transfer from directly bonded \( ^1H \) with a large magnetic moment. They can be applied to identify primary, secondary, tertiary or quarternary carbon.

Two-dimensional spectroscopy can observe all correlation peaks between nuclei through spin-spin coupling or NOE in a single experiment, and there are many techniques for homonuclear and heteronuclear measurements. Representative techniques are described below.

COSY (2D correlation spectroscopy), HOHAHA (homonuclear Hartmann-Hahn spectroscopy) or TOCSY (total correlation spectroscopy): Correlation between protons through scalar spin-spin coupling is obtained and intramolecular connectivities of hydrogen atoms are revealed.

NOESY (2D nuclear Overhauser enhancement and exchange spectroscopy): NOE is measured by a two-dimensional technique. Approximate distances between spatially proximate hydrogen atoms are obtained to analyze the three-dimensional structure.

INADEQUATE (incredible natural abundance double quantum transfer experiment): Although this technique is insensitive because it involves double quantum transfer by \( ^{13}C-^{13}C \) scalar coupling in a sample with natural isotopic abundance, the connectivities of all neighboring \( ^{13}C \) nuclei can be obtained to analyze the carbon skeleton.

HMQC (heteronuclear multiple quantum coherence): This technique observes correlations between \( ^1H \) and \( ^{13}C \) with direct spin-spin coupling using \( ^1H \) detection and reveals intramolecular chemical bonds between hydrogen and carbon atoms.

HMBC (heteronuclear multiple bond connectivity): This technique observes correlations between \( ^1H \) and \( ^{13}C \) with long range spin-spin coupling using \( ^1H \) detection and reveals intramolecular connectivities of hydrogen and carbon atoms.

There are many other techniques such as DQF-COSY (double quantum filtered COSY) and HSQC (heteronuclear single quantum coherence). Furthermore, multidimensional NMR techniques are used to analyze macromolecules.

2.22 Fluorometry

Fluorometry is a method to measure the intensity of fluorescence emitted from a solution of fluorescent substance irradiated with an exciting light in a certain wavelength range. This method is also applied to the phosphorescent substances.

Fluorescence intensity \( F \) in a dilute solution is proportional to the concentration \( c \) in mol per liter of the solution and the pathlength \( l \) of light through the solution in centimeter.

\[
F = kI_0\phi cl
\]

\( k: \) Constant
\( I_0: \) Intensity of exciting light
\( \phi: \) Quantum yield of fluorescence or phosphorescence

\[
\phi = \frac{\text{number of quanta emitted as fluorescence or phosphorescence}}{\text{number of quanta absorbed}}
\]

\( \varepsilon: \) Molar extinction coefficient of the substance at the excitation wavelength

1. Apparatus

Spectrofluorometer is usually used. Generally, a xenon lamp, laser, an alkaline halide lamp, etc. which provide stable exciting light are used as the light source. Usually, a non-fluorescent quartz cell (1 cm × 1 cm) with four transparent sides is used as the container for sample solution.

2. Procedure

Excitation spectrum is obtained by measuring fluorescence intensities of sample solution with varying excitation wavelengths at a fixed emission wavelength (in the vicinity of the fluorescence maximum) and drawing a curve showing the relationship between the excitation wavelength and the fluorescence intensity. Fluorescence spectrum is obtained by measuring fluorescence intensities of sample solution with varying emission wavelengths at a fixed excitation wavelength (in the vicinity of the excitation maximum) and drawing the same curve as described for the excitation spectrum. If necessary, the spectra are corrected with regard to the optical characteristics of the apparatus.

The fluorescence intensity is usually measured at the excitation and the emission wavelengths in the vicinity of excitation and emission maxima of the fluorescent substance. The fluorescence intensity is expressed as a value relative to that of a standard solution, because it is readily affected even by a slight change in the condition for the measurement.

Unless otherwise specified, the instrument is operated as follows with standard, sample, and reference solutions prepared as directed in the monograph: Fix the excitation and fluorescence wavelength scales at the designated positions, adjust the dark current to zero, put the quartz cell containing the standard solution in the light path, and adjust the instrument so that the standard solution shows the fluorescence intensity of 60% to 80% of full scale. Then perform the measurements with cells containing the sample solution and the control solution, and read the fluorescence intensity as % under the same condition. Set the width of the wavelength properly unless otherwise specified.
3. Note
The fluorescence intensity is readily affected by the concentration, temperature and pH of the solution, and nature and purity of solvents or reagents used.

2.23 Atomic Absorption Spectrophotometry

Atomic Absorption Spectrophotometry is a method to determine the amount or the concentration of an element in a sample specimen being examined, by utilizing the phenomenon that atoms being in the ground state absorb the light of specific wavelength, characteristic of the respective atom, when the light passes through an atomic vapor layer of the element to be determined.

1. Apparatus
Usually, the apparatus consists of a light source, a sample atomizer, a spectroscope, a photometer and a recording system. Some are equipped with a background compensation system. As a light source, usually a hollow cathode lamp specified for each element is used and sometimes a discharge lamp is also used. There are three types of sample atomizer: the flame type, the electrothermal type, and the cold-vapor type. The first one is composed of a burner and a gas-flow regulator, the second one is composed of an electric furnace and a power source, and the third one is composed of a mercury generator and an absorption cell. The third one is further classified into two subtypes, which differ in the atomizing method for mercury containing-compounds: one utilizes chemical reduction-vaporization and the other utilizes a thermal reduction-vaporization method.

For the selection of an appropriate analytical wavelength in a spectroscope, a grating for light diffraction or an interference filter can be used. A recording system is composed of a display and a recording device. A background compensation system is employed for the correction of atmospheric effects on the measuring system. Several principles can be utilized for background compensation, using continuous spectrum sources, the Zeeman splitted spectrum, the non-resonance spectrum, or self-inversion phenomena.

Another special options such as a hydride generator and a heating cell, can also be used for analyzing such as selenium. As a hydride generator, a batch method and/or a continuous flow method can be applied. While as a heating cell, there are two kinds of cell: one for heating by flame and the other for heating by electric furnace.

2. Procedure

Unless otherwise specified, proceed by any of the following methods.

2.1. Flame type
Fit the specific light source to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and slit-width. Next, a mixture of a combustible gas and a supporting gas is ignited and the gas flow rate and/or pressure should be adjusted to optimum conditions. The zero adjustment of the detecting system must be done through nebulizing the blank solvent into the flame. After setting up the measuring system, the sample solution prepared by the specified procedure is introduced into the flame and the light absorption at the characteristic wavelength of the element to be determined is measured.

2.2. Electrothermal type
Fit the specific light source to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and slit-width. Further, set an electric furnace to the appropriate temperature, electric current, and heating program, as directed separately in the monograph. When a suitable amount of sample is injected into the heated furnace with an appropriate stream of inert gas, the sample is dried and ashed, simultaneously with atomization of the metallic compound included in the specimen. The atomic absorption specified is observed and the intensity of absorption is measured. Details of the sample preparation method are provided separately in the monograph.

2.3. Cold vapor type
Fit the mercury lamp to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and a slit-width. In the chemical atomization-vaporization method, a mercury containing compound in the sample solution, prepared by the specified procedure, is chemically reduced to metallic mercury by adding a proper reducing reagent to the closed vessel and the generated mercury is vaporized and introduced into the absorption cell with a flow of inert gas. In the thermal atomization-vaporization method, the sample specimen on a quartz dish is heated electrically and the generated atomic mercury is vaporized and introduced into the absorption cell with a flow of inert gas. Thus, in both methods, the generated atomic mercury is carried into the absorption cell as cold vapor and the intensity of the characteristic atomic absorption of mercury is measured.

3. Determination

Usually, proceed by any of the following methods. In the determination, the possibility of interference for various reasons and the background effect must be considered and avoided if possible.

3.1. Calibration curve method
Prepare standard solutions at more than 3 concentration levels, measure the specific absorption due to these standard solutions, and prepare the calibration curve of the atomic absorption against the concentration. Then measure the atomic absorption due to the sample specimen, in which the concentration of the element to be determined should be adjusted to be within the concentration range of the standard solutions, and determine the amount or the concentration of the element to be examined using the calibration curve.

3.2. Standard addition method
To equal volumes of more than 3 sample solutions, prepared as directed in the monograph, add a measured quantity of the standard solutions to produce a series of solutions containing increasing amounts of the element to be examined, and further add a solvent to make up a constant volume. Measure the atomic absorption for the respective solutions, and plot the obtained values on a graph with the added amount or the concentration on the abscissa and the absorbance on the ordinate. Extrapolate the linear plot obtained by linking the data points, and determine the amount or the concentration of the element to be examined from the
distance between the origin and the point where the plot intersects with the abscissa. This method is available only when the calibration curve obtained by Method (1) is confirmed to be linear and to pass through the origin.

3.3. Internal standard method

Prepare a series of standard solutions of the element to be determined, each containing a definite amount of the internal standard element directed in the monograph. For these standard solutions, measure the atomic absorption due to the standard element and the internal standard element separately at the respective wavelengths under the same operating conditions, and obtain the ratio of absorbance by the standard element to that by the internal standard element. Prepare a calibration curve for the element to be determined, with the amount or the concentration of the standard element on the abscissa and the above-mentioned ratio of the absorbance on the ordinate. Then prepare sample solutions, adding the same amount of the internal standard element as contained in the standard solutions. Measure the ratio of the absorbance due to the element to be determined to that due to the internal standard element under the same conditions as employed for preparing the calibration curve, and determine the amount or the concentration of the element being examined by using the calibration curve.

4. Note

Reagents, test solutions, and gases used in this test should not interfere in any process of the measurement.

2.24 Ultraviolet-visible Spectrophotometry

Ultraviolet-visible Spectrophotometry is a method to measure the degree of absorption of light between the wavelengths of 200 nm and 800 nm by substances for the tests of their identity and purity, and for assay. When an atomic absorption spectrophotometer is used for these purposes, proceed as directed under Atomic Absorption Spectrophotometry 2.25. When monochromatic light passes through a substance in the solution, the ratio of transmitted light intensity \( I \) to incident light intensity \( I_0 \) is called transmittance \( T \); transmittance expressed in the percentage is called percent transmittance. The absorbance \( A \) is given by

\[
A = \log \frac{I_0}{I} = k c l
\]

where \( k \) is the molar absorption coefficient, \( c \) is the concentration of the element in molar, and \( l \) is the path length in cm. The difference between absorbance and wavelength. From the absorption spectrum, it is possible to determine the wavelength of maximum absorption \( \lambda_{\text{max}} \) and that of minimum absorption \( \lambda_{\text{min}} \).

The absorption spectrum of a substance in the solution is characteristic, depending on its chemical structure. Therefore, it is possible to identify a substance by comparing the spectrum of a sample within the specified wavelength range with the Reference Spectrum or the spectrum of Reference Standard, by determining the wavelengths of maximum absorption, or by measuring the ratio of absorbances at two specified wavelengths. For the purpose of assay, the absorbance by a sample solution with a certain concentration is measured at the wavelength of the maximum absorption \( \lambda_{\text{max}} \) and compared it with the absorbance of a standard solution with a certain concentration.

1. Apparatus and adjustment

A spectrophotometer or a photoelectric photometer is used for the measurement of absorbance.

After adjusting the spectrophotometer or photoelectric photometer based on the operation manual of the apparatus, it should be confirmed that the wavelength and the transmission rate meet the specifications of the tests described below.

The calibration of wavelength should be carried out as follows. Using an optical filter for wavelength calibration, measure the transmission rate in the vicinity of the standard wavelength value shown in the test results form, under the test conditions given in the test results form attached to each of the filters. When performing a test to determine the wavelength which shows minimal transmission rate, the difference between the measured wavelength and the standard wavelength value should be within ± 0.5 nm. When the measurement is repeated three times, each value obtained should be within the mean ± 0.2 nm. It is also possible to carry out the test using a low-pressure mercury lamp at bright line wavelengths of 253.65 nm, 365.02 nm, 435.84 nm and 546.07 nm, or a deuterium discharge lamp at bright line wavelengths of 486.00 nm and 656.10 nm. In the case of these tests, the difference between the measured wavelength and the wavelength of the bright line should be within ± 0.3 nm. When the measurement is repeated three times, each value obtained should be within the mean ± 0.2 nm.

The calibration of transmission rate or absorbance should be carried out as follows. Using an optical filter for transmission rate calibration, determine the transmission rate at the standard wavelength value under the test conditions given in the test results form attached to each of the filters. The difference between the measured transmission rate and the standard transmission rate value should be within the range of from 1% larger of the upper limit to 1% smaller of the lower limit for the relative accuracy shown in the test results form. When the measurement is repeated three times, each absorbance obtained (or calculated from the transmission rate) should be within the mean ± 0.002 when the absorbance is not more than 0.500, and within the mean ± 0.004 when the absorbance is more than 0.500. In addition, it will be desirable to confirm the linearity of transmission rate at the same wavelength using several optical filters for calibration of transmission rate with different transmission rates.

2. Procedure

After adjusting the apparatus as directed in the Apparatus and adjustment, select and set the light source, detector,
mode of measurement, measuring wavelength or wavelength range, spectrum width and scanning speed.

Subsequently, allow the apparatus to stand for a certain time to confirm its stability. Then, usually adjust the apparatus so that the transmittance is 0% at measuring wavelength or over measuring wavelength range after shutting the sample side of light path. Then open the shutter and adjust the transmittance to 100% (the absorbance is zero). Adjusting the transmittance to 100% is usually done by putting cells containing the control solution in both light paths. For the control solution, unless otherwise specified, blank solvent is used.

Then perform the measurement with the cell containing the sample solution, and read the absorbance at measuring wavelength, or measure the spectrum over measuring wavelength range. Unless otherwise specified, a cell with a path length of 1 cm, made of quartz for ultraviolet range and of quartz or glass for visible range, is used. Special consideration is needed with the absorption of solvents in the ultraviolet range; use a solvent which does not disturb accurate measurement.

3. Specific absorbance

In the Japanese Pharmacopoeia, the absorbance, calculated on the basis that l is 1 cm and c (concentration of a medicament) is 1 w/v%, is called specific absorbance, and is expressed as $E_{1\text{cm}}^{1\%}$.

$$E_{1\text{cm}}^{1\%} = \frac{A}{c \times l}$$

l: Length of the layer of the solution (cm)
A: Absorbance value
c: Concentration of the sample in the solution (w/v%)

The description of, for example, “$E_{1\text{cm}}^{1\%}$ (241 nm): 500 – 530 (after drying, 2 mg, methanol, 200 mL)” in the monograph, indicates that observed $E_{1\text{cm}}^{1\%}$ value is between 500 and 530, when the test is performed in the following manner: The sample is dried under the conditions specified in the Test for Loss on Drying, and about 2 mg of the sample is weighed accurately with a microbalance, and dissolved in methanol to make exactly 200 mL, then the absorbance of the solution is measured as directed in the Procedure at a wavelength of 241 nm using a cell with a path length of 1 cm.

4. Identification

Prepare the sample solution as directed in the monograph, and test as directed in the Procedure. Usually, the test is performed by a single method or in a combination of a few methods in the following methods using the absorbance or absorption spectrum obtained from the sample solution. Subtle differences in the absorption spectrum arising from differences in the apparatus used may be neglected.

4.1. Identification using Reference Spectrum

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the Reference Spectrum, the identity of the sample and the reference may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

Reference spectrum: Reference spectra are specified under the Ultraviolet-visual Reference Spectra, which are used as the reference for the test of identification specified in the monograph.

4.2. Identification using Reference Standard

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the spectrum obtained from the Reference Standard, the identity of the sample and the reference may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum. When the relevant Reference Spectrum is not available, the range is that specified in the monograph.

4.3. Identification using absorption wavelength

When maximum absorption wavelengths of the spectrum obtained from the sample solution match the wavelengths specified in the monograph, the identity of the substance may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

4.4. Identification using the ratio of the absorbances obtained at two or more wavelengths

When the ratios of absorbances at the specified wavelengths in the spectrum obtained from the sample solution meet the specifications in the monograph, the identity of the substance may be confirmed.

5. Assay

Prepare the control solution, the sample solution and the standard solution as directed in the monograph, measure the absorbances of the sample solution and the standard solution according to the method described in the Procedure, and determine the amount of the substance to be assayed in the sample by comparing the absorbances.

2.25 Infrared Spectrophotometry

Infrared Spectrophotometry is a method of measurement of the extent, at various wave numbers, of absorption of infrared radiation when it passes through a layer of a substance. In the graphic representation of infrared spectra, the plot usually shows units of wave numbers as the abscissa and units of transmittance or absorbance as the ordinate. Wave number and transmittance or absorbance at each absorption maximum may be read graphically on an absorption spectrum and/or obtained by a data-processor. Since the wave number and the respective intensity of an absorption maximum depend on the chemical structure of a substance, this measurement can be used to identify or determine a substance.

1. Instrument and adjustment

Several models of dispersive infrared spectrophotometers or Fourier-transform infrared spectrophotometers are available.

The instruments, adjusted according to the instruction manual of each individual instrument, should comply with the following test for resolving power, transmittance reproducibility and wave number reproducibility. When the spectrum of a polystyrene film about 0.04 mm thick is recorded, the depth of the trough from the maximum absorption at about 2850 cm$^{-1}$ to the minimum at about 2870 cm$^{-1}$ should not be less than 18% transmittance and that from the maximum at about 1583 cm$^{-1}$ to the minimum at about 1589 cm$^{-1}$ should be not less than 12% transmittance.

The wave number (cm$^{-1}$) scale is usually calibrated by the
use of several characteristic absorption wave numbers (cm\(^{-1}\)) of a polystyrene film shown below. The number in parentheses indicates the permissible range.

\[
\begin{align*}
3060.0 \pm 1.5 & \quad 2849.5 \pm 1.5 & \quad 1942.9 \pm 1.5 \\
1601.2 \pm 1.0 & \quad 1583.0 \pm 1.0 & \quad 1154.5 \pm 1.0 \\
1028.3 \pm 1.0 & \\
\end{align*}
\]

When the dispersive infrared spectrophotometer is used, the permissible range of the absorption wave numbers at 1601.2 cm\(^{-1}\) and at 1028.3 cm\(^{-1}\) should be both within ± 2.0 cm\(^{-1}\).

As the repeatability of transmittance and wave number, the difference of transmittance should be within 0.5% when the spectrum of a polystyrene film is measured twice at several wave numbers from 3000 to 1000 cm\(^{-1}\), and the difference of wave number should be within 5 cm\(^{-1}\) at about 3000 cm\(^{-1}\) and within 1 cm\(^{-1}\) at about 1000 cm\(^{-1}\).

2. Preparation of samples and measurement

Unless otherwise specified, when it is directed to perform the test "after drying the sample", use a sample dried under the conditions specified in the monograph. Prepare the specimen for the measurement according to one of the following procedures so that the transmittance of most of the absorption bands is in the range of 5% to 80%. Single crystals of sodium chloride, potassium bromide, etc. are available for the optical plate. Generally, the reference cell or material is placed in the reference beam for double-beam instruments, while for single-beam instruments, it is placed in the same optical path in place of the specimen and measured separately under the same operating conditions. The composition and preparation of the reference depend on the sample preparation methods, and sometimes the background absorption of the atmosphere can be utilized.

Unless otherwise specified in the monograph, the spectrum is usually recorded between 4000 cm\(^{-1}\) and 400 cm\(^{-1}\). The spectrum should be scanned using the same instrumental conditions as were used to ensure compliance with the requirements for the resolving power and for the precision of wave number scale and of wave numbers.

2.1. Potassium bromide disk or potassium chloride disk method

Powder 1 to 2 mg of a solid sample in an agate mortar, triturate rapidly with 0.10 to 0.20 g of potassium bromide or potassium chloride for infrared spectrophotometry with precautions against moisture absorption, and compress the mixture with a press in a suitable die (disk-forming container) to make the sample disk. If necessary to obtain a transparent disk, press the mixture under vacuum in a die with pressure applied to the die of 50 to 100 kN per cm\(^{2}\) for 5 to 8 minutes. Prepare a potassium bromide reference disk or a potassium chloride reference disk in the same manner as the sample disk.

2.2. Solution method

Place the sample solution prepared by the method directed in each monograph in a fixed cell for liquid, and usually measure the spectrum against the reference solvent used for preparing the sample solution. The solvent used in this method should not show any interaction or chemical reaction with the specimen to be examined and should not damage the optical plate. The thickness of the fixed cell is usually 0.1 mm or 0.5 mm.

2.3. Paste method

Powder 5 to 10 mg of a solid specimen in an agate mortar, and, unless otherwise specified, triturate the specimen with 1 to 2 drops of liquid paraffin to give a homogeneous paste. After spreading the paste to make a thin film in the center of an optical plate, place the plate upon another optical plate with precautions against intrusion of air, bubbles in the film, and examine its absorption spectrum.

2.4. Liquid film method

Examine 1 to 2 drops of a liquid specimen as a thin film held between two optical plates. When the absorption intensity is not sufficient, place spacers of aluminum foil, etc., between the two optical plates to make a thicker liquid film.

2.5. Film method

Examine a thin film just as it is or a prepared thin film as directed in each monograph.

2.6. Gas sampling method

Put a sample gas in a gas cell previously evacuated under the pressure directed in the monograph, and examine its absorption spectrum. The path length of the gas cell is usually 5 cm or 10 cm, but, if necessary, may exceed 1 m.

2.7. ATR method

Place a specimen in close contact with an attenuated total reflectance (ATR) prism, and examine its reflectance spectrum.

2.8. Diffuse reflectance method

Powder 1 to 3 mg of a solid specimen into a fine powder of not more than about 50 \(\mu\)m particle size in an agate mortar, and triturate rapidly with 0.05 to 0.10 g of potassium bromide or potassium chloride for infrared spectrophotometry with precautions against moisture absorption. Place the mixture in a sample cup, and examine its reflectance spectrum.

3. Identification

When the spectrum of a specimen and the Reference Spectrum of the substance expected to be found or the spectrum of the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the substance expected to be found. Furthermore, when several specific absorption wave numbers are specified in the monograph, the identification of a specimen with the substance expected to be found can be confirmed by the appearance of absorption bands at the specified wave numbers.

3.1. Identification by the use of a Reference Standard

When the spectra of a specimen and the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance as the Reference Standard. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with that of the Reference Standard, treat the specimen being examined and the Reference Standard in the same manner as directed in the monograph, then repeat the measurement.

3.2. Identification by the use of a Reference Spectrum

When the spectra of a specimen and the Reference Spectrum exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance associated with the Reference Spectrum. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with the Reference Spectrum, treat the speci-
men being examined as directed in the monograph, then repeat the measurement.

Infrared Reference Spectra, in the range between 4000 \( \text{cm}^{-1} \) and 400 \( \text{cm}^{-1} \), are shown at the end of this book for the monographs requiring the identification test by Infrared Spectrophotometry, except for monographs in which "Identification by absorption wave number" is specified.

3.3. Identification by the use of absorption wave number

When several specific absorption wave numbers of the substance being examined are specified in the monograph, a specimen can be identified as being the same substance as the expected substance by confirmation of clear appearance of the absorption bands at all the specified wave numbers.

Other Physical Methods

2.41 Loss on Drying Test

Loss on Drying Test is a method to measure the loss in mass of the sample, when dried under the conditions specified in each monograph. This method is applied to determine the amount of water, all or a part of water of crystallization, or volatile matter in the sample, which is removed during the drying.

The description, for example, "not more than 1.0% (1 g, 105°C, 4 hours)" in a monograph, indicates that the loss in mass is not more than 10 mg per 1 g of the substance in the test in which about 1 g of the substance is accurately weighed and dried at 105°C for 4 hours, and "not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours)," indicates that the loss in mass is not more than 5 mg per 1 g of the substance in the test in which about 1 g of the substance is accurately weighed, transferred into a desiccator (phosphorus (V) oxide), and dried in vacuum for 4 hours.

1. Procedure

Weigh accurately a weighing bottle that has been dried for 30 minutes according to the method specified in the monograph. Take the sample within the range of ±10% of the amount directed in the monograph, transfer into the weighing bottle, and, unless otherwise specified, spread the sample so that the layer is not thicker than 5 mm, then weigh it accurately. Place the loaded bottle in a drying chamber, and dry under the conditions specified in the monograph. When the size of the sample is large, convert it to small particles having a size not larger than 2 mm in diameter by quick crushing, and use the crushed sample for the test. After drying, remove from the drying chamber, and reweigh accurately. When the sample is dried by heating, the temperature is within the range of ±2°C of that directed in the monograph, and, after drying the bottle, the sample is allowed to cool in a desiccator (silica gel) before weighing.

If the sample melts at a temperature lower than that specified in the monograph, expose the sample for 1 to 2 hours to a temperature between 5°C and 10°C below the melting temperature, dry under the conditions specified in the monograph. Use a desiccant specified in the monograph, and renew frequently.

2.42 Congealing Point Determination

The congealing point is the temperature measured by the following method.

1. Apparatus

Use the apparatus illustrated in Fig. 2.42-1.

2. Procedure

Transfer the sample into sample container B up to the marked line C. When the sample is solid, melt the sample by heating to a temperature not higher than 20°C above the expected congealing point, and transfer to B. Fill the glass or plastic bath D with water at a temperature about 5°C below the expected congealing point. When the sample is liquid at room temperature, fill bath D with water at a temperature between 10°C and 15°C lower than the expected congealing point.
Insert the sample container B containing the sample into cylinder A. Adjust the immersion line H of thermometer F to the same level of the meniscus of the sample. After cooling the sample to about 5°C above the expected congealing point, move vertically the stirrer E at the rate of about 60 to 80 strokes per minute, and observe the thermometer readings at 30-second intervals. The temperature falls gradually. Discontinue stirring, when an appreciable amount of crystals has formed and the temperature is constant or has begun to rise. Usually, read the maximum temperature (reading of F), that is constant for a while after a rise of temperature. If no rise of temperature occurs, read the temperature that is constant for a while. The average of not less than four consecutive readings that lie within a range of 0.2°C constitutes the congealing point.

3. Note
If a state of super cooling is anticipated, rub the inner wall of bath B or put a small fragment of the solid sample into bath B for promoting the congealment, when the temperature approaches near the expected congealing point.

2.43 Loss on Ignition Test

Loss on Ignition Test is a method to measure the loss in mass when the sample is ignited under the conditions specified in each monograph. This method is usually applied to inorganic drugs which lose a part of the components or impurities during ignition.

The description, for example, “40.0 – 52.0% (1 g, 450 – 550°C, 3 hours)” in a monograph, indicates that the loss in mass is 400 to 520 mg per g of the substance in the test in which about 1 g of the substance is weighed accurately and ignited between 450°C and 550°C for 3 hours.

1. Procedure

Previously ignite a crucible or a dish of platinum, quartz or porcelain to constant mass, at the temperature directed in the monograph, and weigh accurately after cooling.

Take the sample within the range of ±10% of the amount directed in the monograph, transfer into the above ignited container, and weigh it accurately. Ignite under the conditions directed in the monograph, and, after cooling, weigh accurately. Use a desiccator (silica gel) for the cooling.

2.44 Residue on Ignition Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦). ♦The Residue on Ignition Test is a method to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulfuric acid according to the procedure described below. This test is usually used for determining the content of inorganic impurities in an organic substance.

The description, for example, “not more than 0.1% (1 g)” in a monograph, indicates that the mass of the residue is not more than 1 mg per 1 g of the substance in the test in which about 1 g of the substance is weighed accurately and ignited by the procedure described below, and “after drying” indicates that the sample is tested after being dried under the conditions specified in the test for Loss on drying. ♦

1. Procedure

Ignite a suitable crucible (for example, silica, platinum, quartz or porcelain) at 600 ± 50°C for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccant) and weigh it accurately.

Take the amount of test sample specified in the individual monograph in the crucible and weigh the crucible accurately.

Moisten the sample with a small amount (usually 1 mL) of sulfuric acid, then heat gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount (usually 1 mL) of sulfuric acid, heat gently until white fumes are no longer evolved, and ignite at 600 ± 50°C until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Cool the crucible in a desiccator (silica gel or other suitable desiccant), weigh accurately and calculate the percentage of residue.

Unless otherwise specified, if the amount of the residue so obtained exceeds the limit specified in the individual monograph, repeat the moistening with sulfuric acid, heating and ignition as before, using a 30-minute ignition period, until two consecutive weighings of the residue do not differ by more than 0.5 mg or until the percentage of residue complies with the limit in the individual monograph.

2.45 Refractive Index Determination

Refractive Index Determination is a method to measure the ratio of the velocity of light in air to that in the sample. Generally, when light proceeds from one medium into another, the direction is changed at the boundary surface. This phenomenon is called refraction. When light passes from the first isotropic medium into the second, the ratio of the sine of the angle of incidence, \( i \), to that of the angle of refraction, \( r \), is constant with regard to these two media and has no relation to the angle of incidence. This ratio is called the refractive index of the second medium with respect to the first, or the relative refractive index, \( n \).

\[
 n = \frac{\sin i}{\sin r}
\]

The refractive index obtained when the first medium is a vacuum is called the absolute refractive index, \( N \), of the second medium.

In isotropic substances, the refractive index is a characteristic constant at a definite wavelength, temperature, and pressure. Therefore, this measurement is applied to purity test of substances, or to determination of the composition of homogeneous mixtures of two substances.

The measurement is usually carried out at 20°C, and the D line of the sodium spectrum is used for irradiation. This value is expressed as \( nD \).

1. Procedure

For the measurement of refractive index, usually the Abbé
refractometer is used at a temperature in the range of ±0.2°C of that directed in the monograph. Use of the Abbé refractometer permits direct reading of \( n_0 \) under incandescent light, with a measurable range from 1.3 to 1.7, and an attainable precision of 0.0002.

### 2.46 Residual Solvents Test

Residual Solvents Test is a test to determine the amounts of residual organic solvents in pharmaceuticals by using gas chromatography (<2.02> or other suitable methods. If only solvents with low toxic potential to man are present in pharmaceuticals, the test of loss on drying (<2.41>) can be applied in place of gas chromatography.

1. **Procedure and Test Method**

   Perform the test by using gas chromatography (<2.02>) or other suitable methods.

   The items necessary for conducting the test should previously be specified so that the test method is suitable for determining the objective residual solvents. For gas chromatography, the items usually necessary to specify are the quantity of sample and reference standard (reference substance) for the test, method for preparing sample and standard solutions, injection amounts of sample and standard solutions to gas chromatograph, calculation formula, operating conditions for head-space apparatus and gas chromatography, and system suitability.

### 2.47 Osmolarity Determination

Osmolarity Determination is a method for measuring the osmotic concentration of the sample solution from the extent of the freezing-point depression.

When a solution and a pure solvent are separated by a semipermeable membrane, through which the solvent can pass freely, but the solute cannot, a part of the solvent passes into the solution compartment through the membrane. The pressure difference produced between the two compartments concomitantly with the solvent migration through the membrane, is defined as the osmotic pressure \( \Pi \) (Pa). The osmotic pressure is a physical quantity depending on the total of the molecular species present, including neutral molecules and ions, and does not depend on the kind of solute. A solution property, such as osmotic pressure, freezing-point depression, boiling-point elevation etc., which depends not on the kind of solute, but on the total number of all molecular species, is called a colligative property of a solution.

The osmotic pressure of a polymer solution can be measured directly as the hydrostatic pressure difference between two compartments separated by a semipermeable membrane, such as a cellulose membrane. However, this is not applicable to a solution containing low molecular species, which can pass through a semipermeable membrane. Though the osmotic pressure of such a solution cannot be measured directly, the direction and extent of solvent migration through biological membranes can be predicted from the total number of all molecular species present when the solution is placed under physiological conditions. Other colligative properties of a solution such as freezing-point depression, boiling-point elevation, vapor-pressure depression, etc. can be directly obtained by observing changes of temperature and/or pressure, etc. These solution properties depend on the total number of ionic and neutral species in the solution in the same way as the osmotic pressure, and the molecular particle concentration is defined as the osmotic concentration. The osmotic concentration can be defined in two ways, one being mass-based concentration (osmolality, mol/kg) and the other, volume-based concentration (osmolality, mol/L). In practice, the latter is more convenient.

Unless otherwise specified, the freezing-point depression method is used for measuring the osmotic concentration. The method is based on the linear dependency of the freezing-point depression \( \Delta T \) (°C) upon the osmolality \( m \) (mol/kg), as expressed in the following equation,

\[ \Delta T = K \cdot m \]

In this equation, \( K \) is the molal freezing-point depression constant, and it is known to be 1.86°C kg/mol for water. Since the constant \( K \) is defined on the basis of molarity, the molar osmotic concentration can be obtained from the above equation. In the dilute osmotic concentration range, osmolality \( m \) (mol/kg) can be assumed to be numerically equal to osmolality \( c \) (mol/L). Thus, the conventional osmolality (mol/L) and the unit of osmole (Osm) are adopted in this test method. One Osm means that the Avogadro number \((6.022 \times 10^{23})/\text{mol}\) of species is contained in 1 L of solution. Usually the osmotic concentration is expressed as the sub-multiple milliosmole (mOsm, mosmol/L) in the Pharmacopoeia.

1. **Apparatus**

   Usually, the osmotic concentration of a solution can be obtained by measuring the extent of the freezing-point depression. The apparatus (osmometer) is composed of a sample cell for a fixed volume of sample solution and a cell holder, a cooling unit and bath with a temperature regulator, and a thermistor for detecting temperature.

2. **Procedure**

   A fixed volume of the test solution is introduced into the sample cell, as indicated for the individual apparatus.

   The apparatus must first be calibrated by the two-point calibration method by using osmolar standard solutions. For the calibration, select two different standard solutions just covering the expected osmolar concentration of a sample solution. Other than the indicated osmolar standard solutions in the Table below, water can also be used as a standard solution (0 mOsm) for measuring low osmolar sample solutions (0 – 100 mOsm). Next, after washing the sample cell and the thermistor as indicated for the individual apparatus, measure the degree of the freezing-point depression caused by a sample solution. Using the above-mentioned relation of osmolar concentration \( m \) and \( \Delta T \), the osmolality of a sample solution can be obtained, and it is assumed to be numerically equal to the osmolality.

   In the case of higher osmolar solutions over 1000 mOsm, dilute the sample by adding distilled water and prepare \( n \) times diluted sample solution (1 in \( n \)). Measure the osmolality of the diluted solution, as described above. In this case, it is necessary to state that the calculated osmolality for the sample (see below) is an apparent osmolality obtained by the dilution method. When the dilution method is applied,
the dilution number should be selected so that the expected osmolarity is nearly equal to that of physiological saline solution.

In the case of solid samples, such as freeze-dried medicines, prepare a sample solution by dissolving the solid using the indicated solution for dissolution.

3. Suitability of the apparatus

After the calibration of the apparatus, a suitability test must be done by repeating the measurement of osmolarity for one of the standard solutions not less than 6 times. In performing the test, it is advisable that the osmolarity of a sample solution and the selected standard solution are similar to each other. In this test, the repeatability of measured values and the deviation of the average from the indicated value should be less than 2.0% and 3.0%, respectively. When the requirement is not met, calibrate the apparatus again by the two-point calibration method, and repeat the test.

4. Preparation of the osmolar standard solutions

Weigh exactly an amount indicated in Table 2.47-1 of sodium chloride (standard reagent), previously dried between 500°C and 650°C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve the weighed sodium chloride in exactly 100 g of water to make the corresponding osmolar standard solution.

5. Osmolar ratio

In this test method the osmolar ratio is defined as the ratio of osmolarity of a sample solution to that of the isotonic sodium chloride solution. The ratio can be used as a measure of isotonicity of sample solution. Since the osmolarity of the isotonic sodium chloride solution (NaCl 0.900 g/100 mL) \(c_S\) (mOsm) is assumed to be constant (286 mOsm), the osmolar ratio of a sample solution, of which the osmolarity is \(c_T\) (mOsm), can be calculated by means of the following equation,

\[
\text{Osmolar ratio} = \frac{c_T}{c_S}
\]

\(c_S = 286\) mOsm

When the measurement is done by the dilution method, because the sample has an osmolarity over 1000 mOsm, the apparent osmolarity of the sample solution \(c_T\) can be calculated as \(n \cdot c_T' = c_T\), in which \(n\) is the dilution number and \(c_T'\) is the measured osmolarity for the diluted solution. In this calculation, a linear relation between osmolarity and solute concentration is assumed. Thus when the dilution measurement is performed, the dilution number must be stated as (1 in \(n\)).

2.48 Water Determination (Karl Fischer Method)

Water Determination is a method to determine water content in sample materials, utilizing the fact that water reacts with iodine and sulfur dioxide quantitatively in the presence of a lower alcohol such as methanol, and an organic base such as pyridine. The reaction proceeds in the manner shown in the following equation:

\[
\ce{I2 + SO2 + 3C2H5N + CH3OH + H2O \rightarrow 2(C2H5N^+H)I^- + (C2H5N^+H)OSO2OCH3}
\]

In this measurement there are two methods different in iodine-providing principle: one is the volumetric titration method and the other, the coulometric titration method. In the former, iodine is previously dissolved in a reagent for water determination, and water content is determined by measuring the amount of iodine consumed as a result of reaction with water. In the latter, iodine is produced by electrolysis of Karl Fisher reagent containing iodide ion. Based on the quantitative reaction of the generated iodine with water, the water content in a sample specimen can be determined by measuring the quantity of electricity which is required for the production of iodine during the titration.

\[
2I^- \rightarrow I_2 + 2e^-
\]

1. Volumetric titration

1.1. Apparatus

Generally, the apparatus consists of automatic burettes, a titration flask, a stirrer, and equipment for amperometric titration at constant voltage or potentiometric titration at constant current.

The Karl Fischer TS is extremely hygroscopic, so the apparatus should be designed to be protected from atmospheric moisture. Desiccants such as silica gel or calcium chloride for water determination are used for moisture protection.

1.2. Reagents

(i) Chloroform for water determination—To 1000 mL of chloroform add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of chloroform. Preserve the chloroform, protecting it from moisture. The water content of this chloroform should not be more than 0.1 mg per mL.

(ii) Methanol for water determination—To 1000 mL of methanol add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of methanol. Preserve the methanol, protecting it from moisture. The water content of this methanol should not be more than 0.1 mg per mL.

(iii) Propylene carbonate for water determination—To 1000 mL of propylene carbonate add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear propylene carbonate layer. Preserve this protection from moisture. The water content should not be more than 0.3 mg per mL.

(iv) Diethylene glycol monoethyl ether for water determination—To 1000 mL of diethylene glycol monoethyl ether add 30 g of synthetic zeolite for drying, stopper tightly,
allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of diethylene glycol monoethyl ether. Preserve the diethylene glycol monoethyl ether, protecting it from moisture. The water content of this diethylene glycol monoethyl ether should not be more than 0.3 mg per mL.

(v) Pyridine for water determination—Add potassium hydroxide or barium oxide to pyridine, stopper tightly, and allow to stand for several days. Distill and preserve the purified and dried pyridine, protecting it from moisture. The water content of this pyridine should not be more than 1 mg per mL.

(vi) Imidazole for water determination—Use imidazole for thin-layer chromatography, of which the water content should not be more than 1 mg per g.

(vii) 2-Methylaminopyridine for water determination—Distill and preserve 2-methylaminopyridine, protecting it from moisture. The water content of this 2-methylaminopyridine should not be more than 1 mg per mL.

1.3. Preparation of test solutions and standard solutions

1.3.1. Karl Fischer TS for water determination

The Karl Fischer TS is preserved in a cold place, protecting it from light and moisture.

1.3.1.1. Preparation

Prepare according to the following method (i), (ii) or (iii). Additives may be added for the purpose of improving the stability or other performances if it is confirmed that they give almost the same results as those obtained from the specified method.

(i) Preparation 1
Dissolve 63 g of iodine in 100 mL of pyridine for Karl Fischer method, cool the solution in ice bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 32 g. Then make up to 500 mL by adding chloroform for Karl Fischer method or methanol for Karl Fischer method, and allow to stand for more than 24 hours before use.

(ii) Preparation 2
Dissolve 102 g of imidazole for Karl Fischer method in 350 mL of diethylene glycol monoethyl ether for water determination, cool the solution in ice bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 64 g, keeping the temperature between 25°C and 30°C. Then dissolve 50 g of iodine in this solution, and allow to stand for more than 24 hours before use.

(iii) Preparation 3
Pass dried sulfur dioxide gas through 220 mL of propylene carbonate for water determination until the mass increase of the solvent reaches 32 g. To this solution, add 180 mL of propylene carbonate, or diethylene glycol monoethyl ether for water determination, in which 81 g of 2-methylaminopyridine for Karl Fischer method is dissolved and cooled in ice bath. Then dissolve 36 g of iodine in this solution, and allow to stand for more than 24 hours before use.

1.3.1.2. Standardization

According to the procedure described below, take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Then, add exactly 10 mL of Karl Fischer TS to this solution in the flask, and titrate it with the prepared standard water-methanol solution to the end point. Calculate the water concentration in the standard water-methanol solution, $f$(mg/mL), by using the following equation:

$$f$(mg/mL) = \frac{f$(mg/mL) $\times 10 (mL)}{Volume of the standard water-methanol solution consumed for titration (mL)}

1.3.2. Standard water-methanol solution

Standard water-methanol solution is preserved in a cold place, protecting it from light and moisture.

1.3.2.1. Preparation

Take 500 mL of methanol for water determination in a dried 1000-mL volumetric flask, add 2.0 mL of water, and adjust with the methanol to make 1000 mL.

Perform the standardization of this solution, followed by the procedure for Karl Fischer TS. Preserve it in a cool place, protecting it from light and moisture.

1.3.2.2. Standardization

According to the procedure described below, take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Then, add exactly 10 mL of Karl Fischer TS to this solution in the flask, and titrate it with the prepared standard water-methanol solution to the end point. Calculate the water concentration in the standard water-methanol solution, $f$(mg/mL), by using the following equation:

$$f$(mg/mL) = \frac{Amount of water taken (H2O) (mg)}{Volume of Karl Fischer TS consumed for titration of water (H2O) (mL)}

1.4. Procedure

As a rule, the titration of water with a Karl Fischer TS should be performed at the same temperature as that at which the standardization was done, with protection from moisture. The apparatus is equipped with a variable resistor in the circuit, and this resistor is manipulated so as to maintain a constant voltage (mV) between two platinum electrodes immersed in the solution to be titrated. The variable current (mA) can be measured (Amperometric titration at constant voltage). During titration with Karl Fischer TS, the current in the circuit varies noticeably, but returns to the original value within several seconds. At the end of a titration, the current stops changing and persists for a certain time (usually, longer than 30 seconds). When this electric state has been attained, it is designated as the end point of titration.

Otherwise, the manipulation of the resistor serves to pass a definite current between two platinum electrodes. The variable potential (mV) can be measured (Potentiometric titration at constant current). With the progress of titration of water with a Karl Fischer TS, the value indicated by the potentiometer in the circuit decreases suddenly from a polarization state of several hundreds (mV) to the non-polarization state, but it returns to the original value within several seconds. At the end of titration, the non-polarization state persists for a certain time (usually, longer than 30 seconds). When this electric state has been attained, it is designated as the end point of titration.

In the case of back titration, when the amperometric titration method is used at constant voltage, the needle of microammeter is out of scale during excessive presence of Karl Fischer TS, and it returns rapidly to the original position when the titration system has reached the end point.
the case of the potentiometric titration method at constant
current in the back titration mode, the needle of the mil-
vilometer is at the original position during excessive
presence of Karl Fischer TS. Finally a definite voltage is indi-
cated when the titration system has reached the end point.

Unless otherwise specified, the titration of water with Karl
Fischer TS can be performed by either one of the following
methods. Usually, the end point of the titration can be ob-
served more clearly in the back titration method, compared
with the direct titration method.

1.4.1. Direct titration

Unless otherwise specified, proceed by the following
method. Take a suitable quantity of methanol for water
determination in a dried titration flask, and titrate the water
contaminated with Karl Fischer TS to make the content of
the flask anhydrous. Take a quantity of sample specimen
containing 5 to 30 mg of water, transfer it quickly into the
titration flask, dissolve by stirring, and titrate the solution to
be examined with Karl Fischer TS to the end point under
vigorous stirring.

In the case of an insoluble sample specimen, powder the
sample quickly, weigh a suitable amount of the sample con-
taining 5 to 30 mg of water, and transfer it quickly into the
titration vessel, stir the mixture for 5 – 30 minutes, protect-
ing it from moisture, and perform a titration under vigorous
stirring. Alternatively, in the case of a sample specimen
which is insoluble in the solvent for water determination or
which interfere with the Karl Fisher reaction, water in the
sample can be removed by heating under a stream of nitro-
gen gas, and introduced into the titration vessel by using a
blank test must be done under the same conditions as used
for the sample test, and the data must be corrected, accord-
ingly.

\[
\text{Volume of Karl Fischer TS consumed for titration (mL)} \times f'(mg/mL)
\]

Water (H$_2$O) % = \frac{\text{Volume of Karl Fischer TS added (mL)}}{\text{Amount of sample (mg)}} \times 100

1.4.2. Back titration

Unless otherwise specified, proceed by the following
method. Take a suitable quantity of methanol for water de-
termination in the dried titration vessel, and titrate the water
contaminated with Karl Fischer TS to make the content of
the flask anhydrous. Take a suitable quantity of sample
specimen having 5 – 30 mg of water, transfer the sample
quickly into the titration vessel, dissolve it in the solution by
stirring, add an excessive and definite volume of Karl Fisher
TS, and then titrate the solution with the standard water-
methanol solution to the end point under vigorous stirring.

In the case of an insoluble sample specimen, powder the
sample quickly, weigh a suitable amount accurately, transfer
it quickly into the titration vessel, and add an excessive and
definite volume of Karl Fischer TS. After stirring for 5 – 30
minutes, with protection from moisture, perform the titra-
tion under vigorous stirring.

Water (H$_2$O) % = \frac{\left[ \text{Volume of Karl Fischer TS added (mL)} \times f'(mg/mL) \right] - \left[ \text{Volume of the standard water-methanol solution consumed for titration (mL)} \times f'(mg/mL) \right]}{\text{Amount of sample (mg)}} \times 100

2. Coulometric titration

2.1. Apparatus

Usually, the apparatus is comprised of a titration flask
equipped with an electrolytic cell for iodine production, a
stirrer, and a potentiometric titration system at constant cur-
rent. The iodine production system is composed of an anode
and a cathode, separated by a diaphragm. The anode is im-
mersed in the anolyte solution for water determination and
the cathode is immersed in the catholyte solution for water
determination. Both electrodes are usually made of plati-
num-mesh.

Because both the anolyte and the catholyte solutions for
water determination are strongly hygroscopic, the titration
system should be protected from atmospheric moisture. For
this purpose, silica gel or calcium chloride for water determi-
nation can be used.

2.2. Preparation of anolyte and catholyte solutions for
water determination

Electrolytic solutions shall consist of an anolyte solution
and a catholyte solution, the preparations of which are de-
scribed below.

2.2.1. Preparation 1

(i) Anolyte for water determination—Dissolve 102 g of
imidazole for water determination in 900 mL of methanol
for water determination, cool the solution in ice bath, and
pass dried sulfur dioxide gas through the solution, which is
kept below 30°C. When the mass increase of the solution has
reached 64 g, the gas flow is stopped and 12 g of iodine is
dissolved by stirring. Then drop a suitable amount of water
into the solution until the color of liquid is changed from
brown to yellow, and add methanol for water determination
to make up 1000 mL.

(ii) Catholyte for water determination—Dissolve 24 g of
diethanolamine hydrochloride in 100 mL of methanol for
water determination.

2.2.2. Preparation 2

(i) Anolyte for water determination—Dissolve 40 g of
1,3-di(4-pyridyl)propane and 30 g of diethanolamine in
about 200 mL of methanol for water determination, and
pass dried sulfur dioxide gas through the solution. When the
mass increase of the solution has reached 25 g, the gas flow
is stopped. Add 50 mL of propylene carbonate, and dissolve
6 g of iodine in the solution. Then make up the solution to
500 mL by addition of methanol for water determination and
drop in a suitable amount of water until the color of liq-
uid is changed from brown to yellow.

(ii) Catholyte for water determination—Dissolve 30 g of
choline hydrochloride into methanol for water determination
and adjust the volume to 100 mL by adding the methanol.

2.2.3. Preparation 3

(i) Anolyte for water determination—Dissolve 100 g of
diethanolamine in 900 mL of methanol for water determi-
nation or a mixture of methanol and chloroform for water de-
termination (3:1), and pass dried sulfur dioxide gas through the solution. When the mass increase of the solution has reached 64 g, the gas flow is stopped. Dissolve 20 g of iodine in the solution, and drop in a suitable amount of water until the color of liquid is changed from brown to yellow.

(ii) Catholyte for water determination—Dissolve 25 g of lithium chloride in 1000 mL of a mixture of methanol for water determination and nitroethane (4:1).

2.3. Procedure
Take a suitable volume of an anolyte for water determination in the titration vessel, immerse in this solution a pair of platinum electrodes for potentiometric titration at constant current. Then immerse the iodine production system filled with a catholyte for water determination in the anolyte solution. Switch on the electrolytic system and make the content of the titration vessel anhydrous. Next take an accurately weighed amount of a sample specimen containing 0.2 – 5 mg of water, add it quickly to the vessel, dissolve by stirring, and perform the titration to the end point under vigorous stirring.

When a sample specimen cannot be dissolved in the anolyte, powder it quickly, and add an accurately weighed amount of the sample estimated to contain 0.2 – 5 mg of water to the vessel. After stirring the mixture for 5 – 30 minutes, with protection from atmospheric moisture, perform the titration under vigorous stirring. Alternatively, in the case of an insoluble solid or a sample containing a component which interferes with the Karl Fisher reaction, water in the sample can be removed by heating, and carried by a nitrogen gas flow into the titration vessel, by using a water evaporation technique.

Determine the quantity of electricity (C) [i.e. electric current (A) × time (s)] required for the production of iodine during the titration, and calculate the water content (%) in the sample specimen by use of the following equation.

Though the titration procedure should be performed under atmospheric conditions at low humidity, if the effect of atmospheric moisture cannot be avoided, for instance, if a long time is required for extraction and titration of water, a blank test must be done under the same conditions as used for the sample test, and the data must be corrected, accordingly.

\[
\text{Water (H}_2\text{O)} \% = \frac{\text{Quantity of electricity required for iodine production (C)}}{10.72 \text{ (C/mg)} \times \text{Amount of sample (mg)}} \times 100
\]

10.72: quantity of electricity corresponding to 1 mg of water (C/mg)

2.49 Optical Rotation Determination

Optical Rotation Determination is a method for the measurement of the angular rotation of the sample using a polarimeter.

Generally, the vibrations of light take place on planes perpendicular to the direction of the beam. In case of the ordinary light, the directions of the planes are unrestricted, while in case of the plane polarized light, commonly called as polarized light, the vibrations take place on only one plane that includes the advancing direction of the beam. And it is called that these beams have plane of polarization. Some drugs in the liquid state or in solution have a property of rotating the plane of the polarized light either to the right or to the left. This property is referred to as optical activity or optical rotation, and is inherently related to the chemical constitution of the substance.

The optical rotation is a degree of rotation of polarized plane, caused by the optically active substance or its solution, and it is measured by the polarimeter. This value is proportional to the length of the polarimeter tube, and is also related to the solution concentration, the temperature and the measurement wavelength. The character of the rotation is indicated by the direction of the rotation, when facing to the advancing direction of the polarized light. Thus in case of rotation to the right, it is called dextrorotatory and expressed by placing plus sign (+), while in case of rotation to the left, it is called levorotatory and expressed by placing minus sign (−) before the figure of the angular rotation. For example, +20° means 20° of rotation to the right, while −20° means 20° of rotation to the left.

The angular rotation α; means degree of rotation, when it is measured at t°C by using specific monochromatic light \(x\) (expressed by wavelength of light source or the specific beam name). Usually, the measurement is performed at 20°C or 25°C, with a polarimeter tube of 100 mm in length, and with the D line of sodium lamp.

The specific rotation is expressed by the following equation:

\[
[\alpha]_D = \frac{\alpha}{l} \times 100
\]

\(t\): The temperature of measurement.
\(\lambda\): The wavelength or the name of the specific monochromatic light (in the case of the Sodium D line, it is described as D).
\(\alpha\): The angle, in degrees, of rotation of the plane of the polarized light.
\(l\): The thickness of the layer of sample solution, i.e., the length of the polarimeter tube (mm).
\(c\): Drug concentration in g/mL. When an intact liquid drug is used for the direct measurement without dilution by an appropriate solvent, \(c\) equals to its density (g/mL). However, unless otherwise specified, the specific gravity is conventionally used in stead of the density.

The description in the monograph, for example, “\([\alpha]_D = -33.0°\) − 36.0° (after drying, 1 g, water, 20 mL, 100 mm)," means the measured specific rotation \([\alpha]_D\) should be in the range of −33.0° and −36.0°, when 1 g of accurately weighed sample dried under the conditions, specified in the test item of Loss on drying, is taken, and dissolved in water to make exactly 20 mL, then put in the polarimeter tube of 100 mm length, of which temperature is kept at 20°C.
2.50 Endpoint Detection Methods in Titrimetry

Titrimetry is a method or a procedure for volumetric analysis, which is usually classified into acid-base titration (neutralization titration or pH titration), precipitation titration, complexation titration, oxidation-reduction titration, etc., according to the kind of reaction or the nature of the phenomenon occurring between the titrate and the titrant (standard solution for volumetric analysis). Furthermore, titration performed in a noneaqueous solvent is generally called noneaqueous titration, which is frequently used for volumetric analysis of weak acids, weak bases, and their salts. The endpoint in titrimetry can be detected by color changes of indicators and/or by changes of electrical signals such as electrical potential or electrical current.

The indicator method is one of the endpoint detection methods in titrimetry. In this method the color of an indicator dye, dissolved in the titrate, changes dramatically in the vicinity of the equivalence point due to its physico-chemical character, and this property is used for visual endpoint detection. Selection of an indicator and specification of the color change induced in the respective titration system, should be described in the individual monograph. An appropriate indicator should change color clearly, in response to a slight change in physico-chemical properties of the titrate, such as pH, etc., in the vicinity of the equivalence point.

Regarding the electrical endpoint detection methods, there are an electrical potential method and an electrical current method, which are called potentiometric and amperometric titration methods, respectively. They are generically named electrometric titration. In the potentiometric titration method, the endpoint of a titration is usually determined to be the point at which the differential potential change becomes maximum or minimum as a function of the quantity of titrant added. In the amperometric titration method, unless otherwise specified, a bi-amperometric titration method is used, and the endpoint is determined by following the change of microcurrent during the course of a titration. Furthermore, the quantity of electricity (electrical current \( \times \) time) is often used as another electrochemical signal to follow a chemical reaction, as described in Coulometric Titration under Water Determination <2.49>.

The composition of a titration system, such as amount of specimen, solvent, standard solution for volumetric analysis, endpoint detection method, equivalent amount of substance to be examined (mg)/standard solution (mL), should be specified in the individual monograph. Standardization of the standard solution and titration of a specimen are recommended to be done at the same temperature. When there is a marked difference in the temperatures at which the former and the latter are performed, it is necessary to make an appropriate correction for the volume change of the standard solution due to the temperature difference.

1. Indicator Method

Weigh an amount of a specimen in a flask or a suitable vessel as directed in the monograph or in “Standard Solutions for Volumetric Analysis”, and add a specified quantity of solvent to dissolve the specimen. After adding a defined indicator to the solution to prepare the titrate, titrate by adding a standard solution for volumetric analysis by using a buret. In the vicinity of the endpoint, observe the color change induced by the cautious addition of 0.1 mL or less of the titrant. Calculate the quantity of titrant added from the readings on the scale of the buret used for the titration at the starting point and at the endpoint at which the specified color change appears, as directed in the individual monograph or in the “Standard Solutions for Volumetric Analysis”. Although addition of the volumetric standard solution by buret is usually done manually, an automatic buret can also be used.

Unless otherwise specified, perform a blank determination according to the following method, and make any necessary correction.

Measure a specified quantity of solvent, as directed in the monograph or in the “Standard Solutions for Volumetric Analysis”, and titrate as directed. The required quantity of the standard solution added to reach a specified color change, is assumed to be the blank quantity for the titration system. However, when the blank quantity is too small to evaluate accurately, the quantity can be assumed to be zero.

2. Electrical Endpoint Detection Methods

2.1. Potentiometric titration

2.1.1. Apparatus

The apparatus consists of a beaker to contain the specimen, a buret for adding a standard solution, an indicator electrode and a reference electrode, a potentiometer for measuring potential difference between the electrodes or an adequate pH meter, a recorder, and a stirrer for gentle stirring of the solution to be examined. Separately, an automatic titration apparatus assembled from suitable units and/or parts, including a data processing system, can also be used.

In this titration method, unless otherwise specified, indicator electrodes designated in Table 2.50-1 are used according to the kind of titration. As a reference electrode, usually a silver-silver chloride electrode is used. Besides the single indicator electrodes as seen in Table 2.50-1, a combined reference electrode and indicator electrode can also be used.

When the potentiometric titration is carried out by the pH measurement method, the pH meter should be adjusted according to the pH Determination <2.54>.

2.1.2. Procedure

Weigh a defined amount of a specimen in a beaker, and add an indicated quantity of solvent to dissolve the specimen, as directed in the monograph. After the potential difference \( E \) (mV) or the pH value of the solvent to be used for titration has reached a stable value, immerse both reference and indicator electrodes, which have previously been washed with the solvent being used, in the solution to be examined, and titrate with a standard solution for volumetric analysis with gentle stirring of the solution. During the titration, the tip of the buret should be dipped into the solution, to be examined. The endpoint of titration is determined by following the variation of the potential difference between two electrodes as a function of the quantity of titrant added. In the vicinity of the endpoint, the amounts of a titrant added should be 0.1 mL or less for adequate titrimetry. Plot the obtained potential values along the ordinate and the quantity of a titrant added \( V \) (mL) along the abscissa to draw a titration curve, and obtain the endpoint from the
maximum or the minimum value of $\Delta E/\Delta V$ or from the value of electromotive force or pH corresponding to the equivalence point.

Unless otherwise specified, the decision of the endpoint in this method is usually made by either of the following methods.

(i) Drawing method

Usually, draw two parallel tangent lines with a slope of about 45° to the obtained titration curve. Next, draw a 3rd parallel line at the same distance from the previously drawn two parallel lines, and decide the intersection point of this line with the titration curve. Further, from the intersection point, draw a vertical line to the abscissa, and read the quantity of titrant added as the endpoint of the titration.

Separately, the endpoint of the titration can also be obtained from the maximum or the minimum of the differential titration curve ($\Delta E/\Delta V$ vs. $V$).

(ii) Automatic detection method

In the case of potentiometric titration using an automatic titration system, the endpoint can be determined by following the respective instrumental indications. The endpoint is decided either by following the variation of the differential potential change or the absolute potential difference as a function of the quantity of titrant added: in the former case the quantity given by the maximum or the minimum of the differential values, and in the latter the quantity given by the indicator reaching the endpoint previously set for the individual titration system, are assumed to be the endpoint volumes, respectively.

2.2.2. Procedure

Weigh a defined amount of a specimen in a beaker, and add an indicated quantity of solvent to dissolve the specimen, as directed in the individual monograph. Next, after washing the two indicator electrodes with water, immerse both electrodes in the solution to be examined, apply a constant voltage suitable for measurement across two electrodes by using an appropriate device, and titrate the solution with a standard solution for volumetric analysis. During the titration, the tip of the buret should be dipped into the solution to be examined. The endpoint of titration is determined by following the changes of microcurrent between the two electrodes as a function of the quantity of titrant added. In the vicinity of the endpoint, the amounts of the titrant added should be 0.1 mL or less for adequate titrimetry. Plot the obtained current values along the ordinate and the quantity of the titrant added $V$ (mL) along the abscissa to draw a titration curve, and usually take the inflection point of the titration curve (the point of intersection given by the extrapolation of two straight lines before and after the inflection) as the endpoint in amperometric titration.

The blank test in this titration is usually performed as follows: Take a volume of the solvent specified in the individual monograph or in the “Standard Solution for Volumetric Analysis”, and use this as the sample solution. Determine the amount of the volumetric standard solution needed for giving the endpoint, and use this volume as the blank. If this volume is too small to determine accurately, the blank may be considered as 0 (mL).

Unless otherwise specified, the endpoint in this titration is decided by either of the following methods.

(i) Drawing method

Usually, extrapolate the two straight lines before and after the inflection, and obtain the inflection point of the titration curve. Next, read the quantity of titrant added at the inflection point, and assume this point to be the endpoint.

(ii) Automatic detection method

In the case of amperometric titration using an automatic titration system, the endpoint can be determined by following the instrumental indications. The endpoint is decided by following the variation of the indicator current during the course of a titration, and the quantity of titrant added is assumed to be that at which the current has reached the endpoint current set previously for the individual titration system.

When atmospheric carbon dioxide or oxygen is expected to influence the titration, a beaker with a lid should be used, and the procedure should be carried out in a stream of an inert gas, such as nitrogen gas. Further, when a specimen is expected to be influenced by light, use a light-resistant container to avoid exposure of the specimen to direct sunlight.

### Table 2.50-1 Kind of Titration and Indicator Electrode

<table>
<thead>
<tr>
<th>Kind of titration</th>
<th>Indicator electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-base titration (Neutralization titration, pH titration)</td>
<td>Glass electrode</td>
</tr>
<tr>
<td>Precipitation titration (Titration of halogen ion by silver nitrate)</td>
<td>Silver electrode</td>
</tr>
<tr>
<td>Oxidation-reduction titration (Diazo titration, etc.)</td>
<td>Mercury-mercury chloride</td>
</tr>
<tr>
<td>Complexation titration (Chelometric titration)</td>
<td>(I) electrode</td>
</tr>
<tr>
<td>Nonaqueous titration (Perchloric acid titration, Tetramethylammonium hydroxide titration)</td>
<td>(II) electrode</td>
</tr>
<tr>
<td></td>
<td>Glass electrode</td>
</tr>
</tbody>
</table>

### 2.51 Conductivity Measurement

Conductivity Measurement is a method for the measuring the flowability of electric current in an aqueous solution.
The measurement is made with a conductivity meter or a resistivity meter, and is used, for example, in the purity tests in monographs. The method is applied to evaluate the test item “Conductivity (Electrical Conductivity)” specified in the monographs. Further it is also used for monitoring the quality of water in the preparation of highly purified water. However, when applying this method for monitoring the quality of water, the details of measurement should be specified by the user, based on the principles described here.

Conductivity of a solution \[ \kappa \, (\text{S} \cdot \text{m}^{-1}) \] is defined as the reciprocal of resistivity \[ \rho \, (\Omega \cdot \text{m}) \], which is an indicator of the strength of ionic conductivity for a fluid conductor. Resistivity is defined as the product of electrical resistance per unit length and cross-sectional area of a conductor. When resistivity is defined as the product of electrical resistance per unit length and cross-sectional area of a conductor. When resistivity is \[ \rho \], cross-section area \[ A \, (\text{m}^2) \], and length \[ l \, (\text{m}) \], resistance \[ R \, (\Omega) \] can be expressed by the following equation.

\[
R = \rho \left( \frac{l}{A} \right)
\]

Thus, conductivity \[ \kappa \] is expressed as follows,

\[
\kappa = \frac{1}{\rho} = \left( \frac{1}{R} \right) \left( \frac{l}{A} \right)
\]

If \( l/A \) is known, the conductivity \( \kappa \) can be obtained by measuring resistance \( R \) or conductance \( G = \frac{1}{R} \).

In the International System (SI), the unit of conductivity is the Siemens per meter (S·m⁻¹). In practice, conductivity of a solution is generally expressed by \( \mu \text{S} \cdot \text{cm}^{-1} \), and resistivity by \( \Omega \cdot \text{cm} \).

Unless otherwise specified, the reference temperature for the expression of conductivity or resistivity is 20°C.

Items such as the sample preparation method, the necessity of blank correction, the calculation method, the specification value, and the measuring temperature should be described in the monograph, if necessary.

1. Apparatus

A conductivity meter or a resistivity meter is composed of an indicator part (operating panel, display, recording unit) and a detector part, the latter of which includes a conductivity cell. In the conductivity cell a pair of platinum electrodes is embedded. The cell is immersed in a solution, and the resistance or the resistivity of the liquid column between the electrodes is measured. Alternating current is supplied to this apparatus to avoid the effects of electrode polarization. Further, a temperature compensation system is generally contained in the apparatus.

Conductivity measurement is generally performed by using an immersion-type cell. A pair of platinum electrodes, the surfaces of which are coated with platinum black, is fixed in parallel. Both electrodes are generally protected by a glass tube to prevent physical shocks.

When the surface area of the electrode is \( A \, (\text{cm}^2) \), and the separation distance of the two electrodes is \( l \, (\text{cm}) \), the cell constant \( C \, (\text{cm}^{-1}) \) is given by the following equation.

\[
C = \alpha \left( \frac{l}{A} \right)
\]

\( \alpha \) is a dimensionless numerical coefficient, and it is characteristic of the cell design.

In addition to the immersion-type cell, there are flow-through-type and insert-in-pipe-type cells. These cells are set or inserted in an appropriate position in the flow system for monitoring the quality of water continuously or intermittently, during the preparation of highly purified water.

### Table 2.51-1  Conductivity and Resistivity of the Standard Solutions of Potassium Chloride at 20°C

<table>
<thead>
<tr>
<th>Concentration (g/1000.0 g)</th>
<th>Conductivity ( \kappa ) (( \mu \text{S} \cdot \text{cm}^{-1} ))</th>
<th>Resistivity ( \rho ) (( \Omega \cdot \text{cm} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7455</td>
<td>1330</td>
<td>752</td>
</tr>
<tr>
<td>0.0746</td>
<td>133.0</td>
<td>7519</td>
</tr>
<tr>
<td>0.0149</td>
<td>26.6</td>
<td>37594</td>
</tr>
</tbody>
</table>

2. Standard Solution of Potassium Chloride

After pulverizing an appropriate amount of potassium chloride for conductivity measurement, dry it at 500 – 600°C for 4 hours. For the preparation of the standard solutions, take the amount of the dried potassium chloride indicated in Table 2.51-1, dissolve it in distilled water previously boiled and cooled, or water with a conductivity less than 2 \( \mu \text{S} \cdot \text{cm}^{-1} \), and adjust to make 1000.0 g. The conductivity and the resistivity of the respective standard solutions at 20°C are shown in Table 2.51-1. These standard solutions should be kept in tightly closed polyethylene or hard glass bottles.

When measurement at 20°C can not be performed, the value of conductivity for the respective standard solution (shown in Table 2.51-1), can be corrected by using the following equation. However, this equation is valid only within the range of 15 – 30°C.

\[ \kappa_T = \kappa_{20} \left[ 1 + 0.021(T - 20) \right] \]

\( T \): Measuring temperature specified in the monograph

\( \kappa_{20} \): Calculated conductivity of the KCl standard solution at \( T^\circ \text{C} \)

\( \kappa_{20} \): Conductivity of the KCl standard solution at 20°C

3. Operating Procedure

3.1. Cell Constant

An appropriate conductivity cell should be chosen according to the expected conductivity of the sample solution. The higher the expected conductivity, the larger the cell constant required for the conductivity cell, so that the electrical resistance is within the measuring range of the apparatus being used. Conductivity cells with a cell constant of the order of 0.1 cm⁻¹, 1 cm⁻¹, or 10 cm⁻¹, are generally used.

For determination or confirmation of the cell constant, an appropriate KCl standard solution should be chosen and prepared, taking account of the expected conductivity of the sample solution to be measured. Rinse the cell several times with distilled water. Next, after rinsing the cell 2 – 3 times with the standard solution used for the cell constant determination, immerse the cell in the standard solution contained in a measuring vessel. After confirming that the temperature of the standard solution is maintained at 20 ± 0.1°C or at the temperature specified in the monograph, measure the resistance \( R_{\text{KCl}} \) or the conductance \( G_{\text{KCl}} \) of the standard solution, and calculate the cell constant \( C \, (\text{cm}^{-1}) \) by use of the following equation.

\[
C = R_{\text{KCl}} \cdot \kappa_{\text{KCl}} \quad \text{or} \quad C = \kappa_{\text{KCl}} / G_{\text{KCl}}
\]

\( R_{\text{KCl}} \): Measured resistance (MΩ)
\( G_{\text{KCl}} \): Measured conductance (\( \mu \text{S} \))

\( \kappa_{\text{KCl}} \): Conductivity of the standard solution being used (\( \mu \text{S} \cdot \text{cm}^{-1} \))
The measured cell constant should be consistent with the given value within 5%. If it is not consistent, coat the electrodes with platinum black again, or replace the cell with a new one.

3.2. Suitability Test for the Apparatus

Using an appropriate KCl standard solution according to the expected conductivity of the sample solution, perform the suitability test for the apparatus. Rinse the conductivity cell several times with distilled water, and rinse again 2 – 3 times with the selected standard solution. Fill the standard solution in the measuring vessel. After confirming that the temperature of the measuring system is maintained at 20 ± 0.1°C, measure the conductivity of the standard solution. When this measuring procedure is repeated several times, the average conductivity should be consistent with an indicated value in Table 1 within 5%. Further, the relative standard deviation should be less than 2%.

3.3. Measurement

After confirmation of the suitability of the apparatus, perform the conductivity measurement for the sample solution. Unless otherwise specified, the preparation method for sample solution should be as specified in the respective monograph. Rinse the conductivity cell several times with distilled water, and rinse again 2 – 3 times with sample solution. Immerse the cell in the sample solution placed in a measuring vessel. If necessary, agitate gently the sample solution. After confirming that the temperature of the sample solution is maintained at 20 ± 0.1°C or at the temperature specified in the monograph, measure the resistance $R_T$ (MΩ) or conductance $G_T$ ($\mu$S) of the sample solution, and calculate the conductivity $\kappa_T$ by using the following equation.

$$\kappa_T = CG_T \quad \text{or} \quad \kappa_T = C/R_T$$

2.52 Thermal Analysis

"Thermal Analysis" is a generic term for a variety of techniques to measure the physical properties of a substance as a function of temperature and/or time. Among the physical properties, phase transitions such as solid/liquid phase transition (melting, freezing) and crystal polymorphism or thermal behavior such as heat evolution or absorption accompanying thermal degradation or chemical reaction can be detected by the techniques of differential thermal analysis (DTA) or differential scanning calorimetry (DSC). DTA is a method for detecting the thermal behavior of a specimen in terms of the temperature change, while DSC employs the heat quantity (enthalpy) change. There is also a method, thermogravimetry (TG), in which the mass change of a specimen caused by dehydration, adsorption, elimination or oxidation etc., is detected as a function of temperature and/or time.

Among the above three different methods, TG can be used as an alternative method for "Loss on Drying <2.45%" or "Water Determination <2.49%". However, it must be confirmed beforehand that no volatile component except for water is included in the test specimen when TG is used as an alternative method for "Water Determination".

1. Method 1 Differential Thermal Analysis (DTA) or Differential Scanning Calorimetry (DSC)

1.1. Apparatus

Apparatus for DTA or DSC is usually composed of a heating furnace, a temperature-controller, a detector, a device for controlling the atmosphere, and an indicator/ recorder.

In a DTA apparatus, a sample specimen and an inert reference material placed in the heating furnace are heated or cooled at a constant rate, and the temperature difference between the sample and the reference, which is detected by a device such as a thermocouple and recorded as a function of time and/or temperature. As an inert reference material, α-Alumina for thermal analysis is usually adopted.

Two kinds of DSC apparatus, based upon different principles are available as shown below.

(i) Input compensation-type differential scanning calorimetry (Input compensation DSC): A sample specimen and the reference material in twin furnaces are programmed to be heated or cooled at a constant rate, and the temperature difference between the sample and the reference, which is detected by a platinum resistance thermometer, is kept at null by controlling the heating unit with a compensation feed-back circuit. The instrument is designed to measure and record continuously the balance of thermal energy applied to each furnace as a function of temperature and/or time.

(ii) Heat flux-type differential scanning calorimetry (Heat flux DSC): A sample specimen and the reference material in twin furnaces are programmed to be heated or cooled at a constant rate, and the temperature difference between the sample and the reference is detected as a difference of heat flux and recorded as a function of temperature and/or time. In heat flux DSC, thermal conductors are adopted so that the heat flux between the sample and the heat reservoir is proportional to the temperature difference between them.

In usual DSC analysis, α-Alumina is used as a reference material, both in Input compensation DSC and in Heat flux DSC. But in some cases, an empty sample container can also be used without any reference material.

1.2. Procedure

A sample specimen and the reference material are put in sample pans, and the furnace is heated or cooled under a controlled temperature program. As the temperature changes, the temperature difference (DTA) or heat quantity change (DSC) that develops between the specimen and the reference is detected and recorded continuously. Apparatus equipped with a data-processor is operated according to the instruction manual provided with the instrument.

A preliminary experiment is needed to determine the appropriate temperature range of measurement, within which a predicted physical change such as melting or polymorphic phase transition will occur, and to confirm that unpredicted thermal changes are not induced in a specimen in that temperature range. In this preliminary test, a wide temperature range (room temperature—the temperature at which degradation begins) can be scanned at a rapid heating rate (10 – 20°C/min). Thereafter, tests by DSC or DTA should be performed at a low heating rate, usually 2°C/min, in the chosen temperature range. However, when a clear heat change cannot be observed, such as in a case of glass-transition, the heating rate may be changed to a higher or a lower rate, as
appropriate for the kind of physical change being observed. By analyzing the measured DTA-curve or DSC-curve, a quantity of heat change and/or a specific temperature (ignition, peak or end temperature) that accompanies a physical change, such as melting or polymorphic phase transition, can be obtained.

1.3. Calibration of the apparatus

1.3.1. Temperature calibration for DTA and DSC

Temperature calibration for DTA and/or DSC apparatus can be performed by using reference substances having an intrinsic thermal property, such as melting point of pure metals or organic substances, or phase transition point of crystalline inorganic salts or oxides. Melting points of Indium for thermal analysis and/or Tin for thermal analysis are usually employed for calibration.

1.3.2. Heat-quantity calibration for DSC

For accurate estimation of a quantity of heat change (enthalpic change) of a sample specimen, caused by a certain physical change accompanying a temperature change, it is necessary to calibrate the apparatus by using appropriate reference substances. As indicated in the section of Temperature calibration, heat-quantity calibration for DSC apparatus can be performed by using appropriate reference substances having a known definite enthalpic change caused by such physical changes as melting of pure metals and/or organic substances, or phase transition of crystalline inorganic salts. Melting points of Indium for thermal analysis and/or Tin for thermal analysis are usually employed for calibration.

1.4. Notes on operating conditions

When DTA or DSC measurements are made, the following items must be recorded: sample size, discrimination of open- or closed-type sample container, heating or cooling rate, measuring temperature range, and kind and flow rate of atmospheric gas.

2. Method 2 Thermogravimetry (TG)

2.1. Apparatus

The construction of a TG apparatus is fundamentally similar to that of DTA or DSC apparatus. However, the detector for TG is a balance, called a thermobalance, which can be classified to hanging- or Roberval's-, and horizontal-type balances. The TG apparatus is designed to detect small mass changes of a specimen, placed at a fixed position on a thermobalance, caused by temperature change of the furnace under a controlled temperature program. Mass change with time and/or temperature is recorded continuously.

2.2. Procedure

A specimen is put in a sample container, which is placed at a fixed position of the thermobalance, then the heating furnace is run under a controlled temperature program. During this temperature change of the furnace, the mass change of a specimen with time and/or temperature is recorded continuously. Apparatus equipped with a data-processor is operated according to the instruction manual provided with the instrument.

When TG is used as an alternative method for “Loss on Drying” or “Water Determination”, the measurement starts at room temperature and ends at a temperature above which no further mass change due to drying and/or vaporization of water can be observed. The standard heating rate is usually 5°C/min, and a linear heating program is recommended. However heating conditions (rate and time span) can be changed as necessary, depending on the kind of specimen and the extent of the measuring temperature range. Further, in TG measurement, dry air or dry nitrogen is usually passed through the heating furnace to ensure rapid elimination of evolved water or other volatile components and to avoid the occurrence of any chemical reaction, such as oxidation. By analyzing the TG curve plotted against time and/or temperature, absolute mass change and/or relative mass change with respect to the initial quantity(%) is obtained.

When the mass change caused by oxidation or degradation of a specimen is measured, a specific temperature range has to be determined beforehand so that stable baselines can be obtained before and after a targetted chemical reaction. Subsequent operating procedures are the same as described above.

2.3. Calibration of the apparatus

2.3.1. Temperature calibration

The Curie temperature of a ferromagnetic substance such as pure Nickel can be used for temperature calibration for TG, based on the occurrence of an apparent mass change at the Curie point. In the case of a TG apparatus capable of simultaneously conducting DSC and DTA, the same reference substances as those for DTA and DSC can be adopted.

2.3.2. Scale calibration and confirmation

The scale calibration for TG must be done by using reference masses for chemical balances and/or semimicrobalances in the appropriate range. This is called a primary scale calibration, and is performed under ordinary temperature and pressure when the apparatus is set up initially and periodically, thereafter.

In usual measurement by TG, scale calibration or confirmation is done by using Calcium Oxalate Monohydrate Reference Standard to take account of such effects as buoyancy and convection due to atmospheric gas flow in the real measurement state. This is called secondary scale calibration, and is performed under the standard operation conditions stated below by using the above-mentioned Reference Standard, with a certified water content. When the difference of water content between the measured value and the certified one for the Reference Standard is less than 0.3%, normal operation of the apparatus is confirmed. However, when the difference is more than 0.3%, scale calibration for TG must be done, based on the certified water content of the Reference Standard.

The standard operation conditions are as follows:

Amount of Calcium Oxalate Monohydrate Reference Standard: 10 mg

Heating rate: 5°C/min

Temperature range: from room temperature to 250°C

Atmospheric gas: dried Nitrogen or dried Air

Flow rate of atmospheric gas, hanging- or Roberval's-type balance: 40 mL/min

horizontal-type balance: 100 mL/min

2.4. Notes on operating conditions

In TG measurement, the following operation conditions must be recorded: sample size, heating rate, temperature range, kind and flow rate of atmospheric gas, etc.
2.53 Viscosity Determination

Viscosity Determination is a method to determine the viscosity of liquid samples using a viscometer.

When a liquid moves in a definite direction, and the liquid velocity has a gradient with respect to the direction rectangular to that of flow, a force of internal friction is generated along both sides of a hypothetical plane parallel to the movement. This flow property of a liquid is expressed in terms of viscosity. The internal friction per unit area on the parallel plane is called slip stress or shear stress, and the velocity gradient with respect to the direction rectangular to that of flow is called slip velocity or shear velocity. A liquid of which the slip velocity is proportional to its slip stress is called a Newtonian liquid. Since the viscosity of a liquid at a certain temperature and is called viscosity. The viscosity is expressed in the unit of meters squared per second (m²/s), and usually millimeters squared per second (mm²/s).

A liquid whose slip velocity is not proportional to its slip stress is called a non-Newtonian liquid. Since the viscosity of a sample of a non-Newtonian liquid changes with its slip velocity, the viscosity measured at a certain slip velocity is called an apparent viscosity. In that case, the value of slip velocity divided by the corresponding slip stress is called an apparent viscosity. Thus, the relationship between apparent viscosity and slip velocity will permit characterization of the flow properties of a given non-Newtonian liquid.

The value of the viscosity, η, divided by the density, ρ, at the same temperature is defined as a kinematic viscosity, ν, which is expressed in the unit of meters squared per second (m²/s), and usually millimeters squared per second (mm²/s).

The viscosity of a liquid is determined either by the following Method I or Method II.

1. Method I Viscosity measurement by capillary tube viscometer

For measuring the viscosity of a Newtonian liquid, a capillary tube viscometer is usually used, in which the downflowing time of a liquid, t (s), required for a definite volume of the liquid to flow through a capillary tube is measured and the kinematic viscosity, ν, is calculated according to the following equation:

\[ \nu = Kt \]

Further, the viscosity, η, is calculated from the next equation:

\[ \eta = \nu \rho = Kt \rho \]

where ρ (g/mL) is the density of the liquid measured at the same temperature, t (°C).

The parameter K (mm²/s) represents the viscometer constant and is previously determined by using the Standard Liquids for Calibrating Viscometers with known kinematic viscosity. In the case of a liquid having a similar viscosity to water, water itself can be used as a reference standard liquid for the calibration. The kinematic viscosity of water is 1.0038 mm²/s at 20°C. In the cases of liquids having a slightly higher viscosity than water, the Standard Liquids for Calibrating Viscometers should be used for the calibration.

The intrinsic viscosity, \([n]\) (dL/g), of a polymer solution is obtained by plotting the relation of viscosity versus concentration and extrapolating the obtained straight line to zero concentration. Intrinsic viscosity shows the degree of molecular expansion of a polymer substance in a given solvent (sample solution) and is also a measure of the average molecular mass of the polymer substance.

The downflowing time, t (s), for a polymer solution, whose concentration is \(c (g/dL)\), and \(t_0\) (s) for the solvent used for dissolving the polymer, are measured by using the same viscometer, and then the intrinsic viscosity of a given polymer substance, \([\eta]\), is calculated according to the following equation:

\[ [\eta] = \lim_{c \to 0} \frac{(t - t_0)}{c} - 1 \]

or

\[ [\eta] = \lim_{c \to 0} \frac{\ln \frac{t}{t_0}}{c} \]

When the concentration dependency of \(\frac{(t/t_0) - 1}{c}\) is not large, the value of \(\frac{(t/t_0) - 1}{c}\) at a concentration directed in the respective monograph can be assumed to be the intrinsic viscosity for a given substance.

Unless otherwise specified, the viscosity of a sample solution is measured with the following apparatus and procedure.

1.1. Apparatus

For measurement of the kinematic viscosity in the range of 1 to 100,000 mm²/s, the Ubbelohde-type viscometer illustrated in Fig. 2.53-1 can be used. The approximate relations between kinematic viscosity range and inside diameter of the capillary tube suitable for the measurement of various liquids with different viscosity, are given in Table 2.53-1. Although a capillary tube viscometer other than the Ubbelohde-type one specified in Table 2.53-1 can also be used, a viscometer should be selected in which the downflowing time, t (s), of a sample solution to be determined would be
between 200 s and 1000 s.

1.2. Procedure

Place a sample solution in a viscometer from the upper end of tube 1, so that the meniscus of the solution is at a level between the two marked lines of bulb A. Place the viscometer vertically in a thermostatted bath maintained at a specified temperature within ±0.1°C, until bulb C is fully immersed, and let it stand for about 20 minutes to attain the specified temperature. Close bulb C with a cork and pull the sample solution up to the middle part of tube 2, and stop the suction. Open the end of tube 3, and immediately close the end of tube 2. After confirming that the liquid column is cut off at the lowest end of the capillary tube, open the end of tube 2 to make the sample solution flow down through the capillary tube. Record the time, \( t \) (s), required for the meniscus of the sample solution to fall to the upper to the lower marked line of bulb B.

Determine the viscometer constant \( K \) previously, using the Standard Liquids for Calibrating Viscometers under the same conditions. The temperature at which the calibration is conducted must be identical with that specified in the monograph.

2. Method II Viscosity measurement by rotational viscometer

A rotational viscometer is usually used for measuring the viscosity of Newtonian or non-Newtonian liquids. The measuring principle of a rotational viscometer generally consists in the detection and determination of the force acting on a rotor (torque), when it rotates at a constant angular velocity in a liquid. The extent of torque generated by the rotation can be detected in terms of the torsion of a spring and the liquid viscosity is calculated from the scale-indicated value corresponding to the degree of torsion.

The viscosity of a sample solution is measured with the following apparatus and procedure.

### Table 2.53-1 Specifications of the Ubbelohde-type viscometer

<table>
<thead>
<tr>
<th>Viscometer constant ( K ) (mm²/s²)</th>
<th>Inner diameter of capillary tube (mm)</th>
<th>Volume of bulb B (mL)</th>
<th>Permissible tolerance ±10%</th>
<th>Measuring range of kinematic viscosity (mm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.005</td>
<td>0.46</td>
<td>3.0</td>
<td>10%</td>
<td>1–5</td>
</tr>
<tr>
<td>0.01</td>
<td>0.58</td>
<td>4.0</td>
<td>10%</td>
<td>2–10</td>
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<tr>
<td>0.03</td>
<td>0.73</td>
<td>4.0</td>
<td>10%</td>
<td>6–30</td>
</tr>
<tr>
<td>0.05</td>
<td>0.88</td>
<td>4.0</td>
<td>10%</td>
<td>10–50</td>
</tr>
<tr>
<td>0.1</td>
<td>1.03</td>
<td>4.0</td>
<td>10%</td>
<td>20–100</td>
</tr>
<tr>
<td>0.3</td>
<td>1.36</td>
<td>4.0</td>
<td>10%</td>
<td>60–300</td>
</tr>
<tr>
<td>0.5</td>
<td>1.55</td>
<td>4.0</td>
<td>10%</td>
<td>100–500</td>
</tr>
<tr>
<td>1.0</td>
<td>1.83</td>
<td>4.0</td>
<td>10%</td>
<td>200–1,000</td>
</tr>
<tr>
<td>3.0</td>
<td>2.43</td>
<td>4.0</td>
<td>10%</td>
<td>600–3,000</td>
</tr>
<tr>
<td>5.0</td>
<td>2.75</td>
<td>4.0</td>
<td>10%</td>
<td>1,000–5,000</td>
</tr>
<tr>
<td>10.0</td>
<td>3.27</td>
<td>4.0</td>
<td>10%</td>
<td>2,000–10,000</td>
</tr>
<tr>
<td>30.0</td>
<td>4.32</td>
<td>4.0</td>
<td>10%</td>
<td>6,000–30,000</td>
</tr>
<tr>
<td>50.0</td>
<td>5.20</td>
<td>5.0</td>
<td>10%</td>
<td>10,000–50,000</td>
</tr>
<tr>
<td>100</td>
<td>6.25</td>
<td>5.0</td>
<td>10%</td>
<td>20,000–100,000</td>
</tr>
</tbody>
</table>

2.1. Apparatus

Viscosity measurement is performed by using any one of the following three types of rotational viscometers.

2.1.1. Coaxial double cylinder-type rotational viscometer (Couette type viscometer)

In the coaxial double cylinder-type rotational viscometer, viscosity is determined by placing a liquid in the gap between the inner and the outer cylinders, which share the same central axis and rotate separately, and the generated torque acting on one cylinder surface when the other cylinder is rotated, and the corresponding angular velocity, are measured.

As shown in Fig. 2.53-2a, the inner cylinder is hung by a wire whose twist constant is designated as \( k \). In Fig. 2.53-2a, half the outer diameter of the inner cylinder and inner diameter of the outer cylinder are designated as \( R_i \) and \( R_o \), respectively, and the length of the inner cylinder immersed in a liquid is designated as \( l \). When a liquid is introduced into the gap between the two cylinders, and the outer cylinder is made to rotate at a constant angular velocity, \( \omega \), the inner cylinder is also forced to rotate due to the viscosity of the liquid. Consequently, torque, \( T \), is generated by the forced rotation in a viscous liquid, and in the steady state the torque is balanced by the torsion of the wire, as indicated by the degree of rotation \( \theta \). Then, the relationship can be expressed by \( T = k \theta \) and the viscosity of a liquid, \( \eta \), is determined from the following equation by measuring the relationship between \( \omega \) and \( \theta \). Conversely, viscosity measurement can also be performed by rotating the inner cylinder, and the same relationship holds.

\[
\eta = \frac{100T}{4\pi l\omega} \left( \frac{1}{R_i^2} - \frac{1}{R_o^2} \right)
\]

\( \eta \): viscosity of a liquid (mPa·s)
\( \pi \): circumference/diameter ratio
\( l \): length of the inner cylinder (cm)
\( \omega \): angular velocity (rad/s)
\( T \): torque acting on cylinder surface (10⁻⁷ N·m)
\( R_i \): 1/2 of outer diameter of the inner cylinder (cm)
\( R_o \): 1/2 of inner diameter of the outer cylinder (cm)

2.1.2. Single cylinder-type rotational viscometer (Brookfield type viscometer)

In the single cylinder-type rotational viscometer, viscosity is determined by measuring the torque acting on the cylinder
Fig. 2.53-2b  Single cylinder-type rotational viscometer

surface when the cylinder immersed in a liquid is rotated at a given angular velocity. Use an apparatus of the type illustrated in Fig. 2.53-2b. If the apparatus constant $K_B$ is previously determined experimentally by using the Standard Liquids for Calibrating Viscometers, the viscosity of a liquid, $\eta$, can be obtained from the following equation.

$$\eta = \frac{K_B T}{\omega}$$

where, $\eta$: viscosity of a liquid (mPa·s)  
$K_B$: apparatus constant of viscometer (rad/cm³)  
$\omega$: angular velocity (rad/s)  
$T$: torque acting on cylinder surface ($10^{-7}$ N·m)  

2.1.3. Cone-flat plate-type rotational viscometer (Cone-plate type viscometer)

In the cone-flat plate-type rotational viscometer, viscosity is determined by placing a liquid in the gap between a flat disc and a cone with a large vertical angle sharing the same rotational axis, and the torque and the corresponding angular velocity are measured, when either the disc or the cone is rotated in a viscous liquid.

As shown in Fig. 2.53-2c, a liquid is introduced to fill the gap between a flat disc and a cone forming an angle $\alpha$ (rad). When either the flat disc or the cone is rotated at a constant angular velocity or a constant torque, the torque acting on the disc or cone surface rotated by the viscous flow and the corresponding angular velocity in the steady state, are measured. The viscosity of the liquid, $\eta$, can be calculated from the following equation.

$$\eta = \frac{3\pi}{2\pi R^3} \cdot \frac{100T}{\omega}$$

$\eta$: viscosity of a liquid (mPa·s)  
$\pi$: circumference/diameter ratio  
$R$: radius of cone (cm)  
$\alpha$: angle between flat disc and cone (rad)  
$\omega$: angular velocity (rad/s)  
$T$: torque acting on flat disc or cone surface ($10^{-7}$ N·m)  

2.2. Procedure

Set up the viscometer so that its rotational axis is perpendicular to the horizontal plane. Place a sufficient quantity of a sample solution in the viscometer, and allow the measuring system to stand until a specified temperature is attained, as directed in the monograph. Where it is desired to measure the viscosity within a precision of 1%, measuring temperature should be controlled within 0.1°C. Next, after confirming that the sample solution is at the designated temperature, start operating the rotational viscometer. After the forced rotation induced by the viscous flow has reached a steady state and the indicated value on the scale, which corresponds to the rotational frequency or the torque, has become constant, read the value on the scale. Then, calculate the viscosity $\eta$ by using the respective equation appropriate to the type of viscometer being used. Determination or confirmation of the apparatus constant should be conducted beforehand by using the Standard Liquids for Calibrating Viscometers, and the validation of the apparatus and operating procedures should also be performed by using those standard liquids.

In the case of a non-Newtonian liquid, repeat the procedure for measuring the viscosity of the liquid with variation of the rotation velocity or torque from one measurement to another. From a series of such viscosity measurements, the relationship between the slip velocity and the slip stress of a non-Newtonian liquid, i.e., the flow characteristics of a non-Newtonian liquid, can be obtained.

Calibration of a rotational viscometer is conducted by using water and the Standard Liquids for Calibrating Viscometers. These standard liquids are used for the determination or confirmation of the apparatus constant of the rotational viscometer. They are also used for periodic recalibration of the viscometer to confirm maintenance of a specified precision.

2.54 pH Determination

pH is defined as the reciprocal of the common logarithm of hydrogen ion activity, which is the product of hydrogen ion concentration and the activity coefficient. Conventionally it is used as a scale of hydrogen ion concentration of a sample solution.

pH of a sample solution is expressed by the following equation in relation to the pH of a standard solution (pHs), and can be measured by a pH meter using a glass electrode.

$$\text{pH} = \text{pHs} + \frac{E - E_s}{2.3026\frac{RT}{F}}$$
pHs: pH value of a pH standard solution.

$E$: Electromotive force (volt) induced on the following galvanic cell composed of a glass electrode and suitable reference electrode in a sample solution:

Glass electrode | sample solution | reference electrode

$E$: Electromotive force (volt) induced on the following galvanic cell composed of a glass electrode and suitable reference electrode in a pH standard solution:

Glass electrode | standard pH solution | reference electrode

$R$: Gas constant

$T$: Absolute temperature

$F$: Faraday’s constant

The value of 2.3026 $RT/F$ (V) in the above equation means the degree of electromotive force (V) per one pH unit and it is dependent on the temperature as shown in Table 2.54-1:

### 1. pH Standard Solution

The pH standard solutions are used as a standard of pH, for standardization of a pH meter. To prepare the pH standard solutions, use distilled water or water with a conductivity not more than 0.50 mg/L, boiled for not less than 15 minutes and cooled in a container fitted with a carbon dioxide-absorbing tube. Since the pH may change gradually during storage over a long period, it is necessary to ascertain whether the expected pH value is held or not by comparison with newly prepared standard, when the solution is used after long storage.

(i) Oxalate pH standard solution—Pulverize potassium trihydrogen dioxalate dihydrate for pH determination, and dry in a desiccator (silica gel). Weigh 12.71 g (0.05 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(ii) Phthalate pH standard solution—Pulverize potassium phthalate for pH determination, and dry at 110°C to constant mass. Weigh 10.21 g (0.05 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(iii) Phosphate pH standard solution—Pulverize potassium dihydrogen phosphate for pH determination and disodium hydrogen phosphate for pH determination, and dry at 110°C to constant mass. Weigh 3.40 g (0.025 mole) of potassium dihydrogen phosphate and 3.55 g (0.025 mole) of disodium hydrogen phosphate accurately, and dissolve in water to make exactly 1000 mL.

(iv) Borate pH standard solution—Allow sodium tetraborate for pH determination to stand in a desiccator (saturated sodium bromide aqueous solution) until it reaches constant mass. Weigh 3.81 g (0.01 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(v) Carbonate pH standard solution—Dry sodium hydrogen carbonate for pH determination in a desiccator (silica gel) to constant mass, and weigh 2.10 g (0.025 mole) of it accurately. Dry sodium carbonate for pH determination between 300°C and 500°C to constant mass, and weigh 2.65 g (0.025 mole) of it accurately. Dissolve both reagents in water to make exactly 1000 mL.

(vi) Calcium hydroxide pH standard solution—Reduce calcium hydroxide for pH determination to a fine powder, transfer 5 g to a flask, add 1000 mL of water, shake well, and allow the solution to become saturated at a temperature between 23°C and 27°C. Then filter the supernatant at the same temperature and use the clear filtrate (about 0.02 mol/L).

The pH values of these pH standard solutions at various temperatures are shown in the Table below. pH values at an arbitrary temperature not indicated in Table 2.54-2 can be calculated by the interpolation method.

### 2. Apparatus

A pH meter generally consists of an electrode system of a glass electrode and a reference electrode, an amplifier and an indicating unit for controlling the apparatus and for displaying the measured value of electromotive force. The indicating unit is usually fitted with dials for zero and span (sensitivity) adjustment. Sometimes a temperature compensation dial is included.

The reproducibility of a pH meter should be within 0.05 pH unit, when measurements for an arbitrary pH standard solution are repeated five times, following the procedure described below. After each measurement it is necessary to wash the detecting unit well with water.

### 3. Procedure

Immerse the glass electrode previously in water for more than several hours. Start the measurement after confirming stable running of the apparatus. Rinse well the detecting unit with water, and remove the remaining water gently with a piece of filter paper.

---

**Table 2.54-1** Temperature dependency of the electromotive force (V)

<table>
<thead>
<tr>
<th>Temperature of solution (°C)</th>
<th>2.3026 $RT/F$ (V)</th>
<th>Temperature of solution (°C)</th>
<th>2.3026 $RT/F$ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.05519</td>
<td>35</td>
<td>0.06114</td>
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<tr>
<td>10</td>
<td>0.05618</td>
<td>40</td>
<td>0.06213</td>
</tr>
<tr>
<td>15</td>
<td>0.05717</td>
<td>45</td>
<td>0.06313</td>
</tr>
<tr>
<td>20</td>
<td>0.05817</td>
<td>50</td>
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</tr>
<tr>
<td>25</td>
<td>0.05916</td>
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<td>0.06511</td>
</tr>
<tr>
<td>30</td>
<td>0.06015</td>
<td>60</td>
<td>0.06610</td>
</tr>
</tbody>
</table>

**Table 2.54-2** pH values of six pH standard solutions

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Oxalate pH standard solution</th>
<th>Phthalate pH standard solution</th>
<th>Phosphate pH standard solution</th>
<th>Borate pH standard solution</th>
<th>Carbonate pH standard solution</th>
<th>Calcium hydroxide pH standard solution</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>1.67</td>
<td>4.01</td>
<td>6.98</td>
<td>9.46</td>
<td>10.32</td>
<td>13.43</td>
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<td>1.67</td>
<td>4.01</td>
<td>6.95</td>
<td>9.39</td>
<td>10.25</td>
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<td>10</td>
<td>1.67</td>
<td>4.00</td>
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<td>9.33</td>
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<td>6.84</td>
<td>8.96</td>
<td>9.73</td>
<td>11.45</td>
</tr>
</tbody>
</table>
To standardize the pH meter, two pH standard solutions are usually used as follows. Immers the detection unit in the phosphate pH standard solution and adjust the indicated pH to the pH value shown in the Table. Next, immerse the detection system in the second pH standard solution, which should be selected so that the expected pH of the sample solution to be determined is between the pH values of the two pH standard solutions, and measure the pH under the same conditions as used for the first pH standard solution. Adjust the indicated pH to the defined pH value using the span adjustment dial, when the observed pH is not identical with that tabulated. Repeat the above standardization procedure until both pH standard solutions give observed pH values within 0.02 pH unit of the tabulated value without further adjustments. When a pH meter is fitted with a temperature compensation dial, the standardization procedure is done after the setting of the temperature to that of the pH standard solution to be measured.

In the case of using an apparatus having an auto-calibration function, it is necessary to confirm periodically that the pH values of two pH standard solutions are identical with the tabulated values within 0.05 pH unit.

After finishing the standardization procedure described above, rinse well the electrodes with water, remove the attached water using a filter paper, immerse the electrode system in the sample solution, and read the indicated pH value after confirming the value is stable. If necessary, a sample solution can be agitated gently.

In the pH determination, the temperature of a sample solution must be controlled to be the same as that of the pH standard solutions with which the pH meter was standardized (within 2°C). When a sample solution is alkaline, the measurement should be done in a vessel with a cover and if necessary, in a stream of inert gas such as nitrogen. Furthermore, for a strongly alkaline solution above pH 11 containing alkali metal ions, an alkali error may be induced in the pH measurement. Thus, in such a case, an electrode with less alkali error should be used and an appropriate correction should be applied to the measured value.

4. Note
Construction and treatment in detail are different for different pH meters.

### 2.55 Vitamin A Assay

Vitamin A Assay is a method to determine vitamin A in Retinol Acetate, Retinol Palmitate, Vitamin A Oil, Cod Liver Oil and other preparations. Method 1 is for the assay of synthetic vitamin A esters, using the ultraviolet-visible spectrophotometry (Method 1-1) or the liquid chromatography (Method 1-2). Method 2 is for the assay of vitamin A of natural origin, containing many geometrical isomers, using the ultraviolet-visible spectrophotometry to determine vitamin A as vitamin A alcohol obtained by saponification in an alkaline solution and extraction.

One Vitamin A Unit (equal to 1 vitamin A I.U.) is equivalent to 0.300 µg of vitamin A (all-trans vitamin A alcohol).

1. Procedure
All procedures should be carried out quickly and care should be taken as far as possible to avoid exposure to light, air, oxidants, oxidizing catalysts (e.g. copper, iron), acids and heat. If necessary, light-resistant vessels may be used.

Generally, for synthetic vitamin A esters apply Method 1-1 or Method 1-2, but if the assay conditions required for Method 1-1 are not suitable, apply Method 2.

#### 1. Method 1-1
Weigh accurately about 0.1 g of the sample, and dissolve in 2-propanol for vitamin A assay to make exactly 50 mL. Dilute this solution with 2-propanol for vitamin A assay to make a solution so that each mL contains 10 to 15 vitamin A Units, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution between 220 nm and 400 nm as directed under Ultraviolet-visible Spectrophotometry to obtain the wavelength of the maximum absorption and the absorbances at 300 nm, 310 nm, 320 nm, 326 nm, 330 nm, 340 nm and 350 nm. When the maximum absorption lies between 325 nm and 328 nm, and the ratios, $A_{326}/A_{328}$, of each absorbance, $A_{326}$ at 300 nm, 310 nm, 320 nm, 330 nm, 340 nm and 350 nm to the absorbance, $A_{326}$ at 326 nm are within the range of ±0.030 of the values in the Table, the potency of vitamin A in Units per g of the sample is calculated from the following equation.

$$
\text{Units of vitamin A in 1 g} = \frac{A_{326}}{M} \times \frac{V}{100} \times 1900
$$

$A_{326}$: Absorbance at 326 nm
$V$: Total volume (mL) of the sample solution
$M$: Amount (g) of sample in V mL of the sample solution
1900: Conversion factor from specific absorbance of retinol ester to IU (Unit/g)

This method is applied to drugs or preparations containing vitamin A esters (retinol acetate or retinol palmitate) as the main component. However, when the wavelength of maximum absorption does not lie between 325 nm and 328 nm, or when the absorbance ratio $A_{326}/A_{328}$ is not within the range of ±0.030 of the values in Table 2.55-1, apply Method 2.

#### 1.2. Method 1-2
Proceed with an appropriate amount of sample as directed under Liquid Chromatography.

For the assay of retinol acetate and retinol palmitate use Retinol Acetate Reference Standard and Retinol Palmitate Reference Standard, respectively, and fix appropriately the operating procedure, the operating conditions and the system suitability based on the characteristics of the substance to be tested and the species and amount of coexisting substances.

1.3. Method 2

Unless otherwise specified, weigh accurately a sample con-
2.56 Determination of Specific Gravity and Density / General Tests

The density \( \rho \) (g/mL or g/cm\(^3\)) means the mass per unit volume, and the relative density means the ratio of the mass of a sample specimen to that of an equal volume of a standard substance. The relative density is also called the specific gravity.

The specific gravity, \( d' \), means the ratio of the mass of the sample specimen at \( t \)°C to that of an equal volume of water (H\(_2\)O) at \( t \)°C. Unless otherwise specified, the measurement is to be performed by Method 1, Method 2 or Method 4. When the specified value is accompanied with the term “about” in the monograph, Method 3 is also available.

1. Method 1. Measurement using a pycnometer

A pycnometer is a glass vessel with a capacity of usually 10 mL to 100 mL, having a ground-glass stopper fitted with a thermometer, and a side inlet-tube with a marked line and a ground-glass cap.

Weigh a pycnometer, previously cleaned and dried, to determine its mass \( M \). Remove the stopper and the cap. Fill the pycnometer with the sample solution, keeping them at a slightly lower temperature by 1°C to 3°C than the specified temperature \( t \)°C, and stopper them, taking care not to leave bubbles. Raise the temperature gradually, and when the thermometer shows the specified temperature, remove the portion of the sample solution above the marked line through the side tube, cap the side tube, and wipe the outside surface thoroughly. Measure the mass \( M_t \) of the pycnometer filled with the sample solution. Perform the same procedure, using the same pycnometer containing water, and note the mass \( M_z \) at the specified temperature \( t \)°C.

The specific gravity \( d' \) can be calculated by use of the following equation.

\[
d' = \frac{M_t - M}{M_z - M}
\]

Further, when measurements for a sample solution and water are performed at the same temperature \( t \)°C, the density of the sample solution at the temperature \( t \)°C \((\rho_t)\) can be calculated from the measured specific gravity \( d' \) and the density of water at the temperature \( t \)°C \((\rho_0)\) indicated in Table 2.56-1 by using the following equation.

\[
\rho_t = \rho_0 d'
\]


A Sprengel-Ostwald pycnometer is a glass vessel with a capacity of usually 1 mL to 10 mL. As shown in Fig. 2.56-1, both ends are thick-walled fine tubes (inside diameter: 1-

<table>
<thead>
<tr>
<th>Table 2.56-1 Density of water</th>
</tr>
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<tbody>
<tr>
<td>Temp. °C</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
</tbody>
</table>

* In this Table, although the unit of density is represented by g/mL in order to harmonize with the unit expression in the text, it should be expressed in g/cm\(^3\) seriously.
Take the pycnometer out of the water bath, wipe thoroughly and then, by attaching a piece of filter paper to the end of B, sample solution is performed at the same temperature. Attach a hydrometer, the reading must be in accordance with the instructions. Further, when measurements of specific gravity for a sample solution and water are performed at the same temperature $t^\circ C$, the density of sample solution at temperature $t^\circ C$ can be calculated by using the equation described in Method 1.

Further, when measurements of specific gravity for a sample solution and water are performed at the same temperature ($t^\circ C = r^\circ C$), the density of sample solution at temperature $t^\circ C$ can be calculated by using the equation described in Method 1.

Clean a hydrometer with ethanol (95) or diethyl ether. Stir the sample well with a glass rod, and float the hydrometer in the well. When the temperature is adjusted to the specified temperature $t^\circ C$ and the hydrometer comes to a standstill, read the specific gravity $d'_i$ or the density $\rho'_i$ at the upper brim of the meniscus. Here the temperature $t^\circ C$ indicates the temperature at which the hydrometer is calibrated. If specific instructions for reading the meniscus are supplied with the hydrometer, the reading must be in accordance with the instructions.

Further, when measurement of the specific gravity for a sample solution is performed at the same temperature ($t^\circ C = t^\circ C$), at which the hydrometer is calibrated, the density of a sample solution at $t^\circ C$, $\rho'_i$, can be calculated by using the specific gravity $d'_i$ and the equation shown in Method 1.

Density measurement with an oscillator-type density meter is a method for obtaining the density of liquid or gas by measuring the intrinsic vibration period $T$ (s) of a glass tube cell filled with sample specimen. When a glass tube containing a sample is vibrated, it undergoes a vibration with an intrinsic vibration period $T$ in proportion to the mass of the sample specimen. If the volume of the vibrating part of the sample cell is fixed, the relation of the square of intrinsic oscillation period and density of the sample specimen shall be linear.

Before measuring a sample density, the respective intrinsic oscillation periods $T_{S1}$ and $T_{S2}$ for two reference substances (density: $\rho_{S1}$, $\rho_{S2}$) must be measured at a specified temperature $t^\circ C$, and the cell constant $K_r$ (g cm$^{-3}$ s$^{-2}$) must be determined by using the following equation.

$$K_r = \frac{\rho'_{S1} - \rho'_{S2}}{T_{S1}^2 - T_{S2}^2}$$

Usually, water and dried air are chosen as reference substances. Here the density of water at $t^\circ C$, $\rho'_{S1}$, is taken from Table 2.56-1, and that of dried air $\rho'_{S2}$ is calculated by using the following equation, where the pressure of dried air is at $p$ kPa.

$$\rho'_{S2} = 0.0012932 \times \{273.15/(273.15 + t')\} \times (p/101.325)$$

Next, introduce a sample specimen into a sample cell having a cell constant $K_s$, the intrinsic vibration period, $T_s$, for the sample under the same operation conditions as employed for the reference substances. The density of a sample specimen at $t^\circ C$, $\rho'_s$, is calculated by use of the following equation, by introducing the intrinsic oscillation period $T_{S1}$ and the density of water at a specified temperature $t^\circ C$, $\rho'_{S1}$, into the equation.

$$\rho'_s = \rho'_{S1} + K_r (T_{S1}^2 - T_s^2)$$

Further, the specific gravity of a sample specimen $d'_s$ against water at a temperature $t^\circ C$ can be obtained by using the equation below, by introducing the density of water at a temperature $t^\circ C$, $\rho'_{S1}$, indicated in Table 2.56-1.

$$d'_s = \frac{\rho'_s}{\rho'_{S1}}$$

4.1. Apparatus
An oscillator-type density meter is usually composed of a glass tube cell of about 1 mL capacity, the curved end of which is fixed to the vibration plate, an oscillator which ap-
2.57 Boiling Point and Distilling Range Test

The boiling point and distilling range are determined by Method 1 or Method 2 as described herein, unless otherwise specified. Boiling point is the temperature shown between the first 5 drops of distillate leave the tip of the condenser and when the last liquid evaporates from the bottom of the flask. Distilling range test is done to determine the volume of the distillate which has been collected in the range of temperature directed in the monograph.

1. Method 1 This method is applied to a sample for which the permissible range of boiling temperature is smaller than 5°C.

1.1. Apparatus

Use the apparatus illustrated in Fig. 2.57-1.

1.2. Procedure

Measure 25 mL of the sample, whose temperature is previously noted, using a volumetric cylinder graduated in 0.1 mL, and transfer it to a distilling flask A of 50- to 60-mL capacity. Use this cylinder as the receiver for the distillate without rinsing out any of the adhering liquid. Put boiling chips into the distilling flask A, insert a thermometer B with an immersion line so that its immersion line C is on a level with the lower end of cork stopper D and the upper end of its mercury bulb is located in the center of the delivery tube, and connect condenser E with the distilling flask A and adapter F with the condenser E. Insert the open end of F into the mouth of cylinder G (receiver) so that air can pass through slightly. Use a hood with a height sufficient to shield A, and heat A with a suitable heat source. When direct flame is applied as the heat source, put A on a hole of a fire-resistant, heat-insulating board [a board consisting of a fire-resistant, heat-insulating material, 150 mm square and about 6 mm thick (or a wire gauge of 150 mm square bonded to fire-resistant, heat-insulation materials in about 6 mm thickness), having an its center a round hole 30 mm in diameter].

Unless otherwise specified, distill the liquid sample by the application of heat, at a rate of 4 to 5 mL per minute of distillate in the case of liquids whose boiling temperature to be determined is lower than 200°C and at a rate of 3 to 4 mL per minute in the case of liquids whose boiling temperature is 200°C or over, and read the boiling point. For the distilling range test, bring the temperature of distillate to the temperature at which the volume was originally measured, and measure the volume of distillate.

Liquids that begin to distill below 80°C are cooled to between 10°C and 15°C before measuring the volume, and the receiving cylinder is kept immersed in ice up to a point 25 mm from the top during the distillation.

Correct the observed temperature for any variation in the barometric pressure from the normal (101.3 kPa), by allowing 0.1 degree for each 0.36 kPa of variation, adding if the pressure is lower, or subtracting if higher than 101.3 kPa.

2. Method 2 This method is applied to the sample for which the permissible range of boiling temperature is 5°C or more.

2.1. Apparatus

The same apparatus as described in Method 1 is used. However, use a 200-mL distilling flask A with a neck 18 to 24 mm in inside diameter having a delivery tube 5 to 6 mm in inside diameter. The fire-resistant, heat-insulating board used for direct flame heating should have in its center a round hole 50 mm in diameter.
2.2. Procedure
Measure 100 mL of the sample, whose temperature is previously noted, using a volumetric cylinder graduated in 1 mL, and carry out the distillation in the same manner as in Method 1.

2.58 X-Ray Powder Diffraction Method

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (*). *X-Ray Powder Diffraction Method is a method for measuring characteristic X-ray diffraction angles and intensities from randomly oriented powder crystallites irradiated by a monochromated X-ray beam.

Every crystalline phase of a given substance produces a characteristic X-ray diffraction pattern. Diffraction patterns can be obtained from a randomly oriented crystalline powder composed of crystallites or crystal fragments of finite size. Essentially 3 types of information can be derived from a powder diffraction pattern: angular position of diffraction lines (depending on geometry and size of the unit cell); intensities of diffraction lines (depending mainly on atom type and arrangement, and particle orientation within the sample); and diffraction line profiles (depending on instrumental resolution, crystallite size, strain and specimen thickness).

Experiments giving angular positions and intensities of lines can be used for applications such as qualitative phase analysis (for example, identification of crystalline phases) and quantitative phase analysis of crystalline materials. An estimate of the amorphous and crystalline fractions(1) can also be made. The X-ray powder diffraction (XRPD) method provides an advantage over other means of analysis in that it is usually non-destructive in nature (specimen preparation is usually limited to grinding to ensure a randomly oriented sample). XRPD investigations can also be carried out under in situ conditions on specimens exposed to non-ambient conditions, such as low or high temperature and humidity.

1. Principle
X-ray diffraction results from the interaction between X-rays and electron clouds of atoms. Depending on the atomic arrangement, interferences arise from the scattered X-rays. These interferences are constructive when the path difference between 2 diffracted X-ray waves differs by an integral number of wavelengths. This selective condition is described by the Bragg equation, also called Bragg’s law (see Fig. 2.58-1)

\[ 2d_{hkl} \sin \theta_{hkl} = n \lambda \]

The wavelength \( \lambda \) of the X-rays is of the same order of magnitude as the distance between successive crystal lattice planes, or \( d_{hkl} \) (also called ‘d-spacings’). \( \theta_{hkl} \) is the angle between the incident ray and the family of lattice planes, and \( \sin \theta_{hkl} \) is inversely proportional to the distance between successive crystal planes or d-spacings.

The direction and spacing of the planes with reference to the unit cell axes are defined by the Miller indices \([hkl]\). These indices are the reciprocals, reduced to the next-lower integer, of the intercepts that a plane makes with the unit cell axes. The unit cell dimensions are given by the spacings, \( a, b \) and \( c \) and the angles between them, \( \alpha, \beta \) and \( \gamma \). The interplanar spacing for a specified set of parallel \( hkl \) planes is denoted by \( d_{hkl} \). Each such family of planes may show higher orders of diffraction where the \( d \) values for the related families of planes, \( nh, nk, nl \) are diminished by the factor \( 1/n \) (\( n \) being an integer: \( 2,3,4, \) etc.). Every set of planes throughout a crystal has a corresponding Bragg diffraction angle, \( \theta_{hkl} \), associated with it (for a specific wavelength \( \lambda \)).

A powder specimen is assumed to be polycrystalline so that at any angle \( \theta_{hkl} \) there are always crystallites in an orientation allowing diffraction according to Bragg’s law(2). For a given X-ray wavelength, the positions of the diffraction peaks (also referred to as ‘lines’, ‘reflections’ or ‘Bragg reflections’) are characteristic of the crystal lattice (d-spacings), their theoretical intensities depend on the crystallographic unit cell content (nature and positions of atoms), and the line profiles on the perfection and extent of the crystal lattice. Under these conditions the diffraction peak has a finite intensity arising from atomic arrangement, type of atoms, thermal motion and structural imperfections, as well as from instrument characteristics. The intensity is dependent upon many factors such as structure factor, temperature factor, crystallinity, polarization factor, multiplicity and Lorentz factor. The main characteristics of diffraction line profiles are at position, peak height, peak area and shape (characterized by, for example, peak width or asymmetry, analytical function, empirical representation). An example of the type of powder patterns obtained for 5 different solid phases of a substance are shown in Fig. 2.58-2.

In addition to the diffraction peaks, an X-ray diffraction experiment also generates a more-or-less uniform background, upon which the peaks are superimposed. Besides specimen preparation, other factors contribute to the background, for instance the sample holder, diffuse scattering from air and equipment, other instrumental parameters such as detector noise, general radiation from the X-ray tube, etc. The peak to background ratio can be increased by minimizing background and by choosing prolonged exposure times.
2. Instrument
2.1. Instrument set-up

X-ray diffraction experiments are usually performed using powder diffractometers or powder cameras. A powder diffractometer generally comprises 5 main parts: an X-ray source; incident beam optics, which may perform monochromatization, filtering, collimation and/or focusing of the beam; a goniometer; diffraction beam optics, which may perform monochromatization, filtering, collimation and focusing or parallelising of the beam; and a detector. Data collection and data processing systems are also required and are generally included in current diffraction measurement equipment.

Depending on the type of analysis to be performed (phase identification, quantitative analysis, lattice parameters determination, etc.), different XRPD instrument configurations and performance levels are required. The simplest instruments used to measure powder patterns are powder cameras. Replacement of photographic film as the detection method by photon detectors has led to the design of diffractometers in which the geometric arrangement of the optics is not truly focusing but parafocusing, such as in the Bragg-Brentano geometry. The Bragg-Brentano parafocusing configuration is currently the most widely used and is therefore briefly described here.

A given instrument may provide a horizontal or vertical \( \theta/2\theta \) geometry or a vertical \( \theta/\theta \) geometry. For both geometries, the incident X-ray beam forms an angle \( \theta \) with the specimen surface plane and the diffracted X-ray beam forms an angle \( 2\theta \) with the direction of the incident X-ray beam (an angle \( 0 \) with the specimen surface plane). The basic geometric arrangement represented in Fig. 2.58-3. The divergent beam of radiation from the X-ray tube (the so-called ‘primary beam’) passes through the parallel plate collimators and a divergence slit assembly and illuminates the flat surface of the specimen. All the rays diffracted by suitably oriented crystallites in the specimen at an angle \( 2\theta \) converge to a line at the receiving slit. A second set of parallel plate collimators and a scatter slit may be placed either behind or before the receiving slit. The axes of the line focus and of the receiving slit are at equal distances from the axis of the goniometer. The X-ray quanta are counted by a radiation detector, usually a scintillation counter, a sealed-gas proportional counter, or a position-sensitive solid-state detector such as imaging plate or CCD detector. The receiving slit assembly and the detector are coupled together and move tangentially to the focusing circle. For \( \theta/2\theta \) scans the goniometer rotates the specimen about the same axis as that of the detector, but at half the rotational speed, in \( \theta/2\theta \) motion. The surface of the specimen thus remains tangential to the focusing circle. The parallel plate collimator limits the axial divergence of the beam and hence partially controls the shape of the diffracted line profile.

A diffractometer may also be used in transmission mode. The advantage with this technology is to lessen the effects due to preferred orientation. A capillary of about 0.5 – 2 mm thickness can also be used for small sample amounts.

2.2. X-ray radiation

In the laboratory, X-rays are obtained by bombarding a metal anode with electrons emitted by the thermionic effect and accelerated in a strong electric field (using a high-voltage generator). Most of the kinetic energy of the electrons is converted to heat, which limits the powder of the tubes and
requires efficient anode cooling. A 20- to 30-fold increase in brilliance can be obtained using rotating anodes and by using X-ray optics. Alternatively, X-ray photons may be produced in a large-scale facility (synchrotron).

The spectrum emitted by an X-ray tube operating at sufficient voltage consists of a continuous background of polychromatic radiation and additional characteristic radiation that depends on the type of anode. Only this characteristic radiation is used in X-ray diffraction experiments. The principal radiation sources utilized for X-ray diffraction are vacuum tubes utilizing copper, molybdenum, iron, cobalt or chromium as anodes; copper, molybdenum or cobalt X-rays are employed most commonly for organic substances (the use of cobalt anodes can be especially preferred to separate distinct X-ray lines). The choice of radiation to be used depends on the absorption characteristics of the specimen and possible fluorescence by atoms present in the specimen. The wavelengths used in powder diffraction generally correspond to the Kα radiation from the anode. Consequently, it is advantageous to make the X-ray beam ‘monochromatic’ by eliminating all the other components of the emission spectrum. This can be partly obtained using Kα filters, i.e. metal filters selected as having an absorption edge between the Kα and Kβ wavelengths emitted by the tube.

Such a filter is usually inserted between the X-ray tube and the specimen. Another, more-and-more-commonly used way to obtain a monochromatic X-ray beam is via a large monochromator crystal (usually referred to as a ‘monochromator’). This crystal is placed before or behind the specimen and diffracts the different characteristic peaks of the X-ray beam (i.e. Kα and Kβ) at different angels, so that only one of them may be selected to enter into the detector. It is even possible to separate Kα1 and Kα2 radiations by using a specialized monochromator. Unfortunately, the gain in getting a monochromatic beam by using a filter or a monochromator is counteracted by a loss in intensity. Another way of separating Kα and Kβ wavelengths is by using curved X-rays mirrors that can simultaneously monochromate and focus or parallelize the X-ray beam.

2.3. Radiation protection

Exposure of any part of the human body to X-rays can be injurious to health. It is therefore essential that whenever X-ray equipment is used, adequate precautions are taken to protect the operator and any other person in the vicinity. Recommended practice for radiation protection as well as limits for the levels of X-radiation exposure are those established by national legislation in each country. If there are no official regulations or recommendations in a country, the latest recommendations of the International Commission on Radiological Protection should be applied.

3. Specimen preparation and mounting

The preparation of the powdered material and mounting of the specimen in a suitable holder are critical steps in many analytical methods, and are particularly so for X-ray powder diffraction analysis, since they can greatly affect the quality of the data to be collected. The main sources of error due to specimen preparation and mounting are briefly discussed here for instruments in Bragg-Brentano parafocusing geometry.

3.1. Specimen preparation

In general, the morphology of many crystalline particles tends to give a specimen that exhibits some degree of preferred orientation in the specimen holder. This is particularly evident for needle-like or plate-like crystals when size reduction yields finer needles or platelets. Preferred orientation in the specimen influences the intensities of various reflections, so that some are more intense and others are less intense, compared to what would be expected from a completely random specimen. Several techniques can be employed to improve randomness in the orientation of crystallites (and therefore to minimize preferred orientation), but further reduction of particle size is often the best and simplest approach. The optimum number of crystallites depends on the diffractometer geometry, the required resolution and the specimen attenuation of the X-ray beam. In some cases, particle sizes as large as 50 μm will provide satisfactory results in phase identification. However, excessive milling (crystallite sizes less than approximately 0.5 μm) may cause line broadening and significant changes to the sample itself such as:

(i) specimen contamination by particles abraded from the milling instruments (mortar, pestle, balls, etc.);
(ii) reduced degree of crystallinity;
(iii) solid-state transition to another polymorph;
(iv) chemical decomposition;
(v) introduction of internal stress;
(vi) solid-state reactions.

Therefore, it is advisable to compare the diffraction pattern of the non-ground specimen with that corresponding to a specimen of smaller particle size (e.g. a milled specimen). If the X-ray powder diffraction pattern obtained is of adequate quality considering its intended use, then grinding may not be required. It should be noted that if a sample contains more than one phase and if sieving is used to isolate particles to a specific size, the initial composition may be altered.

4. Control of the instrument performance

Goniometers and the corresponding incident and diffracted X-ray beam optics have many mechanical parts that need adjustment. The degree of alignment or misalignment directly influences the quality of the results of an XRPD investigation. Therefore, the different components of the diffractometer must be carefully adjusted (optical and mechanical systems, etc.) to adequately minimize systematic errors, while optimizing the intensities received by the detector. The search for maximum intensity and maximum resolution is always antagonistic when aligning a diffractometer. Hence, the best compromise must be sought whilst performing the alignment procedure. There are many different configurations and each supplier’s equipment requires specific alignment procedures.

The overall diffractometer performance must be tested and monitored periodically using suitable certified reference materials. Depending on the type of analysis, other well-defined reference materials may also be employed, although the use of certified reference materials is preferred.

5. Qualitative phase analysis (Identification of phases)

The identification of the phase composition of an unknown sample by XRPD is usually based on the visual or computer-assisted comparison of a portion of its X-ray diffraction powder pattern to the experimental or calculated pattern of a reference material. Ideally, these reference patterns are collected on well-characterized single-phase specimens. This approach makes it possible in most cases to identify a crystalline substance by its 2θ diffraction angles or d-
spacings and by its relative intensities. The computer-aided comparison of the diffraction pattern of the unknown sample to the comparison data can be based either on a more-or-less extended 2θ-range of the whole diffraction pattern or on a set of reduced data derived from the pattern. For example, the list of d-spacings and normalized intensities \( I_{\text{norm}} \), a so-called \((d, I_{\text{norm}})\)-list extracted from the pattern, is the crystallographic fingerprint of the material, and can be compared to \((d, I_{\text{norm}})\)-lists of single-phase samples compiled in databases.

For most organic crystals, when using Cu Kα radiation, it is appropriate to record the diffraction pattern in a 2θ-range from near 0° to possibly to at least 40°. The agreement in the 2θ-diffraction angles between specimen and reference is within 0.2° for the same crystal form, while relative intensities between specimen and reference may vary considerably due to preferred orientation effects. By their very nature, variable hydrates and solvates are recognized to have varying unit cell dimensions and as such shifting occurs in peak positions of the measured XRPD patterns for these materials. In these unique materials, variance in 2θ-positions of greater than 0.2° is not unexpected. As such, peak position variances such as 0.2° are not applicable to these materials. For other types of samples (e.g. inorganic salts), it may be necessary to extend the 2θ-region scanned to well beyond 40°. It is generally sufficient to scan past the 10 strongest reflections identified in single phase X-ray powder diffraction database files.

It is sometimes difficult or even impossible to identify phases in the following cases:

(i) non-crystallized or amorphous substances;
(ii) the components to be identified are present in low mass fractions of the analyte amounts (generally less than 10 per cent \(m/m\));
(iii) pronounced preferred orientation effects;
(iv) the phase has not been filed in the database used;
(v) formation of solid solutions;
(vi) presence of disordered structures that alter the unit cell;
(vii) the specimen comprises too many phases;
(viii) presence of lattice deformations;
(ix) structural similarity of different phases.

6. Quantitative phase analysis

If the sample under investigation is a mixture of 2 or more known phases, of which not more than 1 is amorphous, the percentage (by volume or by mass) of each crystalline phase and of the amorphous phase can, in many cases, be determined. Quantitative phase analysis can be based on the integrated intensities, on the peak heights of several individual diffraction lines\(^6\), or on the full pattern. These integrated intensities, peak heights or full-pattern data points are compared to the corresponding values of reference materials. These reference materials shall be single-phase or a mixture of known phases. The difficulties encountered during quantitative analysis are due to specimen preparation (the accuracy and precision of the results require in particular homogeneity of all phases and a suitable particle size distribution in each phase) and to matrix effects. In favorable cases, amounts of crystalline phases as small as 10 per cent may be determined in solid matrices.

6.1. Polymorphic samples

For a sample composed of 2 polymorphic phases \(a\) and \(b\), the following expression may be used to quantify the fraction \(F_a\) of phase \(a\):

\[
F_a = \frac{1}{1 + K(I_a/I_b)}
\]

The fraction is derived by measuring the intensity ratio between the 2 phases, knowing the value of the constant \(K\). \(K\) is the ratio of the absolute intensities of the 2 pure polymorphic phases \(I_a/I_b\). Its value can be determined by measuring standard samples.

6.2. Methods using a standard

The most commonly used methods for quantitative analysis are:

– the ‘external standard method’;
– the ‘internal standard method’;
– the ‘spiking method’ (often also called the ‘standard addition method’).

The ‘external standard method’ is the most general method and consists of comparing the X-ray diffraction pattern of the mixture, or the respective line intensities, with those measured in a reference mixture or with the theoretical intensities of a structural model, if it is fully known.

To limit errors due to matrix effects, an internal reference material is compared to the sample. With a diffraction pattern that does not overlap at all that of the sample to be analyzed, can be used. A known quantity of this reference material is added to the sample to be analyzed and to each of the reference mixtures. Under these conditions, a linear relationship between line intensity and concentration exists. This application, called the ‘internal standard method’, requires a precise measurement of diffraction intensities.

In the ‘spiking method’ (or ‘standard addition method’), some of the pure phase \(a\) is added to the mixture containing the unknown concentration of \(a\). Multiple additions are made to prepare an intensity-versus-concentration plot in which the negative \(x\) intercept is the concentration of the phase \(a\) in the original sample.

7. Estimate of the amorphous and crystalline fractions

In a mixture of crystalline and amorphous phases, the crystalline and amorphous fractions can be estimated in several ways. The choice of the method used depends on the nature of the sample:

(i) if the sample consists of crystalline fractions and an amorphous fraction of different chemical compositions, the amounts of each of the individual crystalline phases may be estimated using appropriate standard substances as described above; the amorphous fraction is then deduced indirectly by subtraction;
(ii) if the sample consists of one amorphous and one crystalline fraction, either as a 1-phase or a 2-phase mixture, with the same elemental composition, the amount of the crystalline phase (‘the degree of crystallinity’) can be estimated by measuring 3 areas of the diffractogram:

\[
\begin{align*}
A &= \text{total area of the peaks arising from diffraction from the crystalline fraction of the sample;} \\
B &= \text{total area below area } A; \\
C &= \text{background area (due to air scattering, fluorescence, equipment, etc.)}
\end{align*}
\]

When these areas have been measured, the degree of
crystallinity can be roughly estimated using the following formula:
\[
\% \text{crystallinity} = \frac{100A}{(A + B - C)}
\]

It is noteworthy that this method does not yield absolute degree-of-crystallinity values and hence is generally used for comparative purposes only. More sophisticated methods are also available, such as the Ruland method.

8. Single crystal structure

In general, the determination of crystal structures is performed from X-ray diffraction data obtained using single crystals. However, crystal structure analysis of organic crystals is a challenging task, since the lattice parameters are comparatively large, the symmetry is low and the scattering properties are normally very low. For any given crystalline form of a substance, knowledge of the crystal structure allows the calculation of the corresponding XRPD pattern, thereby providing a ‘preferred-orientation-free’ reference XRPD pattern, which may be used for phase identification.

There are many other applications of the X-ray powder diffraction technique that can be applied to crystalline pharmaceutical substances such as: determination of crystal structures, refinement of crystal structures, determination of crystallographic properties, characterization of crystallographic texture, etc. These applications are not described in this chapter.

An ‘ideal’ powder for diffraction experiments consists of a large number of small, randomly oriented spherical crystallites (coherently diffracting crystalline domains). If this number is sufficiently large, there are always enough crystallites in any diffusing orientation to give reproducible diffraction patterns.

Similarly, changes in the specimen can occur during data collection in the case of a non-equilibrium specimen (temperature, humidity).

If the crystal structures of all components are known, the Rietveld method can be used to quantify them with good accuracy. If the crystal structures of the components are not known, the Pawley or least squares methods can be used.

### 2.59 Test for Total Organic Carbon

Test for Total Organic Carbon is a method for measuring the amount of organic carbon, which forms organic compounds, in water. Normally, organic carbon can be oxidized to carbon dioxide by a dry decomposition method, where organic compounds are oxidized by combustion, or by a wet decomposition method, where organic compounds are oxidized by applying ultraviolet rays or by adding oxidizing agent. The amount of carbon dioxide generated in the decomposition process is measured using an appropriate method such as infrared gas analysis, electric conductivity measurement, or resistivity measurement. The amount of organic carbon in water can be calculated from the amount of carbon dioxide measured in one of the above methods.

There are two types of carbon in water: organic carbon and inorganic carbon. For measuring the amount of organic carbon, two approaches can be taken. One method is to measure the amount of total carbon in water, then to subtract the amount of inorganic carbon from that of total carbon. The other method is to remove inorganic carbon from the test water, then to measure the amount of remaining organic carbon.

1. Instrument

The instrument consists of a sample injection port, a decomposition device, a carbon dioxide separation block, a detector, and a data processor or a recorder. The instrument should be capable of measuring the amount of organic carbon down to 0.050 mg/L.

The sample injection port is designed to be able to accept a specific amount of sample injected by a microsyringe or other appropriate sampling devices. The decomposition device for the dry decomposition method consists of a combustion tube and an electric furnace to heat the sample. Both devices are adjusted to operate at specified temperatures. The decomposition device for the wet decomposition method consists of an oxidizing reaction box, an ultraviolet ray lamp, a decomposition aid injector, and a heater. The decomposition device for either method should be capable of generating not less than 0.450 mg/L of organic carbon when using a solution of sodium dodecylbenzenesulfonate (theoretical value of total organic carbon in this solution is 0.806 mg/L) as the sample. The carbon dioxide separation block removes water from carbon dioxide formed in the decomposition process or separates carbon dioxide from the decomposed gas. An infrared gas analyzer, electric conductivity meter or specific resistance meter is used as the detector which converts the concentration of carbon dioxide into electric signal. The data processor calculates the concentration of the total organic carbon in the sample based on the electric signal converted by the detector. The recorder records the electric signal intensity converted by the detector.

2. Reagents and standard solutions

(i) Water used for measuring organic carbon (water for measurement): This water is used for preparing standard solutions or decomposition aid or for rinsing the instrument. The amount of organic carbon in this water, when collected into a sample container, should be not more than 0.250 mg/L.

(ii) Standard potassium hydrogen phthalate solution: The concentration of this standard solution is determined as specified for the instrument. Dry potassium hydrogen phthalate (standard reagent) at 105°C for 4 hours, and allow it to cool in a desiccator (silica gel). Weigh accurately a prescribed amount of dried potassium hydrogen phthalate, and dissolve it in the water for measurement to prepare the standard solution.

(iii) Standard solution for measuring inorganic carbon: The concentration of this standard solution is determined as specified for the instrument. Dry sodium hydrogen carbonate in a desiccator (sulfuric acid) for not less than 18 hours. Dry sodium carbonate decahydrate separately between 500°C and 600°C for 30 minutes, and allow to cool in a desiccator (silica gel). Weigh accurately prescribed amounts of these compounds so that the ratio of their carbon content is 1:1, and dissolve them in the water for measurement to prepare the standard solution.

(iv) Decomposition aid: Dissolve a prescribed amount of potassium peroxodisulfate or other substances that can be used for the same purpose, in the water for measurement up to the concentration as specified for the instrument.

(v) Gas for removing inorganic carbon or carrier gas:
Nitrogen, oxygen, or other gases that can be used for the same purpose.
(vi) Acid for removing inorganic carbon: Dilute hydrochloric acid, phosphoric acid or other acids that can be used for the same purpose, with the water for measurement down to the concentration as specified for the instrument.

3. Apparatus
(i) Sample container and reagent container: Use a container made of the material which does not release organic carbon from its surface, such as hard glass. Soak the container before use in a mixture of diluted hydrogen peroxide solution (1 in 3) and dilute nitric acid (1:1), and wash well with the water for measurement.
(ii) Microsyringe: Wash a microsyringe with a mixture of a solution of sodium hydroxide (1 in 20) and ethanol (99.5) (1:1), or diluted hydrochloric acid (1 in 4), and rinse well with the water for measurement.

4. Procedure
Employ an analytical method suitable for the instrument used. Calibrate the instruments using the standard potassium hydrogen phthalate solution with the test procedure specified for the instrument.
It is recommended that this instrument be incorporated into the manufacturing line of the water to be tested.
Otherwise, this test should be performed in a clean circumstance where the use of organic solvents or other substances that may affect the result of this test is prohibited, using a large sample container to collect a large volume of the water to be tested. The measurement should be done immediately after the sample collection.

4.1. Measurement of organic carbon by subtracting inorganic carbon from total carbon
According to the test procedure specified for the instrument used, inject a suitable volume of the sample for measuring the expected amount of total carbon into the instrument from sample injection port, and decompose organic and inorganic carbon in the sample. Detect the generated carbon dioxide with the detector, and calculate the amount of total carbon in the sample using a data processor or a recorder. Change the setting of the instrument for measuring inorganic carbon exclusively, and measure the amount of inorganic carbon in the same manner as total carbon. The amount of organic carbon can be obtained by subtracting the amount of inorganic carbon from that of total carbon.

4.2. Measurement of organic carbon after removing inorganic carbon
Remove inorganic carbon by adding the acid for removing inorganic carbon to the sample, followed by bubbling the gas for removing inorganic carbon (e.g. nitrogen) into the sample. According to the test procedure specified for the instrument used, inject a suitable volume of the sample for measuring the expected amount of organic carbon into the instrument from sample injection port, and decompose organic carbon in the sample using a data processor or a recorder.

For the instrument where the removal of inorganic carbon is performed in the instrument, first inject a suitable volume of the sample for measuring the expected amount of organic carbon into the instrument from sample injection port, according to the test procedure specified for the instrument used. Then, remove inorganic carbon by adding the acid for removing inorganic carbon to the sample in the decomposition device, followed by bubbling the gas for removing inorganic carbon into the sample. Decompose organic carbon, detect the generated carbon dioxide with the detector, and calculate the amount of organic carbon using a data processor or a recorder.

2.60 Melting Point Determination
The melting point is defined to be the temperature at which a crystalline substance melts during heating, when the solid phase and the liquid phase are in an equilibrium. However, in this Pharmacopoeia it is conventionally defined to be the temperature at which the remaining solid sample melts completely when it is subjected to continuous heating and the change of the sample state that accompanies heating is accurately observed. Since a pure substance has an intrinsic melting point, it is used for the identification and/or confirmation of a substance and also as an indicator of the purity of a substance.
The melting point is determined by either of the following methods: Method 1 is applied to those substances of which the purity is comparably high and which can be pulverized, Method 2 to those substances which are insoluble in water and can not be readily pulverized, and Method 3 to petrolatum.
Unless otherwise specified, measurement is performed by Method 1.

1. Method 1
This method is applied to those substances of which the purity is comparably high and which can be pulverized.

1.1. Apparatus
Use the apparatus illustrated in the Fig. 2.60-1.
Alternatively, apparatus in which some of the procedures, such as stirring, heating, and cooling are automated, can be used.

(i) Bath fluid: Usually use clear silicone oil having a viscosity of 50 to 100 mm2/s at an ordinary temperature.
(ii) Thermometer with an immersion line: There are six types of thermometers, Type 1—Type 6, which are specified by an appropriate measuring temperature range. For melting points lower than 50°C, use a thermometer Type 1; for 40°C to 100°C, Type 2; for 90°C to 150°C, Type 3; for 140°C to 200°C, Type 4; for 190°C to 250°C, Type 5; for 240°C to 320°C, Type 6.
(iii) Capillary tube: Use a hard glass capillary tube 120 mm long, 0.8 to 1.2 mm in inner diameter and 0.2 to 0.3 mm thick, with one end closed.

1.2. Procedure
Pulverize the sample to a fine powder, and, unless otherwise specified, dry in a desiccator (silica gel) for 24 hours. When it is specified to do the test after drying, dry the sample under the conditions specified in the monograph before measurement. Place the sample in a dried capillary tube H, and pack it tightly so as to form a layer about 2.5 – 3.5 mm high by dropping the capillary repeatedly, with the closed end of H down, through a glass tube, about 70 cm long, held vertically on a glass or porous plate.
Heat the bath fluid B until the temperature rises to about 10°C below the expected melting point, place the thermometer D in the bath with the immersion line at the same level as
Fig. 2.60-1 Melting point determination apparatus

A: Heating vessel of hard glass
B: Bath fluid
C: Teflon stopper
D: Thermometer with an immersion line
E: Thermometer-fastening spring
F: Vent for adjustment of the bath fluid volume
G: Coil spring
H: Capillary tube
J: Spring for fastening Teflon stopper

The figures are in mm.

the meniscus of the bath fluid, and insert capillary tube H into a coil spring G so that the packed sample is placed in a position corresponding to the center of the mercury bulb of the thermometer D. Continue heating to raise the temperature at a rate of approximately 3°C per minute until the temperature rises to 5°C below the expected melting point, then carefully regulate the rate of temperature increase to 1°C per minute.

Read the thermometer indication of the instantaneous temperature at which the sample liquefies completely and no solid is detectable in the capillary, and designate the indicated temperature as the melting point of the sample specimen.

1.2.1. System suitability test

Confirmation of the system suitability of the apparatus should be done periodically by using the Melting Point Standards. The Reference Standard is prepared for the suitability test of the apparatus when it is used with Type 2—Type 5 thermometers, and consists of 6 highly purified substances: acetonilide, acetophenetidin, caffeine, sulfanilamide, sulfapyridine, and vanillin. The label shows the certified melting points of the respective substances (the end point of the melting change), MPf.

After selecting one of the thermometers and the appropriate Melting Point Standard based upon the expected melting point of a sample specimen, perform a melting point measurement of the selected Reference Standard, according to the above procedure. When the value of the obtained melting point of the Reference Standard is within MPf ± 0.5°C in the case of vanillin and acetonilide, within MPf ± 0.8°C in the case of acetophenetidin and sulfanilamide, and within MPf ± 1.0°C in the case of sulfapyridine and caffeine, the apparatus is assumed to be suitable.

The above-mentioned measurement is repeated 3 times and the average is determined to be the melting point of the Reference Standard tested. When the above suitability test criteria are not met in a certain melting point measurement system of an apparatus and a Reference Standard, do the test again, after checking the packing of the sample specimen into the capillary tube, the locations and positioning of the thermometer and the capillary tube, the heating and stirring of the bath fluid, and the control of the temperature increasing rate. When a melting point measurement system does not meet the suitability test criteria again after checking these measuring conditions, the thermometer with an immersion line should be calibrated again or replaced with a new one.

2. Method 2

This method is applied to substances such as fats, fatty acids, paraffins or waxes.

2.1. Apparatus

Instead of the apparatus specified in Method 1, use a water-containing beaker as a bath fluid and a heating vessel. In this measurement, total immersion mercury-filled thermometers can also be used in place of the thermometer with an immersion line. Furthermore, the capillary tube should be the same as specified in Method 1, except that both ends of the tube are open.

2.2. Procedure

Carefully melt the sample at as low a temperature as possible, and, taking care to prevent bubbles, introduce it into a capillary tube to a height of about 10 mm. Allow the capillary containing the sample to stand for 24 hours at below 10°C, or for at least 1 hour in contact with ice, holding the capillary so that the sample can not flow out. Then attach the capillary to the thermometer by means of a rubber band so that the absorbed sample is located at a position corresponding to the center of the mercury bulb. Adjust the capillary tube in a water-containing beaker to such a position that the lower edge of the sample is located 30 mm below the water surface. Heat the beaker with constant stirring until the temperature rises to 5°C below the expected melting point. Then regulate the rate of temperature increase to 1°C per minute. The temperature at which the sample begins floating in the capillary is taken as the melting point of the sample specimen.

3. Method 3

This method is applied to petrolatums.

3.1. Apparatus

Instead of the apparatus specified in Method 1, use a water-containing beaker as a bath fluid and a heating vessel. When the value of the obtained melting point of the Reference Standard is within MPf ± 0.5°C in the case of vanillin and acetonilide, within MPf ± 0.8°C in the case of acetophenetidin and sulfanilamide, and within MPf ± 1.0°C in the case of sulfapyridine and caffeine, the apparatus is assumed to be suitable.

The above-mentioned measurement is repeated 3 times and the average is determined to be the melting point of the Reference Standard tested. When the above suitability test criteria are not met in a certain melting point measurement system of an apparatus and a Reference Standard, do the test again, after checking the packing of the sample specimen into the capillary tube, the locations and positioning of the thermometer and the capillary tube, the heating and stirring of the bath fluid, and the control of the temperature increasing rate. When a melting point measurement system does not meet the suitability test criteria again after checking these measuring conditions, the thermometer with an immersion line should be calibrated again or replaced with a new one.
3.2. Procedure
Melt the sample slowly by heating, with thorough stirring, until the temperature reaches 90–92°C. Discontinue the heating, and allow the sample to cool to 8 – 10°C above the expected melting point. Chill the bulb of the thermometer to held at a temperature about 16°C, while still cold, stick half of the thermometer bulb into the melted sample. Withdraw it immediately, hold vertically, cool until the attached sample becomes turbid, then dip the sample-bearing bulb for 5 minutes in water having a temperature below 16°C. Next, fix the thermometer securely in a test tube by means of a cork stopper so that the lower end is located 15 mm above the bottom. Suspend the test tube in a water-containing beaker held at a temperature about 16°C, and raise the temperature of the water bath to 30°C at a rate of 2°C per minute, then continue heating carefully at a rate of 1°C per minute until it reaches the melting point. Read the thermometer indication of the instantaneous temperature at which the first drop of the sample leaves the thermometer. If the variations between three repeated determinations are not more than 1°C, take the average of the three as the melting point. If any variation is greater than 1°C, make two additional measurements, and take the average of the five as the melting point.

3. Powder Property Determinations

3.01 Determination of Bulk and Tapped Densities

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the test that are not harmonized are marked with symbols (•).

• Determination of Bulk and Tapped Densities is a method to determine the bulk densities of powdered drugs under loose and tapped packing conditions respectively. Loose packing is defined as the state obtained by pouring a powder sample into a vessel without any consolidation, and tapped packing is defined as the state obtained when the vessel containing the powder sample is to be repeatedly dropped a specified distance at a constant drop rate until the apparent volume of sample in the vessel becomes almost constant.

1. Bulk density
The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per milliliter (g/mL) although the international unit is kilogram per cubic meter (1 g/mL = 1000 kg/m³) because the measurements are made using cylinders. It may also be expressed in grams per cubic centimeter (g/cm³).

The bulking properties of a powder are dependent upon the preparation, treatment and storage of the sample, i.e. how it was handled. The particles can be packed to have a range of bulk densities and, moreover, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made.

The bulk density of a powder is determined by measuring the volume of a known mass of powder sample, that may have been passed through a sieve into a graduated cylinder (Method 1), or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (Method 2) or a measuring vessel (Method 3). Method 1 and Method 3 are favoured.

1.1. Method 1: Measurement in a graduated cylinder
1.1.1. Procedure
Pass a quantity of powder sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated cylinder of 250 mL (readable to 2 mL), gently introduce, without compacting, approximately 100 g of the test sample (m) weighed with 0.1 per cent accuracy. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume (V₀) to the nearest graduated unit. Calculate the bulk density in g per mL by the formula m/V₀.

Generally, replicate determinations are desirable for the determination of this property.

If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as test sample, such that its untapped apparent volume is 150 mL to 250 mL (apparent volume greater than or equal to 60 per cent of the total volume of the cylinder); the mass of the test sample is specified in the expression of results.

For test samples having an apparent volume between 50 mL and 100 mL, a 100 mL cylinder readable to 1 mL can be used; the volume of the cylinder is specified in the expression of results.

1.2. Method 2: Measurement in a volumeter
1.2.1. Apparatus
The apparatus (Fig. 3.01-1) consists of a top funnel fitted with a 1.0 mm sieve. The funnel is mounted over a baffle box containing four glass baffle plates over which the powder slides and bounces as it passes. At the bottom of the baf-


**Determination of Bulk and Tapped Densities**

**3.01 Determination of Bulk and Tapped Densities**

**1.3. Method 3: Measurement in a vessel**

**1.3.1. Apparatus**

The apparatus consists of a 100 mL cylindrical vessel of stainless steel with dimensions as specified in Fig. 3.01-2.

**1.3.2. Procedure**

Pass a quantity of powder sufficient to complete the test through a 1.0 mm sieve, if necessary, to break up agglomerates that may have formed during storage and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of the vessel as described for Method 2. Determine the mass (m) of the powder to the nearest 0.1 per cent. Calculate the bulk density in g per mL by the formula $m/V_0$, in which $V_0$ is the volume of the cup and record the average of 3 determinations using 3 different powder samples.

**2. Tapped density**

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample.

The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped, and volume or mass readings are taken until little further volume or mass change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop, under its own mass, a specified distance by either of 3 methods as described below. Devices that rotate the cylinder or vessel during tapping may be preferred to minimize any possible separation of the mass during tapping down.

**2.1. Method 1**

**2.1.1. Apparatus**

The apparatus (Fig. 3.01-3) consists of the following:

(i) a 250 mL graduated cylinder (readable to 2 mL) with a mass of 220 ± 44 g.

(ii) a settling apparatus capable of producing, in 1 min, either nominally 250 ± 15 taps from a height of 3 ± 0.2 mm, or nominally 300 ± 15 taps from a height of 14 ± 2 mm. The support for the graduated cylinder, with its holder, has a mass of 450 ± 10 g.

**2.1.2. Procedure**

Proceed as described above for the determination of the bulk volume ($V_f$).

Secure the cylinder in the holder. Carry out 10, 500 and 1250 taps on the same powder sample and read the corresponding volumes $V_{10}$, $V_{500}$ and $V_{1250}$ to the nearest graduated unit. If the difference between $V_{500}$ and $V_{1250}$ is less than or equal to 2 mL, $V_{1250}$ is the tapped volume. If the difference between $V_{500}$ and $V_{1250}$ exceeds 2 mL, repeat increments such as 1250 taps, until the difference between succeeding measurements is less than or equal to 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/mL) using the formula $m/V_f$, in which $V_f$ is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results.

If it is not possible to use a 100 g test sample, use a reduced amount and a suitable 100 mL graduated cylinder (readable to 1 mL) weighing 130 ± 16 g and mounted on a holder weighing 240 ± 12 g. The modified test conditions are specified in the expression of the results.

**2.2. Method 2**

**2.2.1. Procedure**

Proceed as directed under Method 1 except that the mechanical tester provides a fixed drop of 3 ± 0.2 mm at a nominal rate of 250 taps per minute.

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**Fig. 3.01-2** Measuring vessel (left) and cap (right)

**Fig. 3.01-3**
3.02 Specific Surface Area by Gas Adsorption

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (• •).

The specific surface area determination method is a method to determine specific surface area (the total surface area of powder per unit mass) of a pharmaceutical powder sample by using gas adsorption method. The specific surface area of a powder is determined by physical adsorption of a gas on the surface of the solid and by calculating the amount of adsorbate gas corresponding to a monomolecular layer on the surface. Physical adsorption results from relatively weak forces (van der Waals forces) between the adsorbate gas molecules and the adsorbent surface of the test powder. The determination is usually carried out at the temperature of liquid nitrogen. The amount of gas adsorbed can be measured by a volumetric or continuous flow procedure.

1. Measurements

1.1. Multi-point measurement

When the gas is physically adsorbed by the powder sample, the following relationship (Brunauer, Emmett and Teller (BET) adsorption isotherm) holds when the relative pressure ($P/P_0$) is in the range of 0.05 to 0.30 for pressure $P$ of the adsorbate gas in equilibrium for the volume of gas adsorbed, $V_0$.

$$\frac{1}{V_0} = \frac{1}{V_m} + \frac{C}{V_m P} + \frac{(1/C)}{\left(\frac{V_m}{P}\right)}$$

$P$: Partial vapour pressure of adsorbate gas in equilibrium with the surface at $-195.8^\circ C$ (b. p. of liquid nitrogen), in pascals

$P_0$: Saturated pressure of adsorbate gas, in pascals

$V_0$: Volume of gas adsorbed at standard temperature and pressure (STP) [0°C and atmospheric pressure (1.013 × 10^5 Pa)], in milliliters

$V_m$: Volume of gas adsorbed at STP to produce an apparent monolayer on the sample surface, in milliliters

$C$: Dimensionless constant that is related to the enthalpy of adsorption of adsorbate gas on the powder sample

A value of $V_0$ is measured at each of not less than 3 values of $P/P_0$. Then the BET value, $1/[V_m((P_0/P) - 1)]$, is plotted against $P/P_0$ according to equation (1). This plot should yield a straight line usually in the approximate relative pressure range 0.05 to 0.3. The data are considered acceptable if the correlation coefficient, $r$, of the linear regression is not less than 0.9975; that is, $r^2$ is not less than 0.995. From the resulting linear plot, the slope, which is equal to $(C - 1)/V_m$, and the intercept, which is equal to $1/(V_m C)$, are evaluated by linear regression analysis. From these values, $V_m$ is calculated as $1/(slope + intercept)$, while $C$ is calculated as $(slope/intercept) + 1$. From the value of $V_m$ so determined, the specific surface area, $S$, in m²g⁻¹, is calculated by the equation:

$$S = \frac{(V_m N a)}{(m \times 22400)}$$

$N$: Avogadro constant ($6.022 \times 10^{23}$ mol⁻¹),

$a$: Effective cross-sectional area of one adsorbate molecule, in square meters ($0.162 \times 10^{-18}$ m² for nitrogen and $0.195 \times 10^{-18}$ m² for krypton)

$m$: Mass of test powder, in grams

22,400: Volume, in milliliters, occupied by one mole of the adsorbate gas at STP allowing for minor departures from the ideal

A minimum of 3 data points is required. Additional meas-
urements may be carried out, especially when non-linearity is obtained at a $P/P_0$ value close to 0.3. Because non-linearity is often obtained at a $P/P_0$ value below 0.05, values in this region are not recommended. The test for linearity, the treatment of the data, and the calculation of the specific surface area of the sample are described above.

1.2. Single-point measurement

Normally, at least 3 measurements of $V_s$ each at different values of $P/P_0$ are required for the determination of specific surface area by the dynamic flow gas adsorption technique (Method I) or by volumetric gas adsorption (Method II). However, under certain circumstances described below, it may be acceptable to determine the specific surface area of a powder from a single value of $V_s$ measured at a single value of $P/P_0$, such as 0.300 (corresponding to 0.300 mole of nitrogen or 0.001038 mole fraction of krypton), using the following equation for calculating $V_m$:

$$V_m = V_i[1 - (P/P_0)] \quad (3)$$

The single-point method may be employed directly for a series of very similar powder samples of a given material for which the material constant $C$ is much greater than unity. These circumstances may be verified by comparing values of specific surface area determined by the single-point method with that determined by the multiple-point method for the series of powder samples. Close similarity between the single-point values and multiple-point values suggests that $1/C$ approaches zero. The single-point method may be employed indirectly for a series of very similar powder samples of a given material for which the material constant $C$ is not infinite but may be assumed to be invariant. Under these circumstances, the error associated with the single-point method can be reduced or eliminated by using the multiple-point method to evaluate $C$ for one of the samples from the series from the BET plot, from which $C$ is calculated as $(1 + \text{slope/intercept})$.

Then $V_m$ is calculated from the single value of $V_s$ measured at a single value of $P/P_0$ by the equation:

$$V_m = V_i[(P_0 - P)/P_0] \cdot [(1/C) + [(C - 1)/C \times (P/P_0)] \quad (4)$$

2. Sample preparation

Before the specific surface area of the sample can be determined, it is necessary to remove gases and vapors that may have become physically adsorbed onto the surface during storage and handling. If outgassing is not achieved, the specific surface area may be reduced or may be variable because some parts of surface area are covered with molecules of the previously adsorbed gases or vapors. The outgassing conditions are critical for obtaining the required precision and accuracy of specific surface area measurements on pharmaceuticals because of the sensitivity of the surface of the materials. The outgassing conditions must be demonstrated to yield reproducible BET plots, a constant weight of test powder, and no detectable physical or chemical changes in the test powder.

The outgassing conditions defined by the temperature, pressure and time should be so chosen that the original surface of the solid is reproduced as closely as possible.

Outgassing of many substances is often achieved by applying a vacuum, by purging the sample in a flowing stream of a non-reactive, dry gas, or by applying a desorption-adsorption cycling method. In either case, elevated temperatures are sometimes applied to increase the rate at which the contaminants leave the surface. Caution should be exercised when outgassing powder samples using elevated temperatures to avoid affecting the nature of the surface and the integrity of the sample.

If heating is employed, the recommended temperature and time of outgassing are as low as possible to achieve reproducible measurement of specific surface area in an acceptable time. For outgassing sensitive samples, other outgassing methods such as the desorption-adsorption cycling method may be employed.

The standard technique is the adsorption of nitrogen at liquid nitrogen temperature.

For powders of low specific surface area ($<0.2 \, \text{m}^2\text{g}^{-1}$) the proportion adsorbed is low. In such cases the use of krypton at liquid nitrogen temperature is preferred because the low vapor pressure exerted by this gas greatly reduces error. All gases used must be free from moisture.

Accurately weigh a quantity of the test powder such that the total surface of the sample is at least $1 \, \text{m}^2$ when the adsorbate is nitrogen and $0.5 \, \text{m}^2$ when the adsorbate is krypton. Lower quantities of sample may be used after appropriate validation. Because the amount of gas adsorbed under a given pressure tends to increase on decreasing the temperature, adsorption measurements are usually made at a low temperature. Measurement is performed at $-195.8^\circ \text{C}$, the boiling point of liquid nitrogen.

Adsorption of gas should be measured either by Method I or Method II.

3. Methods

3.1. Method I: The dynamic flow method

In the dynamic flow method (see Fig. 3.02-1), the recommended adsorbate gas is dry nitrogen or krypton, while helium is employed as a diluent gas, which is not adsorbed under the recommended conditions. A minimum of 3 mixtures of...
3.03 Powder Particle Density Determination

The appropriate adsorbate gas with helium are required within the $P/P_0$ range 0.05 to 0.30.

The gas detector-integrator should provide a signal that is approximately proportional to the volume of the gas passing through it under defined conditions of temperature and pressure. For this purpose, a thermal conductivity detector with an electronic integrator is one among various suitable types. A minimum of 3 data points within the recommended range of 0.05 to 0.30 for $P/P_0$ is to be determined.

A known mixture of the gases, usually nitrogen and helium, is passed through a thermal conductivity cell, through the sample again through the thermal conductivity cell and then to a recording potentiometer. Immerse the sample cell in liquid nitrogen, then the sample adsorbs nitrogen from the mobile phase. This unbalances the thermal conductivity cell, and a pulse is generated on a recorder chart.

Remove from the coolant; this gives a desorption peak equal in area and in the opposite direction to the adsorption peak.

Since this is better defined than the adsorption peak, it is the one used for the determination.

To effect the calibration, inject a known quantity of adsorbate into the system, sufficient to give a peak of similar magnitude to the desorption peak and obtain the proportion of gas volume per unit peak area.

Use a nitrogen/helium mixture for a single-point determination and several such mixtures or premixing 2 streams of gas for a multiple-point determination. Calculation is essentially the same as for the volumetric method.

3.2. Method 2: The volumetric method

In the volumetric method (see Fig. 3.02-2), the recommended adsorbate gas is nitrogen is admitted into the evacuated space above the previously outgassed powder sample to give a defined equilibrium pressure, $P$, of the gas. The use of a diluent gas, such as helium, is therefore unnecessary, although helium may be employed for other purposes, such as to measure the dead volume.

Since only pure adsorbate gas, instead of a gas mixture, is employed, interfering effects of thermal diffusion are avoided in this method.

Admit a small amount of dry nitrogen into the sample tube to prevent contamination of the clean surface, remove the sample tube, insert the stopper, and weigh it. Calculate the weight of the sample. Attach the sample tube to the volumetric apparatus. Cautiously evacuate the sample down to the specified pressure (e.g. between 2 Pa and 10 Pa). Alternatively, some instruments operate by evacuating to a defined rate of pressure change (e.g. less than 13 Pa/30 s) and holding for a defined period of time before commencing the next step.

If the principle of operation of the instrument requires the determination of the dead volume in the sample tube, for example, by the admission of a non-adsorbed gas, such as helium, this procedure is carried out at this point, followed by evacuation of the sample. The determination of dead volume may be avoided using difference measurements, that is, by means of reference and sample tubes connected by a differential transducer.

Raise a Dewar vessel containing liquid nitrogen at $-195.8^\circ$C up to a defined point on the sample cell. Admit a sufficient volume of adsorbate gas to give the lowest desired relative pressure. Measure the volume adsorbed, $V_a$. For multipoint measurements, repeat the measurement of $V_a$ at successively higher $P/P_0$ values. When nitrogen is used as the adsorbate gas, $P/P_0$ values of 0.10, 0.20, and 0.30 are often suitable.

4. Reference materials

Periodically verify the functioning of the apparatus using appropriate reference materials of known surface area, such as $\alpha$-alumina for specific surface area determination, which should have a specific surface area similar to that of the sample to be examined.

3.03 Powder Particle Density Determination

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the test that are not harmonized are marked with symbols (•). Powder Particle Density Determination is •a method to determine particle density of powdered pharmaceutical drugs or raw materials of drugs, and generally performed using a gas displacement pycnometer. The gas pycnometric density is determined by measuring the volume occupied by a known mass of powder which is equivalent to the volume of gas displaced by the powder using a gas displacement pycnometer. In gas pycnometric density measurements, the volume determined excludes the volume occupied by open pores; however, it includes the volume occupied by sealed pores or pores inaccessible to the gas.

Usually, helium is used as a test gas due to its high diffusivity into small open pores. If gases other than helium are used, different values would be obtained, since the penetration of the gas is dependent on the size of the pore as well as the cross-sectional area of the gas.

The measured density is a volume weighted average of the densities of individual powder particles. It is called the particle density, distinct from the true density of solid or the bulk density of powder. The density of solids are expressed in
1. **Apparatus**

The schematic diagram of particle density apparatus for gas displacement pycnometric measurement is shown in Fig. 3.03-1. The apparatus consists of a test cell in which the sample is placed, an expansion cell and a manometer (M). The test cell, with an empty cell volume \(V_c\), is connected through a valve (A) to an expansion cell, with a volume \(V_r\). Generally, helium is used as the measurement gas. The apparatus has to be equipped with a system capable of pressuring the test cell to the defined pressure \(P\) through the manometer.

2. **Calibration of apparatus**

The volumes of the test cell \(V_c\) and the expansion cell \(V_r\) must be accurately determined to the nearest 0.001 cm³, and to assure accuracy of the results of volume obtained, calibration of the apparatus is carried out as follows using a calibration ball of known volume for particle density measurement. The final pressures \(P_f\) are determined for the initial empty test cell followed by the test cell placed with the calibration ball for particle density measurement in accordance with the procedures, and \(V_c\) and \(V_r\) are calculated using the equation described in the section of Procedure. Calculation can be made taking into account that the sample volume \(V_s\) is zero in the first run.

3. **Procedure**

The gas pycnometric density measurement is performed at a temperature between 15°C and 30°C and must not vary by more than 2°C during the course of measurement. Volatile contaminants in the powder are removed by degassing the powder under a constant purge of helium prior to the measurement. Occasionally, powders may have to be degassed under vacuum. Because volatiles may be evolved during the measurement, weighing of the sample is done after the pycnometric measurement of volume.

Weigh the mass of the test cell and record it. After weighing out the amount of the sample as described in the individual monograph and placing it in the test cell, seal the cell in the pycnometer.

Open the valve (A) which connects the expansion cell with the test cell, confirm with the manometer (M) that the pressure inside the system is stable, and then read the system reference pressure \(P_i\). Secondly, close the valve that connects to the two cells, and introduce the measurement gas into the test cell to achieve positive pressure. Confirm with the manometer that the pressure inside the system is stable, and then read the initial pressure \(P_i\). Open the valve to connect the test cell with the expansion cell. After confirming that the indicator of the manometer is stable, read the final pressure \(P_f\), and calculate the sample volume \(V_s\) with the following equation:

\[
V_s = V_c - \frac{V_r}{P_f - P_i - 1}
\]

\(V_c\): Expansion volume (cm³)
\(V_r\): Cell volume (cm³)
\(V_s\): Sample volume (cm³)
\(P_i\): Initial pressure (kPa)
\(P_f\): Final pressure (kPa)
\(P_s\): System reference pressure (kPa)

Repeat the measurement sequence for the same powder sample until consecutive measurements of the sample volume agree to within 0.2%, and calculate the mean of sample volumes \(V_s\). Finally, unload the test cell, weigh the mass of the test cell, and calculate the final sample mass \(m\) by deducting the empty cell mass from the test cell mass. The powder particle density \(\rho\) is calculated by the following equation:

\[
\rho = \frac{m}{V_s}
\]

\(\rho\): Powder particle density in g/cm³,
\(m\): Final sample mass in g,
\(V_s\): Sample volume in cm³

If the pycnometer differs in operation or construction from the one shown in Fig. 3.03-1, follow the instructions of the manufacturer of the pycnometer. The sample conditioning is indicated with the results. For example, indicate whether the sample was tested as is or dried under specific conditions such as those described for loss on drying.

### 3.04 Particle Size Determination

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (** •**).

**Particle Size Determination is a method to determine directly or indirectly morphological appearance, shape, size and its distribution of powdered pharmaceutical drugs and excipients to examine their micromeritic properties. Optical microscopy and analytical sieving method may be used depending on the measuring purpose and the properties of test specimen.**

#### 1. **Method 1. Optical Microscopy**

*The optical microscopy is used to observe the morphological appearance and shape of individual particle either directly with the naked eye or by using a microscopic photograph, in order to measure the particle size. The particle size distribution can also be determined by this method. It is also possible with this method to measure the size of the individ-
ual particle even when different kinds of particles mingle if they are optically distinguishable. Data processing techniques, such as image analysis, can be useful for determining the particle size distribution.

This method for particle characterization can generally be applied to particles 1 μm and greater. The lower limit is imposed by the resolving power of the microscope. The upper limit is less definite and is determined by the increased difficulty associated with the characterization of larger particles. Various alternative techniques are available for particle characterization outside the applicable range of optical microscopy. Optical microscopy is particularly useful for characterizing particles that are not spherical. This method may also serve as a base for the calibration of faster and more routine methods that may be developed.

1.1. Apparatus

Use a microscope that is stable and protected from vibration. The microscope magnification (product of the objective magnification, ocular magnification, and additional magnifying components) must be sufficient to allow adequate characterization of the smallest particles to be classified in the test specimen. The greatest numerical aperture of the objective should be sought for each magnification range. Polarizing filters may be used in conjunction with suitable analyzers and retardation plates. Color filters of relatively narrow spectral transmission should be used with achromatic objectives and are preferable with apochromats and are required for appropriate color rendition in photomicrography. Condensers corrected for at least spherical aberration should be used in the microscope substage and with the lamp. The numerical aperture of the substage condenser should match that of the objective under the condition of use; this is affected by the actual aperture of the condenser diaphragm and the presence of immersion oils.

1.1.1. Adjustment

The precise alignment of all elements of the optical system and proper focusing are essential. The focusing of the elements should be done in accordance with the recommendations of the microscope manufacturer. Critical axial alignment is recommended.

1.1.1.1. Illumination

A requirement for good illumination is a uniform and adjustable intensity of light over the entire field of view; Kohler illumination is preferred. With colored particles, choose the color of the filters used so as to control the contrast and detail of the image.

1.1.1.2. Visual Characterization

The magnification and numerical aperture should be sufficiently high to allow adequate resolution of the images of the particles to be characterized. Determine the actual magnification using a calibrated stage micrometer to calibrate an ocular micrometer. Errors can be minimized if the magnification is sufficient that the image of the particle is at least 10 ocular divisions. Each objective must be calibrated separately. To calibrate the ocular scale, the stage micrometer scale and the ocular scale should be aligned. In this way, a precise determination of the distance between ocular stage divisions can be made.

When the particle size is measured, an ocular micrometer is inserted at the position of the ocular diaphragm, and a calibrated stage micrometer is placed at the center of the microscope stage and fixed in place. The ocular is attached to the lens barrel and adjusted to the focus point of the stage micrometer scale. Then, the distance between the scales of the two micrometers is determined, and the sample size equivalent 1 division of the ocular scale is calculated using the following formula:

\[ \text{Particle size equivalent} = \frac{\text{Length on the stage micrometer (μm)}}{\text{Number of scale divisions on the ocular micrometer}} \]

The stage micrometer is removed and the test specimen is placed on the microscope stage. After adjusting the focus, the particle sizes are determined from the number of scale divisions read through the ocular.

Several different magnifications may be necessary to characterize materials having a wide particle size distribution.

1.1.1.3. Photographic Characterization

If particle size is to be determined by photographic methods, take care to ensure that the object is sharply focused at the plane of the photographic emulsion. Determine the actual magnification by photographing a calibrated stage micrometer, using photographic film of sufficient speed, resolving power, and contrast. Exposure and processing should be identical for photographs of both the test specimen and the determination of magnification. The apparent size of a photographic image is influenced by the exposure, development, and printing processes as well as by the resolving power of the microscope.

1.2. Preparation of the Mount

The mounting medium will vary according to the physical properties of the test specimen. Sufficient, but not excessive, contrast between the specimen and the mounting medium is required to ensure adequate detail of the specimen edge. The particles should rest in one plane and be adequately dispersed to distinguish individual particles of interest. Furthermore, the particles must be representative of the distribution of sizes in the material and must not be altered during preparation of the mount. Care should be taken to ensure that this important requirement is met. Selection of the mounting medium must include a consideration of the analyte solubility.

1.3. Characterization

1.3.1. Crystallinity Characterization

The crystallinity of a material may be characterized to determine compliance with the crystallinity requirement where stated in the individual monograph of a drug substance. Unless otherwise specified in the individual monograph, mount a few particles of the specimen in mineral oil on a clean glass slide. Examine the mixture using a polarizing microscope: the particles show birefringence (interference colors) and extinction positions when the microscope stage is revolved.

1.3.2. Limit Test of Particle Size by Microscopy

Weigh a suitable quantity of the powder to be examined (for example, 10 to 100 mg), and suspend it in 10 mL of a suitable medium in which the powder does not dissolve, adding, if necessary, a wetting agent. A homogeneous suspension of particles can be maintained by suspending the particles in a medium of similar or matching density and by providing adequate agitation. Introduce a portion of the homogeneous suspension into a suitable counting cell, and scan under a microscope an area corresponding to not less than 10 μg of the powder to be examined. Count all the particles having a maximum dimension greater than the prescribed size limit. The size limit and the permitted number of particles exceeding the limit are defined for each substance.
1.3.3. Particle Size Characterization

The measurement of particle size varies in complexity depending on the shape of the particle and the number of particles characterized must be sufficient to ensure an acceptable level of uncertainty in the measured parameters\(^1\). For spherical particles, size is defined by the diameter. For irregular particles, a variety of definitions of particle size exist. In general, for irregularly shaped particles, characterization of particle size must also include information on the type of diameter measured as well as information on particle shape. Several commonly used measurements of particle size are defined below (see Fig. 3.04-1).

(i) Feret’s Diameter: The distance between imaginary parallel lines tangent to a randomly oriented particle and perpendicular to the ocular scale.

(ii) Martin’s Diameter: The diameter of the particle at the point that divides a randomly oriented particle into two equal projected areas.

(iii) Projected area Diameter: The diameter of a circle that has the same projected area as the particle.

(iv) Length: The longest dimension from edge to edge of a particle oriented parallel to the ocular scale.

(v) Width: The longest dimension of the particle measured at right angles to the length.

1.3.4. Particle Shape Characterization

For irregularly shaped particles, characterization of particle size must also include information on particle shape. The homogeneity of the powder should be checked using appropriate magnification. The following defines some commonly used descriptors of particle shape (see Fig. 3.04-2).

(i) Acicular: Slender, needle-like particle of similar width and thickness.

(ii) Columnar: Long, thin particle with a width and thickness that are greater than those of an acicular particle.

(iii) Flake: Thin, flat particle of similar length and width.

(iv) Plate: Flat particles of similar length and width but with greater thickness than flakes.


(vi) Equant: Particles of similar length, width, and thickness; both cubical and spherical particles are included.

1.3.5. General Observations

A particle is generally considered to be the smallest discrete unit. A particle may be a liquid or semisolid droplet; a single crystal or polycrystalline; amorphous or an agglomerate. Particles may be associated. This degree of association may be described by the following terms.

(i) Lamellar: Stacked plates.

(ii) Aggregate: Mass of adhered particles.

(iii) Agglomerate: Fused or cemented particles.

(iv) Conglomerate: Mixture of two or more types of particles.

(v) Spherulite: Radial cluster.

(vi) Drusy: Particle covered with tiny particles.

Particle condition may be described by the following terms.

(i) Edges: Angular, rounded, smooth, sharp, fractured.

(ii) Optical: Color (using proper color balancing filters), transparent, translucent, opaque.

(iii) Defects: Occlusions, inclusions.

Surface characteristics may be described by the following terms.

(i) Cracked: Partial split, break, or fissure.

(ii) Smooth: Free of irregularities, roughness, or projections.

(iii) Porous: Having openings or passageways.

(iv) Rough: Bumpy, uneven, not smooth.

(v) Pitted: Small indentations.


The analytical sieving method is a method to estimate the particle size distribution of powdered pharmaceutical drugs by sieving. The particle size determined by this method is shown as the size of a minimum sieve opening through which the particle passes. "Powder" here means a gathering of numerous solid particles.

Sieving is one of the oldest methods of classifying powders and granules by particle size distribution. When using a woven sieve cloth, the sieving will essentially sort the particles by their intermediate size dimension (i.e., breadth or width). Mechanical sieving is most suitable where the majority of the particles are larger than about 75 \(\mu m\). For smaller particles, the light weight provides insufficient force during sieving to overcome the surface forces of cohesion and adhesion that cause the particles to stick to each other and to the sieve, and thus cause particles that would be expected to pass through the sieve to be retained. For such materials other means of agitation such as air-jet sieving or sonic sifting may be more appropriate. Nevertheless, sieving can sometimes be used for some powders or granules having median particle sizes smaller than 75 \(\mu m\) where the method can be validated. In pharmaceutical terms, sieving is usually the method of choice for classification of the coarser grades.
of single powders or granules. It is a particularly attractive method in that powders and granules are classified only on the basis of particle size, and in most cases the analysis can be carried out in the dry state.

Among the limitations of sieving method are the need for an appreciable amount of sample (normally at least 25 g, depending on the density of the powder or granule, and the diameter of test sieves) and difficulty in sieving oily or other cohesive powders or granules that tend to clog the sieve openings. The method is essentially a two-dimensional estimate of size because passage through the sieve aperture is frequently more dependent on maximum width and thickness than on length.

This method is intended for estimation of the total particle size distribution of a single material. It is not intended for determination of the proportion of particles passing or retained on one or two sieves.

Estimate the particle size distribution as described under Dry Sieving Method, unless otherwise specified in the individual monograph. Where difficulty is experienced in reaching the endpoint (i.e., material does not readily pass through the sieves) or when it is necessary to use the finer end of the sieving range (below 75 μm), serious consideration should be given to the use of an alternative particle-sizing method.

Sieving should be carried out under conditions that do not cause the test sample to gain or lose moisture. The relative humidity of the environment in which the sieving is carried out should be controlled to prevent moisture uptake or loss by the sample. In the absence of evidence to the contrary, analytical test sieving is normally carried at ambient humidity. Any special conditions that apply to a particular material should be detailed in the individual monograph.

Principles of Analytical Sieving: Analytical test sieves are constructed from a woven-wire mesh, which is of simple weave that is assumed to give nearly square apertures and is sealed into the base of an open cylindrical container. The basic analytical method involves stacking the sieves on top of one another in ascending degrees of coarseness, and then placing the test powder on the top sieve.

The nest of sieves is subjected to a standardized period of agitation, and then the weight of material retained on each sieve is accurately determined. The test gives the weight percentage of powder in each sieve size range.

This sieving process for estimating the particle size distribution of a single pharmaceutical powder is generally intended for use where at least 80% of the particles are larger than 75 μm. The size parameter involved in determining particle size distribution by analytical sieving is the length of the size of the minimum square aperture through which the particle will pass.

2.1. Procedure

2.1.1. Test Sieves

Test sieves suitable for pharmacopoeial tests conform to the most current edition of International Organisation for Standardization (ISO) Specification ISO 3310-1: Test sieves—Technical requirements and testing (see Table 3.04-1).

Unless otherwise specified in the monograph, use those ISO sieves listed in the Table 3.04-1 as recommended in the particular region.

Sieves are selected to cover the entire range of particle sizes present in the test specimen. A nest of sieves having a \( \sqrt{2} \) progression of the area of the sieve openings is recommended. The nest of sieves is assembled with the coarsest screen at the top and the finest at the bottom. Use micrometers or millimeters in denoting test sieve openings. [Note—Mesh numbers are provided in the table for conversion purposes only.] Test sieves are made from stainless steel or, less preferably, from brass or other suitable non-reactive wire.

2.1.1.1. Calibration of test sieves

Calibration and recalibration of test sieves is in accordance with the most current edition of ISO 3310-1. Sieves should be carefully examined for gross distortions and fractures, especially at their screen frame joints, before use. Sieves may be calibrated optically to estimate the average opening size, and opening variability, of the sieve mesh. Alternatively, for the evaluation of the effective opening of test sieves in the size range of 212 to 850 μm, Standard Glass Spheres are available. Unless otherwise specified in the individual monograph, perform the sieve analysis at controlled room temperature and at ambient relative humidity.

2.1.1.2. Cleaning Test Sieves

Ideally, test sieves should be cleaned using only an air jet or a liquid stream. If some apertures remain blocked by test particles, careful gentle brushing may be used as a last resort.

2.1.2. Test Specimen

If the test specimen weight is not given in the monograph for a particular material, use a test specimen having a weight between 25 and 100 g, depending on the bulk density of the material, and test sieves having a 200 mm diameter. For 76 mm sieves the amount of material that can be accommodated is approximately 1/7th that which can be accommodated on a 200 mm sieve. Determine the most appropriate weight for a given material by test sieving accurately weighed specimens of different weights, such as 25, 50, and 100 g, for the same time period on a mechanical shaker. [Note—If the test results are similar for the 25-g and 50-g specimens, but the 100-g specimen shows a lower percentage through the finest sieve, the 100-g specimen size is too large.] Where only a specimen of 10 to 25 g is available, smaller diameter test sieves conforming to the same mesh specifications may be substituted, but the endpoint must be re-determined. The use of test samples having a smaller mass (e.g. down to 5 g) may be needed. For materials with low apparent particle density, or for materials mainly comprising particles with a highly iso-diametrical shape, specimen weights below 5 g for a 200 mm screen may be necessary to avoid excessive blocking of the sieve. During validation of a particular sieve analysis method, it is expected that the problem of sieve blocking will have been addressed.

If the test material is prone to picking up or losing significant amounts of water with varying humidity, the test must be carried out in an appropriately controlled environment. Similarly, if the test material is known to develop an electrostatic charge, careful observation must be made to ensure that such charging is not influencing the analysis. An antistatic agent, such as colloidal silicon dioxide and/or aluminum oxide, may be added at a 0.5 percent (m/m) level to minimize this effect. If both of the above effects cannot be eliminated, an alternative particle-sizing technique must be selected.

2.1.3. Agitation Methods

Several different sieve and powder agitation devices are
Table 3.04-1. Sizes of Standard Sieve Series in Range of Interest

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commercially available, all of which may be used to perform sieve analyses. However, the different methods of agitation may give different results for sieve analyses and endpoint determinations because of the different types and magnitude of the forces acting on the individual particles under test. Methods using mechanical agitation or electromagnetic agitation, and that can induce either a vertical oscillation or a horizontal circular motion, or tapping or a combination of both tapping and horizontal circular motion are available. Entrainment of the particles in an air stream may also be used. The results must indicate which agitation method was used and the agitation parameters used (if they can be varied), since changes in the agitation conditions will give different results for the sieve analysis and endpoint determinations, and may be sufficiently different to give a failing result under some circumstances.

2.1.4. Endpoint Determination

The test sieving analysis is complete when the weight on any of the test sieves does not change by more than 5% or 0.1 g (10% in the case of 76 mm sieves) of the previous weight on that sieve. If less than 5% of the total specimen weight is present on a given sieve, the endpoint for that sieve is increased to a weight change of not more than 20% of the previous weight on that sieve.

If more than 50% of the total specimen weight is found on any one sieve, unless this is indicated in the monograph, the test should be repeated, but with the addition to the sieve nest of a more coarse sieve intermediate between that carrying the excessive weight and the next coarsest sieve in the original nest, i.e., addition of the ISO series sieve omitted from the nest of sieves.

2.2. Sieving Methods

2.2.1. Mechanical Agitation (Dry Sieving Method)

Tare each test sieve to the nearest 0.1 g. Place an accurately weighed quantity of test specimen on the top (coarsest) sieve, and place the lid. Agitate the nest of sieves for 5 minutes. Then carefully remove each from the nest without loss of material. Reweigh each sieve, and determine the weight of material on each sieve. Determine the weight of material in the collecting pan in a similar manner. Reassemble the nest of sieves, and agitate for 5 minutes. Remove and weigh each sieve as previously described. Repeat these steps until the endpoint criteria are met (see Endpoint Determination under Test Sieves). Upon completion of the analysis, reconcile the weights of material. Total losses must not exceed 5% of the weight of the original test specimen.

Repeat the analysis with a fresh specimen, but using a single sieving time equal to that of the combined times used above. Confirm that this sieving time conforms to the requirements for endpoint determination. When this endpoint has been validated for a specific material, then a single fixed time of sieving may be used for future analyses, providing the particle size distribution falls within normal variation.

If there is evidence that the particles retained on any sieve are aggregates rather than single particles, the use of mechanical dry sieving is unlikely to give good reproducibility; a different particle size analysis method should be used.

2.2.2. Air Entrainment Methods (Air Jet and Sonic Shifter Shaking)

Different types of commercial equipment that use a moving air current are available for sieving. A system that uses a single sieve at a time is referred to as air jet sieving. It uses the same general sieving methodology as that described under the Dry Sieving Method, but with a standardized air jet replacing the normal agitation mechanism. It requires sequential analyses on individual sieves starting with the finest sieve to obtain a particle size distribution. Air jet sieving often includes the use of finer test sieves than used in ordinary dry sieving. This technique is more suitable where only oversize or undersize fractions are needed.

In the sonic sifting method, a nest of sieves is used, and the test specimen is carried in a vertically oscillating column of air that lifts the specimen and then carries it back against the mesh openings at a given number of pulses per minute. It may be necessary to lower the sample amount to 5 g, when sonic shifting is employed.

The air jet sieving and sonic sieving methods may be useful for powders or granules when mechanical sieving techniques are incapable of giving a meaningful analysis.

These methods are highly dependent upon proper dispersion of the powder in the air current. This requirement may be hard to achieve if the method is used at the lower end of the sieving range (i.e., below 75 μm), when the particles tend to be more cohesive, and especially if there is any tendency for the material to develop an electrostatic charge. For the above reasons endpoint determination is particularly critical, and it is very important to confirm that the oversize material comprises single particles and is not composed of aggregates.

2.3. Interpretation

The raw data must include the weight of test specimen, the total sieving time, and the precise sieving methodology and the set values for any variable parameters, in addition to the weights retained on the individual sieves and in the pan. It may be convenient to convert the raw data into a cumulative weight distribution, and if it is desired to express the distribution in terms of a cumulative weight undersize, the range of sieves used should include a sieve through which all the material passes. If there is evidence on any of the test sieves that the material remaining on it is composed of aggregates formed during the sieving process, the analysis is invalid.

4. Biological Tests/Biochemical Tests/Microbial Tests

4.01 Bacterial Endotoxins Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia.

Bacterial Endotoxins Test is a test to detect or quantify bacterial endotoxins of gram-negative bacterial origin using a lysate reagent prepared from blood corpuscle extracts of horseshoe crab (Limulus polyphemus or Tachypleus tridentatus). There are two types of techniques for this test: the gel-clot techniques, which are based on gel formation by the reaction of the lysate TS with endotoxins, and the photometric techniques, which are based on endotoxin-induced optical
changes of the lysate TS. The latter include turbidimetric techniques, which are based on the change in lysate TS turbidity during gel formation, and chromogenic techniques, which are based on the development of color after cleavage of a synthetic peptide-chromogen complex.

Once any one of these techniques for the test. In the event of doubt or dispute, the final decision is made based on gel-clot techniques, unless otherwise indicated.

The test is carried out in a manner that avoids endotoxin contamination.

1. Apparatus

Depyrogenate all glassware and other heat-stable materials in a hot-air oven using a validated process. Commonly used minimum time and temperature settings are 30 minutes at 250°C. If employing plastic apparatus, such as multi-well plates and tips for micropipettes, use only that which has been shown to be free of detectable endotoxin and which does not interfere with the test.

2. Preparation of Solutions

2.1. Preparation of Standard Endotoxin Stock Solution

Prepare Standard Endotoxin Stock Solution by dissolving Endotoxin Reference Standard that has been calibrated to the current WHO International Standard for Endotoxin, using water for bacterial endotoxins test (BET). Endotoxin is expressed in Endotoxin Units (EU). One EU is equal to one International Unit (IU) of endotoxin.

2.2. Preparation of Standard Endotoxin Solution

After mixing Standard Endotoxin Stock Solution thoroughly, prepare appropriate serial dilutions of Standard Endotoxin Solution, using water for BET. Use dilutions as soon as possible to avoid loss of activity by adsorption.

2.3. Preparation of Sample Solutions

Unless otherwise specified, prepare sample solutions by dissolving or diluting drugs, using water for BET. If necessary, adjust the pH of the solution to be examined so that the pH of the mixture of the lysate TS and sample solution falls within the specified pH range for the lysate reagent to be used. This usually applies to a sample solution with a pH in the range of 6.0 to 8.0. TSs or solutions used for adjustment of pH may be prepared using water for BET, and then stored in containers free of detectable endotoxin. The TSs or solutions must be validated to be free of detectable endotoxin and interfering factors.

3. Determination of Maximum Valid Dilution

The Maximum Valid Dilution (MVD) is the maximum allowable dilution of a sample solution at which the endotoxin limit can be determined.

Determine the MVD from the following equation:

\[
\text{MVD} = \frac{(\text{Endotoxin limit} \times \text{Concentration of sample solution})}{\lambda}
\]

Endotoxin limit:

The endotoxin limit for injections, defined on the basis of dose, equals \(K/M\), where \(K\) is a threshold pyrogenic dose of endotoxin per kg body mass (EU/kg), and \(M\) is equal to the maximum dose of product per kg body mass. When the product is to be injected at frequent intervals or infused continuously, \(M\) is the maximum total dose administered in a single hour period.

Concentration of sample solution:

mg/mL in the case of endotoxin limit specified by mass (EU/mg)

mEq/mL in the case of endotoxin limit specified by equivalent (EU/mEq)

Units/mL in the case of endotoxin limit specified by biological unit (EU/Unit)

mL/mL in the case of endotoxin limit specified by volume (EU/mL)

\(\lambda\): the labeled lysate reagent sensitivity in the gel-clot techniques (EU/mL) or the lowest point used (EU/mL) in the standard regression curve of the turbidimetric or chromogenic techniques

4. Gel-clot techniques

The gel-clot techniques detect or quantify endotoxins based on clotting of the lysate TS in the presence of endotoxin. To ensure both the precision and validity of the test, perform the tests for confirming the labeled lysate reagent sensitivity (4.1.1.) and for interfering factors (4.1.2.) as described under Preparatory testing (4.1.).

4.1. Preparatory testing

4.1.1. Test for confirmation of labeled lysate reagent sensitivity

The labeled sensitivity of lysate is defined as the lowest concentration of endotoxin that is needed to cause the lysate TS to clot under the conditions specified for the lysate to be used.

The test for confirmation of the labeled lysate sensitivity is to be carried out when each new lot of lysate is used or when there is any change in the experimental conditions which may affect the outcome of the test.

Prepare standard solutions having four concentrations equivalent to \(2\lambda\), \(\lambda\), \(0.5\lambda\) and \(0.25\lambda\) by diluting the Standard Endotoxin Stock Solution with water for BET. Mix a volume of the lysate TS with an equal volume of one of the standard solutions (usually, 0.1 mL aliquots) in each test tube. When single test vials or ampoules containing lyophilized lysate are used, add solutions directly to the vial or ampoule.

Keep the tubes (or containers such as vials or ampoules) containing the reaction mixture usually at 37 ± 1°C for 60 ± 2 minutes, avoiding vibration. To test the integrity of the gel after incubation, invert each tube or container through approximately 180° in one smooth motion. If a firm gel has formed that remains in place upon inversion, record the result as positive. A result is negative if either a firm gel is not formed, or if a fragile gel has formed but flows out on inversion.

Making the standard solutions of four concentrations one set, test four replicates of the set.

The test is valid when 0.25\(\lambda\) of the standard solution shows a negative result in each set of tests. If the test is not valid, repeat the test after verifying the test conditions.

The endpoint is the last positive test in the series of decreasing concentrations of endotoxin. Calculate the geometric mean endpoint concentration of the four replicate series using the following formula:

\[
\text{Geometric Mean Endpoint Concentration} = \text{antilog} \left( \frac{\Sigma e}{f} \right)
\]

\(\Sigma e\) = the sum of the log endpoint concentrations of the dilution series used

\(f\) = the number of replicates

If the geometric mean endpoint concentration is not less
than 0.5 \( \lambda \) and not more than 2.0 \( \lambda \), the labeled sensitivity is confirmed, and is used in tests performed with this lysate.

4.1.2. Test for interfering factors

This test is performed to check for the presence of enhancing or inhibiting factors in the reaction in sample solutions. Prepare the solutions A, B, C and D according to Table 4.01-1, and test solutions A and B and solutions C and D in quadruplicate and in duplicate, respectively. Concerning the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure described in 4.1.1.

The geometric mean endpoint concentrations of B and C solutions are determined by using the formula described in 4.1.1.

This test must be repeated when there is any change in the experimental conditions which may affect the outcome of the test. The test is valid if solutions A and D show no reaction and the result for solution C confirms the labeled sensitivity. If the geometric mean endpoint concentration of solution B is not less than 0.5 \( \lambda \) and not greater than 2.0 \( \lambda \), the sample solution examined does not contain interfering factors and complies with the test for interfering factors. Otherwise the sample solution interferes with the test.

If the sample under test does not comply with the test at a dilution less than the MVD, repeat the test using a greater dilution, not exceeding the MVD. The use of a more sensitive lysate permits a grater dilution of the sample to be examined. Furthermore, interference of the sample solution or diluted sample solution may be eliminated by suitable treatment, such as filtration, neutralization, dialysis or heat treatment. To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which Standard Endotoxin has been added and which has then been submitted to the chosen treatment.

4.2. Limit test

This method tests whether or not a sample contains endotoxins greater than the endotoxin limit specified in the individual monograph based on the gel formation in the presence of endotoxins at a concentration of more than the labeled lysate sensitivity.

4.2.1. Procedure

Prepare solutions A, B, C and D according to Table 4.01-1. This test is performed to check for the presence of enhancing or inhibiting factors in the reaction in sample solutions. Prepare the solutions A, B, C and D according to Table 4.01-1, and test solutions A and B and solutions C and D in quadruplicate and in duplicate, respectively. Concerning the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure described in 4.1.1.

4.2.2. Interpretation

The test is valid when both replicates of solutions B and C are positive and those of solution D are negative. When a negative result is found for both replicates of solution A, the sample complies with the Bacterial Endotoxins Test. When a positive result is found for both replicates of solution A, the sample does not comply with the test. When a positive result is found for one replicate of solution A and a negative result is found for the other, repeat the test. In the repeat test, the sample complies with the test if a negative result is found for both replicates of solution A. The sample does not comply with the test if a positive result is found for one or both replicates of solution A. However, if the sample does not comply with the test at a dilution less than the MVD, the test may be repeated using a grater dilution, not exceeding the MVD.

4.3. Quantitative Test

This method measures endotoxin concentrations of samples by determining an endpoint of gel formation.

4.3.1. Procedure

Prepare solutions A, B, C and D according to Table 4.01-1. This test is performed to check for the presence of enhancing or inhibiting factors in the reaction in sample solutions. Prepare the solutions A, B, C and D according to Table 4.01-1. This test is performed to check for the presence of enhancing or inhibiting factors in the reaction in sample solutions. Prepare the solutions A, B, C and D according to Table 4.01-1, and test solutions A and B and solutions C and D in quadruplicate and in duplicate, respectively. Concerning the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure described in 4.1.1.

4.3.2. Calculation and interpretation

The test is valid when the following three conditions are met: (a) both replicates of the negative control solution D are negative, (b) both replicates of the positive product control solution B are positive and (c) the geometric mean endpoint concentration of solution C is in the range of 0.5 \( \lambda \) to 2 \( \lambda \). The endpoint is defined as the maximum dilution showing a positive result for both replicates of solution A, the sample complies with the Bacterial Endotoxins Test. When a negative result is found for both replicates of solution A, the sample does not comply with the test. When a positive result is found for one replicate of solution A and a negative result is found for the other, repeat the test. In the repeat test, the sample complies with the test if a positive result is found for both replicates of solution A. The sample does not comply with the test if a negative result is found for one or both replicates of solution A. However, if the sample does not comply with the test at a dilution less than the MVD, the test may be repeated using a grater dilution, not exceeding the MVD.

### Table 4.01-1

<table>
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<tr>
<th>Solution</th>
<th>Endotoxin Concentration/Solution to which endotoxin is added</th>
<th>Dilution</th>
<th>Endotoxin concentration</th>
<th>Number of replicates</th>
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<tr>
<td>A*1</td>
<td>0/Sample solution</td>
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<td>—</td>
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</tr>
<tr>
<td>B*2</td>
<td>2%/Sample solution</td>
<td>Sample solution</td>
<td>1 2 4 8</td>
<td>2l 1l 0.5l 0.25l</td>
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<td>C*3</td>
<td>2%/Water for BET</td>
<td>Water for BET</td>
<td>1 2 4 8</td>
<td>2l 1l 0.5l 0.25l</td>
</tr>
<tr>
<td>D*4</td>
<td>0/Water for BET</td>
<td>—</td>
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</tbody>
</table>

*1 Negative control. Sample solution only.
*2 Sample solutions added with standard endotoxin (for testing interfering factors).
*3 Standard endotoxin solutions for confirmation of the labeled lysate reagent sensitivity.
*4 Negative control. Water for BET only.

### Table 4.01-2

<table>
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<th>Solution</th>
<th>Endotoxin concentration/Solution to which endotoxin is added</th>
<th>Number of replicates</th>
</tr>
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<tbody>
<tr>
<td>A*1</td>
<td>0/Sample solution</td>
<td>2</td>
</tr>
<tr>
<td>B*2</td>
<td>2%/Sample solution</td>
<td>2</td>
</tr>
<tr>
<td>C*3</td>
<td>2%/Water for BET</td>
<td>2</td>
</tr>
<tr>
<td>D*4</td>
<td>0/Water for BET</td>
<td>2</td>
</tr>
</tbody>
</table>

*1 Sample solution for the limit test. The solution may be diluted not to exceed the MVD.
*2 Positive control. Sample solution at the same dilution as solution A, containing standard endotoxin at a concentration of 2l.
*3 Positive control. Standard endotoxin solution containing standard endotoxin concentration of 2l.
*4 Negative control. Water for BET only.
The endotoxin concentration of the sample solution. The sample complies with the Bacterial Endotoxins Test if the endotoxin concentration of the sample solution.

The kinetic-turbidimetric technique is based on the quantitative relationship between the concentration of endotoxins and the turbidity of the reaction mixture at a specified reaction time.

The kinetic-turbidimetric technique is based on the quantitative relationship between the concentration of endotoxins and the turbidity of the reaction mixture or the rate of color development.

The test is usually carried out at 37 ± 1°C.

5.3. Preparatory testing

To assure the precision and validity of the turbidimetric or chromogenic techniques, perform both Test for assurance of criteria for the standard curve (5.3.1.) and Test for interfering factors (5.3.2.), as indicated below.

5.3.1. Test for assurance of criteria for the standard curve

The test is to be carried out when each new lot of lysate reagent is used or when there is any change in the experimental conditions which may affect the outcome of the test.

Using the Standard Endotoxin Solution, prepare at least three endotoxin concentrations to generate the standard curve within the range of endotoxin concentrations indicated by the instructions for the lysate reagent used. Perform the test using at least three replicates of each standard endotoxin concentration according to the optimal conditions for the lysate reagent used (with regard to volume ratios, incubation time, temperature, pH, etc.). If the desired range is greater than two logs, additional standards should be included to bracket each log increase in the range of the standard curve.

If the absolute value of the correlation coefficient, |r|, is greater than or equal to 0.980 for the range of endotoxin concentrations set up, the criteria for the standard curve are valid and the curve complies with the test.

If the standard curve does not comply with the test, repeat the test after verifying the test conditions.

5.3.2. Test for interfering factors

Prepare solutions A, B, C and D according to Table 4.01-4. Perform the test on these solutions following the optimal conditions for the lysate reagent used (with regard to volume of sample solution and lysate TS, volume ratio of sample solution to lysate TS, incubation time, etc.).

The test for interfering factors must be repeated when any condition changes, which is likely to influence the result of the test.

The test is valid when the following conditions are met.

1: The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.

2: The result with solution D does not exceed the limit of the blank value required in the description of the lysate employed, or it is less than the endotoxin detection limit of the lysate employed.

Calculate the recovery of the endotoxin added to solution B from the concentration found in solution B after subtracting the endotoxin concentration found in solution A.

When the recovery of the endotoxin added to solution B is within 50% to 200%, the sample solution under test is considered to be free of interfering factors and the solution complies with the test.

When the endotoxin recovery is out of the specified range,
the sample solution under test is considered to contain interfering factors. If the sample under test does not comply with the test, repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the sample solution or diluted sample solution not to exceed the MVD may be eliminated by suitable treatment, such as filtration, neutralization, dialysis or heat treatment. To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which Standard Endotoxin has been added and which has then been submitted to the chosen treatment.

5.4. Quantitative test

5.4.1. Procedure

Prepare solutions A, B, C and D according to Table 4.01-4, and follow the procedure described in 5.3.2.

5.4.2. Calculation of endotoxin concentration

Calculate the mean endotoxin concentration of solution A using the standard curve generated with solution C. The test is valid when all the following requirements are met.

1. The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.
2. The endotoxin recovery, calculated from the concentration found in solution B after subtracting the concentration of endotoxin found in solution A, is within the range of 50% to 200%.
3. The result with solution D does not exceed the limit of the blank value required in the description of the lysate employed, or it is less than the endotoxin detection limit of the lysate employed.

5.4.3. Interpretation

The sample complies with the Bacterial Endotoxins Test if the endotoxin concentration of the sample calculated from the mean endotoxin concentration of solution A meets the requirement of the endotoxin limit (in EU/mL, EU/mg, EU/mEq or EU/Unit) specified in the individual monograph.

4.02 Microbial Assay for Antibiotics

Microbial Assay for Antibiotics is a method to determine the antimicrobial potency of antibiotics based on their antimicrobial activities. There are three methods for this test: the cylinder-plate, perforated plate, and turbidimetric methods. The former two are based on the measurement of the size of the zones of microbial growth inhibition in a nutrient agar medium, and the turbidimetric method is based on the measurement of the inhibition of turbidity development in a fluid medium with microbial growth. Unless otherwise specified in the individual monograph, tests specified to be carried out by the cylinder-plate method may be conducted under the same test conditions using the perforated plate method instead. If necessary, first sterilize water, isotonic sodium chloride solution, buffer solutions, reagents, test solutions and essential parts of measuring instruments and appliances to be used for the test. In performing the test, precautions must be taken to prevent biohazard.

1. Cylinder-plate method

The cylinder-plate method is a method to determine the antimicrobial potency of the antibiotic to be tested, and is based on the measurement of the size of the zone of growth inhibition of a test organism by the use of cylinder-agar plates.

1.1. Test organisms

Use the test organism specified in the individual monograph.

1.2. Culture media

Unless otherwise specified, use media with the following compositions. When ‘peptone’ is indicated as an ingredient of a medium, either meat peptone or casein peptone is applicable. Use sodium hydroxide TS or 1 mol/L hydrochloric acid TS to adjust the pH of the medium to obtain the specified value after sterilization. In the case of the medium for Bacillus subtilis ATCC 6633, adjust the pH using ammonia TS, potassium hydroxide TS or 1 mol/L hydrochloric acid TS. A different medium to the one specified for each test organism may be used if it has both a similar composition and an equal or better growth efficiency of the test organism in comparison with the specified medium. Unless otherwise specified, sterilize the media to be used in an autoclave.

(i) Agar media for seed and base layer

1. Media for test organism Bacillus subtilis ATCC 6633

i. Peptone 5.0 g
Meat extract 3.0 g
Agar 15.0 g
Water 1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

ii. Peptone 5.0 g
Meat extract 3.0 g
Trisodium citrate dihydrate 10.0 g
Agar 15.0 g
Water 1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

2. Medium for test organism Saccharomyces cerevisiae ATCC 9763
Glucose 10.0 g
Peptone 9.4 g
Meat extract 2.4 g
Yeast extract 4.7 g
Sodium chloride 10.0 g
Agar 15.0 g
Water 1000 mL
Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

3) Media for other organisms
i. Glucose 1.0 g
Peptone 6.0 g
Meat extract 1.5 g
Yeast extract 3.0 g
Agar 15.0 g
Water 1000 mL
Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

ii. Glucose 1.0 g
Meat peptone 6.0 g
Casein peptone 4.0 g
Meat extract 1.5 g
Yeast extract 3.0 g
Agar 15.0 g
Water 1000 mL
Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

iii. Peptone 10.0 g
Meat extract 5.0 g
Sodium chloride 2.5 g
Agar 15.0 g
Water 1000 mL
Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

(2) Agar media for transferring test organisms
1) Medium for test organism Saccharomyces cerevisiae
ATCC 9763
Glucose 15.0 g
Peptone 5.0 g
Yeast extract 2.0 g
Magnesium sulfate heptahydrate 0.5 g
Potassium dihydrogen phosphate 1.0 g
Agar 15.0 g
Water 1000 mL
Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

2) Media for other organisms
i. Glucose 1.0 g
Meat peptone 6.0 g
Casein peptone 4.0 g
Meat extract 1.5 g
Yeast extract 3.0 g
Agar 15.0 g
Water 1000 mL
Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

ii. Peptone 10.0 g
Meat extract 5.0 g
Sodium chloride 2.5 g
Agar 15.0 g
Water 1000 mL
Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

1.3. Preparation of agar slant or plate media

Unless otherwise specified, dispense approximately 9 mL of melted agar medium in each test tube (approximately 16 mm in inside diameter), and make them as slant media, or dispense approximately 20 mL of melted agar medium in each Petri dish (approximately 90 mm in inside diameter), and make them as plate media.

1.4. Preparation of stock suspensions of test spores or organisms

Unless otherwise specified, prepare stock suspensions of test spore or organism cultures as follows. Check the aspects of the test spores or organisms as occasion demands.

(i) Preparation of a stock spore suspension of test organism Bacillus subtilis ATCC 6633

Inoculate the test organism onto the slant or plate of the agar medium which was prepared for transferring the test organisms specified in 2 (2) i. Incubate at 32 to 37°C for 16 to 24 hours. Inoculate the subcultured test organism onto a suitable volume of slant or plate of the agar medium (described above), which was prepared for transferring the test organisms specified in 2 (2) ii. Then incubate at 32 to 37°C for not less than 1 week to prepare spores. Suspend the spores in isotonic sodium chloride solution, heat at 65°C for 30 minutes, and then centrifuge. Wash the spore sediment three times with isotonic sodium chloride solution by means of centrifugation. Re-suspend the spore sediment in water or isotonic sodium chloride solution, and heat again at 65°C for 30 minutes to prepare the stock spore suspension. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock spore suspension at a temperature not exceeding 5°C, and use within 6 months. If the stock spore suspension shows a clear and definite zone of growth inhibition in an antibiotics potency test using adequate antibiotics, it may be used for further 6 months.

(ii) Preparation of a stock suspension of the test organism Saccharomyces cerevisiae ATCC 9763

Inoculate test organism onto the slant or plate agar medium which has been prepared for transferring test organism specified in 2 (2) 1. Incubate at 25 to 26°C for 40 to 48 hours. The subculture should be performed at least three times. Inoculate the subcultured test organism onto another slant or plate of the agar medium (described above), and incubate at 25 to 26°C for 40 to 48 hours. Scrape away and suspend the resulting growth from the agar surface in isotonic sodium chloride solution, and use this as a stock suspension of the test organism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock suspensions of the test organisms at a temperature not exceeding 5°C, and use within 30 days.

(iii) Preparation of a stock suspension of other test organisms

Inoculate the test organism onto the slant or the plate of the agar medium which has been prepared for transferring the test organisms specified in 2 (2) 1. Incubate the inoculated slant at 32 to 37°C for 16 to 24 hours. The subculture should be performed at least three times. Inoculate the subcultured test organism onto another slant or plate agar medium (described above), and incubate the slant at 32 to 37°C for 16 to 24 hours. Scrape away and suspend the resulting growth from the agar surface in isotonic sodium chloride solution, and use this as a stock suspension of the test organism.
ism. The concentration of the test organism is confirmed with turbidity or absorbance, as occasion demands. Store the stock suspensions of the test organisms at a temperature not exceeding 5°C, and use within 5 days.

1.5. Preparation of agar base layer plates

Unless otherwise specified, dispense 20 mL of the melted agar medium for the base layer into each Petri dish, and in the case of a large dish, dispense a quantity of the agar medium to form a uniform layer 2 to 3 mm thick. Distribute the agar evenly in each dish on a flat, level surface, and allow it to harden.

1.6. Preparation of seeded agar layers

Unless otherwise specified, determine the volume of the stock suspension of the spore or the test organism with which the employed standard solution shows a clear and definite zone of growth inhibition. Prepare the seeded agar layer by mixing thoroughly the previously determined volume of stock suspension of spore or test organism with agar medium for the seed layer kept at 48 to 51°C. Usually, the rate of a stock spore suspension and a stock suspension of the test organisms at a temperature not exceeding 5°C, respectively.

1.7. Preparation of cylinder-agar plates

Dispense 4 to 6 mL of the seeded agar layer, which is specified in the individual monograph, on an agar base layer plate in a Petri dish. In the case of large dishes, dispense a quantity of the agar medium to form a uniform layer 1.5 to 2.5 mm thick, and spread evenly over the surface before hardening. After coagulating the agar, allow the plate to stand under a clean atmosphere to exhale moisture vapor of the inside of Petri or large dishes and water on the agar surface. Place 4 cylinders on an agar plate in a Petri dish so that the individual cylinders are equidistant from the center of the plate and equally spaced from one another (the cylinders are set on the circumference of a circle of 25 to 28 mm radius). When large dish plates are used, place cylinders on each plate according to the method of preparation for Petri dish agar plates. A set of 4 cylinders on each large dish plate is considered to be equivalent to one Petri dish plate. Use stainless steel cylinders with the following dimensions: outside diameter 7.9 to 8.1 mm; inside diameter 5.9 to 6.1 mm; length 9.9 to 10.1 mm. The cylinders should not interfere with the test. Prepare the cylinder-agar plates before use.

1.8. Standard solutions

Use both a standard solution of high concentration and one of low concentration, as specified in the individual monograph. Unless otherwise specified, prepare the standard solutions before use.

1.9. Sample solutions

Use both a sample solution of high concentration and one of low concentration, as specified in the individual monograph. Unless otherwise specified, prepare the sample solutions before use.

1.10. Procedure

Unless otherwise specified, use 5 cylinder-agar plates as one assay set when Petri dishes are employed. When large dishes are employed, the number of cylinders for one assay set should be equal to that defined when using Petri dishes. Apply the standard solution of high concentration and that of low concentration to a pair of cylinders set opposite each other on each plate. Apply the high and low concentration sample solutions to the remaining 2 cylinders. The same volume of these solutions must be added to each cylinder.

Incubate the plates at 32 to 37°C for 16 to 20 hours. Using a suitable measuring tool, measure the diameters of circular inhibition zones with a precision that can discriminate differences of at least 0.25 mm. Each procedure should be performed quickly under clean laboratory conditions.

1.11. Estimation of potency

The following correlation between the potency (P) of solution in a cylinder and the diameter (d) of zone of inhibition is established.

\[ d = \alpha \log P + \beta \]

where, \( \alpha \) and \( \beta \) are constants.

If necessary, ascertain the values in the above equation. Based on this equation, estimate the potency of the sample solutions by application of the following equation:

\[ \text{Amount (potency) of sample} = A \times \text{Potency of } S_H \text{ per mL} \times \text{Dilution factor of } U_H \]

where:

\[ \text{log} A = \frac{IV}{W} \]

\[ I = \log (\text{potency of } S_H/\text{potency of } S_L) \]

\[ V = 2(U_H + U_U - U_H - U_L) \]

\[ W = 2(U_H + 2U_U - 2U_L - U_L) \]

The sum of the diameter (mm) of the inhibitory zone measured in each plate is designated as follows:

- for standard solution of high concentration \((S_{SH}) = 2S_H\)
- for standard solution of low concentration \((S_{SL}) = 2S_L\)
- for sample solution of high concentration \((U_{SH}) = 2U_H\)
- for sample solution of low concentration \((U_{SL}) = 2U_L\)

2. Perforated plate method

The perforated plate method is a method to determine the antimicrobial potency of an antibiotic, based on the measurement of the size of the zone of growth inhibition of a test organism by the use of perforated agar plates.

This method is carried out by the use of perforated agar plates in lieu of cylinder-agar plates used in Cylinder-plate method.

Proceed as directed below, but comply with the requirements of Cylinder-plate method, such as test organisms, media, preparation of agar slant or plate media, preparation of stock suspensions of spores or test organisms, preparation of agar base layer plates, preparation of seeded agar layers, standard solutions, sample solutions, and estimation of potency.

2.1. Preparation of perforated agar plates

Dispense 4 to 6 mL of the seeded agar layer specified in the individual monograph on each agar base layer plate of the Petri dish. In the case of large dishes, dispense a quantity of the agar medium to form a uniform layer 1.5 to 2.5 mm thick, and spread evenly over the surface before hardening. After coagulating the agar, allow the plate to stand under a clean atmosphere to exhale moisture vapor of the inside of Petri or large dishes and water on the agar surface. Using a suitable tool, prepare 4 circular cavities having a diameter of 7.9 to 8.1 mm on a Petri dish agar plate so that the individual cylinders are equidistant from the center of the plate and equally spaced from one another on the circumference of a circle with radius 25 to 28 mm, and are deep enough to reach the bottom of dish. When large dish plates are used, prepare the circular cavities on each plate accord-
ing to the method of preparation for Petri dish agar plates. A set of 4 cavities on each large dish plate is considered to be equivalent to one Petri dish plate. Prepare the perforated agar plates before use.

2.2. Procedure

Unless otherwise specified, use 5 perforated agar plates as one assay set when Petri dishes are employed. When large dishes are employed, the number of cavities for one assay set should be equal to that defined when using Petri dishes. Apply the high and low concentration standard solutions to a pair of cavities prepared opposite each other on each plate, and apply the high and low concentration sample solutions to the remaining 2 cavities. The same volume of these solutions must be added to each cavity. Incubate the plates at 32 to 37°C for 16 to 20 hours. Using a suitable measuring tool, measure the diameters of the circular inhibition zones with a precision that can discriminate differences of at least 0.25 mm. Each procedure should be performed quickly under clean laboratory conditions.

3. Turbidimetric method

The turbidimetric method is a method to determine the antimicrobial potency of an antibiotic, based on the measurement of the inhibition of growth of a microbial culture in a fluid medium. The inhibition of growth of a test organism is photometrically measured as changes in turbidity of the microbial culture.

3.1. Test organisms

Use the test organism specified in the individual monograph.

3.2. Culture media

Unless otherwise specified, use media with the following compositions. When peptone is indicated as an ingredient of a medium, either meat peptone or casein peptone is applicable. Use sodium hydroxide TS or 1 mol/L hydrochloric acid TS to adjust the pH of the medium to obtain the specified value after sterilization. A different medium to the one specified for each test organism may be used if it has both a similar composition and an equal or better growth efficiency of the test organism in comparison with the specified medium. Unless otherwise specified, sterilize the media to be used in an autoclave.

(1) Agar media for transferring test organisms

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>6.0 g</td>
</tr>
<tr>
<td>Meat extract</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Water</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

(2) Liquid media for suspending test organisms

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Meat extract</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.5 g</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.32 g</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate*</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Water</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.0 to 7.1 after sterilization.

* Dipotassium hydrogen phosphate (3.68 g) may be used in lieu of disodium hydrogen phosphate (3.0 g).

3.3. Preparation of agar slant or plate media

Unless otherwise specified, proceed as directed in Preparation of agar slant or plate media under Cylinder-plate method.

3.4. Preparation of stock suspensions of test organisms

Unless otherwise specified, inoculate the test organism onto the slant or plate of the agar medium which was prepared for transferring the specified test organism. Incubate the inoculated medium at 32 to 37°C for 16 to 24 hours. The subculture should be performed at least three times. Check the aspects of the test spores or organisms as occasion demands. Inoculate the subcultured test organism onto another slant or plate of the agar medium (described above), and incubate the slant at 32 to 37°C for 16 to 24 hours. After incubation, suspend the test organism in the liquid medium for suspending the test organism, and use as the suspension of the test organism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands.

3.5. Standard solutions

Use the standard solutions specified in the individual monograph. Unless otherwise specified, prepare the standard solutions before use.

3.6. Sample solutions

Use the sample solutions specified in the individual monograph. Unless otherwise specified, prepare the sample solutions before use.

3.7. Procedure

Unless otherwise specified, proceed as follows:

Distribute 1.0 mL of each concentration of the standard solution, the sample solution, and water used as a control, into each set composed of 3 test tubes (about 14 mm in inside diameter and about 13 cm in length). Add 9.0 mL of the suspension of the test organism to each tube, and then incubate in a water bath maintained at 35 to 37°C for 3 to 4 hours. After incubation, add 0.5 mL of dilute formaldehyde (1 in 3) to each tube, and read each transmittance or absorbance at a wavelength of 530 nm.

3.8. Estimation of potency

Average the transmittance or absorbance values of each concentration of the standard solution, the sample solution and water used as a control, respectively. Generate the standard curve based on the average values of transmittance or absorbance of each concentration of the standard solution, and estimate the potency of the sample solution from its average value of transmittance or absorbance using the obtained standard curve.

If the standard dilutions of five concentrations in geometric progression are used, calculate the L and H values from the following equations. Plot point L and point H on graph paper and construct a straight line for the standard curve.

\[
L = \frac{3a + 2b + c - e}{5}
\]

\[
H = \frac{3e + 2d + c - a}{5}
\]

where:

L = calculated value of transmittance or absorbance for the lowest concentration of the standard curve.

H = calculated value of transmittance or absorbance for
4.03 Digestion Test

Digestion Test is a test to measure the activity of digestive enzymes, as crude materials or preparations, on starch, protein and fat.

1. Assay for Starch Digestive Activity

The assay for starch digestive activity is performed through the measurement of starch saccharifying activity, dextrinizing activity, and liquefying activity.

1.1. Measurement of starch saccharifying activity

The starch saccharifying activity can be obtained by measuring an increase of reducing activity owing to the hydrolysis of the glucoside linkages when amylase acts on the starch. Under the conditions described in Procedure, one starch saccharifying activity unit is the amount of enzyme that catalyzes the increase in reducing activity equivalent to 1 mg of glucose per minute.

1.1.1. Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the reducing activity increases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.4 to 0.8 starch saccharifying activity unit/mL. Filter if necessary.

1.1.2. Preparation of Substrate Solution

Use potato starch TS for measuring the starch digestive activity. If necessary, add 10 mL of buffer or salts solution specified in the monograph, instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0.

1.1.3. Procedure

Pipet 10 mL of the substrate solution, stand at 37 ± 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Allow this solution to stand at 37 ± 0.5°C for exactly 10 minutes, add exactly 2 mL of alkaline trtarate solution of the Fehling’s TS for amylolytic activity test, shake gently, heat the solution in a water bath for exactly 15 minutes, and then immediately cool to below 25°C. Then, add exactly 2 mL of concentrated potassium iodide TS and 2 mL of diluted sulfuric acid (1 in 6), and titrate the released iodine with 0.05 mol/L sodium thiosulfate VS to the disappearance of the blue color produced by addition of 1 to 2 drops of soluble starch TS (a mL). Separately, pipet 10 mL of water instead of the substrate solution and titrate the starch saccharifying activity (unit/g)

\[
\text{Starch saccharifying activity} = \frac{1}{10} \times \frac{1}{M} \times (b - a)
\]

Amount (mg) of glucose = (b - a) × 1.6

\[M: \text{Amount (g) of sample in 1 mL of sample solution}\]

1.2. Measurement of starch dextrinizing activity

The starch dextrinizing activity can be obtained by measuring a decrease in starch coloration by iodine resulting from hydrolysis of the straight chain component (amylose) in starch when amylase acts on the starch. Under the conditions described in Procedure, one starch dextrinizing activity unit is the amount of enzyme required to reduce the coloration of potato starch by iodine by 10% per minute.

1.2.1. Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water or a buffer or salts solution specified in the monograph so that the coloration of starch by iodine decreases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.2 to 0.5 starch dextrinizing activity unit/mL. Filter if necessary.

1.2.2. Preparation of Substrate Solution

Prepare the substrate solution in the same manner as the substrate solution in the measurement of starch saccharifying activity.

1.2.3. Procedure

Pipet 10 mL of the substrate solution, stand at 37 ± 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Allow this solution to stand at 37 ± 0.5°C for exactly 10 minutes. Pipet 1 mL of this solution, add it to 10 mL of 0.1 mol/L hydrochloric acid TS, and shake immediately. Pipet 0.5 mL of this solution, add exactly 10 mL of 0.0002 mol/L iodine TS, and shake. Determine the absorbance \(A_r\) of this solution at the wavelength of 660 nm as directed under Ultraviolet-visible Spectrophotometry (2.24). Separately, using 1 mL of water instead of the sample solution, determine the absorbance \(A_b\) in the same manner.

\[
\text{Starch dextrinizing activity} = \frac{(A_b - A_r)}{A_b} \times \frac{1}{M}
\]

\[M: \text{Amount (g) of sample in 1 mL of sample solution}\]

1.3. Measurement of starch liquefying activity

The starch liquefying activity can be obtained by measuring a decrease in the viscosity of starch solution resulting from the hydrolysis of molecules when amylase acts on the starch. Under the conditions described in Procedure, one starch liquefying activity unit is the amount of enzyme required to reduce the viscosity of the substrate solution equivalent to 1 g of potato starch from 200% to 100% of that of the 50% sucrose standard solution.

1.3.1. Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the viscosity decreases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.15 to 0.25 starch liquefying activity unit/mL. Filter if necessary.
1.3.2. Preparation of Substrate Solution

Weigh accurately about 1 g of potato starch, and measure the loss of drying at 105°C for 2 hours. Weigh exactly potato starch equivalent to 15.00 g calculated on the dried basis, add 300 mL of water, then add gradually 25 mL of 2 mol/L sodium hydroxide TS under thorough shaking, until the mixture forms a paste. Heat the mixture in a water bath for 10 minutes, shaking it occasionally. After cooling, neutralize the mixture with 2 mol/L hydrochloric acid TS, and add 50 mL of the buffer solution specified in the monograph and water to make exactly 500 g. Prepare before use.

1.3.3. Preparation of 50% Standard Sucrose Solution

Dissolve 50.0 g of sucrose in 50.0 mL of water.

1.3.4. Procedure

Put 50 mL of the 50% standard sucrose solution in a 100-mL conical flask, and allow it to stand in a thermostat at 37 ± 0.5°C for 15 minutes. Fix a viscometer shown in Fig. 4.03-1 so that its lower end almost touches the bottom of the flask and that the water in the thermostat circulates around the outer cylinder of the viscometer. After slowly pulling up the 50% standard sucrose solution by suction to the middle of the upper bulb of the viscometer, let it flow down by gravity, measuring the time taken for the solution to fall from the upper to the lower indicators (t1; seconds). Take exactly 50 g of the substrate solution in another 100-mL conical flask, and stand it in another thermostat at 37 ± 0.5°C for 20 minutes. Add exactly 1 mL of the sample solution to it, and shake the flask immediately. Fix a viscometer vertically so that its lower end almost touches the bottom of the flask and that the water in the thermostat circulates around the outer cylinder of the viscometer. Occasionally pull the reaction solution up by suction to the middle of the upper bulb slowly, then let it flow down by gravity, measuring the time taken for the solution to fall from the upper to the lower indicators (t seconds).

Repeat this operation until t becomes shorter than t1. At each measurement, record the time (T′) from the moment that the sample solution is added to the moment that the solution surface in the flask passes the upper indicator. (T′ + t/2) is the reaction time (T) corresponding to t. Draw a curve for both t and T. Obtain T1 and T2 that correspond to t1 and (2 × t1) by interpolation.

Starch liquefying activity (unit/g) = \[ \frac{60}{(T1 - T2)} \times \frac{1.5}{M} \]

M: Amount (g) of sample in 1 mL of sample solution

2. Assay for Protein Digestive Activity

The protein digestive activity can be obtained by the colorimetric measurement, making use of Folin’s reaction, of the amount of acid-soluble low-molecular products, which is increased owing to the hydrolysis of the peptide linkages when protease acts on casein. One protein digestive activity unit is the amount of enzymes that produces Folin’s TS-colorable substance equivalent to 1 μg of tyrosine per minute under the conditions described in Procedure.

2.1. Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the amount of non-protein, Folin’s TS-colorable substances increase in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 15 to 30 protein digestive activity unit/mL.

2.2. Tyrosine Calibration Curve

Weigh exactly 50 mg of Tyrosine Reference Standard, previously dried at 105°C for 3 hours, and dissolve in 0.2 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 1 mL, 2 mL, 3 mL and 4 mL of this solution separately, and add 0.2 mol/L hydrochloric acid TS to each solution to make them exactly 100 mL. Pipet 2 mL of each solution, and add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin’s TS (1 in 3) to each solution, shake immediately, then stand them at 37 ± 0.5°C for 30 minutes. Determine the absorbances, A1, A2, A3, and A4, of these solutions at 660 nm as directed under Ultraviolet-visible Spectrophotometry <2.2A>, using a solution prepared with exactly 2 mL of 0.2 mol/L hydrochloric acid TS in the same manner as the blank. Then, draw a calibration curve with the absorbances, A1, A2, A3, and A4 as the ordinate, and with the amount (μg) of tyrosine in 2 mL of each solution as the abscissa. Obtain the amount (μg) of tyrosine for the absorbance 1.

2.3. Preparation of Substrate Solution

(i) Substrate solution 1: Weigh accurately about 1 g of milk casein, and measure the loss on drying at 105°C for 2 hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 12 mL of lactic acid TS and 150 mL of water, and warm to dissolve in a water bath. After cooling in running water, adjust to the pH specified in the monograph with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200 mL. Prepare before use.

(ii) Substrate solution 2: Weigh accurately about 1 g of milk casein, and measure the loss on drying at 105°C for 2
hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 160 mL of 0.05 mol/L disodium hydrogenphosphate TS, and warm to dissolve in a water bath. After cooling in running water, adjust to the pH specified in the monograph with the 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200 mL. Prepare before use.

2.4. Preparation of Precipitation Reagent
(i) Trichloroacetic acid TS A: Dissolve 7.20 g of trichloroacetic acid in water to make 100 mL.
(ii) Trichloroacetic acid TS B: Dissolve 1.80 g of trichloroacetic acid, 1.80 g of anhydrous sodium acetate and 5.5 mL of 6 mol/L acetic acid TS in water to make 100 mL.

2.5. Procedure
Pipet 5 mL of the substrate solution specified in the monograph, stand at 37 ± 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. After standing this solution at 37 ± 0.5°C for exactly 10 minutes, add exactly 5 mL of trichloroacetic acid TS A or B as specified in the monograph, shake, stand it at 37 ± 0.5°C for 30 minutes, and then filter. Discard the first 3 mL of the filtrate, exactly measure the subsequent 2 mL of the filtrate, and determine the absorbance \( A_T \) of this solution at 660 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS A or B to the solution as specified in the monograph, and shake. To this solution add exactly 5 mL of the substrate solution specified in the monograph, shake immediately, and stand it at 37 ± 0.5°C for 30 minutes. Follow the same procedure for the sample solution, and determine the absorbance \( A_B \) at 660 nm.

**Protein digestive activity (unit/g)**

\[
\text{Protein digestive activity (unit/g)} = \frac{(A_T - A_B) \times F \times 11/2 \times \frac{1}{10} \times \frac{1}{M}}{M}
\]

**M:** Amount (g) of sample in 1 mL of sample solution
**F:** Amount (\( \mu g \)) of tyrosine for absorbance 1 determined from Tyrosine Calibration Curve

3. Assay for Fat Digestive Activity

The fat digestive activity can be obtained by back titration of the amount of fatty acid produced from the hydrolysis of the ester linkage, when lipase acts on olive oil. One fat digestive activity unit is the amount of enzymes that produces 1 \( \mu \)mole of fatty acid per minute under the conditions described in Procedure.

3.1. Preparation of Sample Solution

Dissolve or suspend the sample in an appropriate amount of cold water, or a buffer or salts solution specified in the monograph so that the amount of fatty acid increases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 1 to 5 fat digestive activity unit/mL.

3.2. Preparation of Substrate Solution

Take 200 to 300 mL of a mixture of emulsifier and olive oil (3:1) in a blender (see Fig. 4.03-2), and emulsify it at 12,000 to 16,000 revolutions per minute for 10 minutes, while cooling the solution to a temperature below 10°C.

Stand this solution in a cool place for 1 hour, and make sure before use that the oil does not separate.

3.3. Preparation of Emulsifier

Dissolve 20 g of polyvinyl alcohol specified in the monograph in 800 mL of water by heating between 75°C and 80°C for 1 hour while stirring. After cooling, filter the solution if necessary, and add water to make exactly 1000 mL.

3.4. Procedure

Pipet 5 mL of the substrate solution and 4 mL of the buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing the mixture at 37 ± 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Stand this solution at 37 ± 0.5°C for exactly 20 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Then add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Titrate \( <2.30>\) the excess sodium hydroxide with 0.05 mol/L hydrochloric acid VS (b mL) (indicator: 2 to 3 drops of phenolphthalein TS). Separately, pipet 5 mL of the substrate solution and 4 mL of buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing it at 37 ± 0.5°C for 10 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), then add exactly 1 mL of the sample solution, and shake. Add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, and titrate \( <2.30>\) in the same manner (\( b \) mL).

**Fat digestive activity (unit/g)**

\[
\text{Fat digestive activity (unit/g)} = \frac{50 \times (a - b) \times \frac{1}{20} \times \frac{1}{M}}{M}
\]

**M:** Amount (g) of sample in 1 mL of sample solution
4.04 Pyrogen Test

Pyrogen Test is a method to test the existence of pyrogens by using rabbits.

1. Test animals

Use healthy mature rabbits, each weighing not less than 1.5 kg, which have not lost body mass when kept on a constant diet for not less than one week. House the rabbits individually in an area free from disturbances likely to excite them. Keep the temperature of the area constant between 20°C and 27°C for at least 48 hours before and throughout the test. Before using a rabbit that has not previously been used for a pyrogen test, condition it 1 to 3 days prior to the test by conducting a sham test omitting the injection. Do not use a rabbit for pyrogen tests more frequently than once every 48 hours, or after it has been given a test sample that was adjudged pyrogen-positive or that contained an antigen present commonly in the test sample to be examined.

2. Apparatus, instruments

(i) Thermometer—Use a rectal thermometer or temperature-measuring apparatus with an accuracy of ±0.1°C or less.

(ii) Syringe and injection needle—Depyrogenate the syringes and needles in a hot-air oven using a validated process, usually by heating at 250°C for not less than 30 minutes. Sterilized syringes with needles are also available provided that they have been validated to assure that they are free of detectable pyrogens and do not interfere with the test.

3. Test procedures

3.1. Quantity of injection

Unless otherwise specified, inject 10 mL of the sample per kg of body mass of each rabbit.

3.2. Procedure

Perform the test in a separate area at an environmental temperature similar to that of the room wherein the animals were housed and free from disturbances likely to excite them. Withhold food from the rabbits for several hours before the first record of the temperature and throughout the testing period. The test animals are usually restrained with loosely fitting neck stocks that allow the rabbits to assume a natural resting posture. Determine the temperature of each rabbit by inserting the thermometer or temperature-measuring probe into the rectum of the test animal to a constant depth within the range of 60 mm to 90 mm. The “control temperature” of each rabbit is the mean of two temperature readings recorded for that rabbit at an interval of 30 min in the 40 min immediately preceding the injection of the sample to be examined. Rabbits showing a temperature variation greater than 0.2°C between the two successive temperature readings or rabbits having an initial temperature higher than 39.8°C are withdrawn from the test.

Warm the test solution to a temperature of 37 ± 2°C before injection, and inject the solution slowly into the marginal vein of the ear of each rabbit over a period not exceeding 10 min. Hypotonic test sample may be made isotonic by the addition of pyrogen-free sodium chloride. Record the temperature of each rabbit during a period of 3 hours after the injection, taking the measurements at intervals of not more than 30 min. The difference between the control temperature and the maximum temperature of each rabbit is taken to be the rise in body temperature. Consider any temperature decreases as zero rise.

4. Interpretation of results

The test is carried out on a group of three rabbits and the result is judged on the basis of the sum of the three temperature rises. Repeat if necessary on further groups of three rabbits to a total of three groups, depending on the results obtained. If the summed response of the first group does not exceed 1.3°C, the sample is judged to be pyrogen-negative. If the summed response exceeds 2.5°C, the sample is judged to be pyrogen-positive. If the summed response exceeds 1.3°C but does not exceed 2.5°C, repeat the test on another group of three rabbits. If the summed response of the first and second group does not exceed 3.0°C, the sample is judged to be pyrogen-negative. If the summed response of the 6 rabbits exceeds 4.2°C, the sample is judged to be pyrogen-positive. If the summed response exceeds 3.0°C but does not exceed 4.2°C, repeat the test on one more group of three rabbits. If the summed response of the 9 rabbits does not exceed 5.0°C, the sample is judged to be pyrogen-negative. If the summed response exceeds 5.0°C, the sample is judged to be pyrogen-positive.

When the test sample is judged to be pyrogen-negative, the sample passes the pyrogen test.

4.05 Microbial Limit Test

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

This chapter includes microbial enumeration tests and tests for specified micro-organisms. For the test, use a mixture of several portions selected at random from the bulk or from the contents of a sufficient number of containers. If test specimens are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard.

1. Microbiological Examination of Non-sterile Products: Total viable aerobic count

These tests are harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The tests described hereafter will allow quantitative enumeration of mesophilic bacteria and fungi which may grow under aerobic conditions.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results as stated below.

The methods are not applicable to products containing viable micro-organisms as active ingredients.

Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

1. General Procedures

Carry out the determination under conditions designed to avoid extrinsic microbial contamination of the product to be examined. The precautions taken to avoid contamination
must be such that they do not affect any micro-organisms which are to be revealed in the test. If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized. If inactivators are used for this purpose their efficacy and their absence of toxicity for micro-organisms must be demonstrated.

2. Enumeration Methods

Use the membrane filtration method, or the plate-count methods, as prescribed. The most probable number (MPN) method is generally the least accurate method for microbial counts, however, for certain product groups with very low bioburden, it may be the most appropriate method.

The choice of a method is based on factors such as the nature of the product and the required limit of micro-organisms. The method chosen must allow testing of a sufficient sample size to judge compliance with the specification. The suitability of the chosen method must be established.

3. Growth Promotion Test, Suitability of the Counting Method and Negative Controls

The ability of the test to detect micro-organisms in the presence of product to be tested must be established.

Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

3.1. Preparation of test strains

Use standardised stable suspensions of test strains or prepare as stated below.

Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial and fungal test strains separately as described in Table 4.05-I-1.

Use Buffered Sodium Chloride-Peptone Solution (pH 7.0), or Phosphate Buffer (pH 7.2) to make test suspensions; to suspend *Aspergillus brasiliensis* spores, 0.05 per cent of polysorbate 80 may be added to the buffer. Use the suspensions within 2 h or within 24 h if stored at 2 – 8°C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Aspergillus brasiliensis* or *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 – 8°C for a validated period of time.

3.2. Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described under 4. Testing of Products. A failed negative control requires an investigation.

3.3. Growth promotion of the media

Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described.

Inoculate portions/plates of Fluid Soybean-Casein Digest Medium and Soybean-Casein Digest Agar Medium with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 4.05-I-1, using a separate portion/plate of medium for each. Inoculate plates of Sabouraud Glucose Agar Medium with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 4.05-I-1, using a separate plate of medium for each. Incubate in the conditions described in Table 4.05-I-1.

For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Liquid media are suitable if clearly visible growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

3.4. Suitability of the counting method in the presence of product

3.4.1. Preparation of the sample

The method for sample preparation depends on the physical characteristics of the product to be tested. If none of the procedures described below can be demonstrated to be satisfactory, an alternative procedure must be developed.

(i) Water-soluble products: Dissolve or dilute (usually a 1 in 10 dilution is prepared) the product to be examined in Buffered Sodium Chloride-Peptone Solution (pH 7.0), Phosphate Buffer (pH 7.2) or Fluid Soybean-Casein Digest Medium. If necessary adjust to pH 6 – 8. Further dilutions, where necessary, are prepared with the same diluent.

(ii) Non-fatty products insoluble in water: Suspend the product to be examined (usually a 1 in 10 dilution is prepared) in Buffered Sodium Chloride-Peptone Solution (pH 7.0), Phosphate Buffer (pH 7.2) or Fluid Soybean-Casein Digest Medium. A surface-active agent such as 1 g/L of polysorbate 80 may be added to assist the suspension of poorly wettable substances. If necessary adjust to pH 6 – 8. Further dilutions, where necessary, are prepared with the same diluent.

(iii) Fatty products: Dissolve in isopropyl myristate, sterilised by filtration or mix the product to be examined with the minimum necessary quantity of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent, heated if necessary to not more than 40°C, or in exceptional cases to not more than 45°C. Mix carefully and if necessary maintain the temperature in a water-bath. Add sufficient of the pre-warmed chosen diluent to make a 1 in 10 dilution of the original product. Mix carefully whilst maintaining the temperature for the shortest time necessary for the formation of an emulsion. Further serial tenfold dilutions may be prepared using the chosen diluent containing a suitable concentration of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent.

(iv) Fluids or solids in aerosol form: Aseptically transfer the product into a membrane filter apparatus or a sterile container for further sampling. Use either the total contents or a defined number of metered doses from each of the containers tested.

(v) Transdermal patches: Remove the protective cover sheets ("release liner") of the transdermal patches and place them, adhesive side upwards, on sterile glass or plastic trays. Cover the adhesive surface with sterile porous material, for example sterile gauze, to prevent the patches from sticking together, and transfer the patches to a suitable volume of the chosen diluent containing inactivators such as polysorbate 80 and/or lecithin. Shake the preparation vigorously for at least
Table 4.05-I-1 Preparation and use of test micro-organisms

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Preparation of test strain</th>
<th>Growth promotion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total aerobic microbial count</td>
<td>Total yeasts and moulds count</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h</td>
<td>Soybean-Casein Digest Agar Medium and Fluid Soybean-Casein Digest Medium 3 ≤ 100 CFU 30 – 35°C 3 ≤ 3 days</td>
</tr>
<tr>
<td>such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276</td>
<td>Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h</td>
<td>Soybean-Casein Digest Agar Medium and Fluid Soybean-Casein Digest Medium 3 ≤ 100 CFU 30 – 35°C 3 ≤ 3 days</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h</td>
<td>Soybean-Casein Digest Agar Medium and Fluid Soybean-Casein Digest Medium 3 ≤ 100 CFU 30 – 35°C 3 ≤ 3 days</td>
</tr>
<tr>
<td>such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275</td>
<td>Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h</td>
<td>Soybean-Casein Digest Agar Medium and Fluid Soybean-Casein Digest Medium 3 ≤ 100 CFU 30 – 35°C 3 ≤ 3 days</td>
</tr>
<tr>
<td><strong>Bacillus subtilis</strong></td>
<td>Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h</td>
<td>Soybean-Casein Digest Agar Medium and Fluid Soybean-Casein Digest Medium 3 ≤ 100 CFU 30 – 35°C 3 ≤ 3 days</td>
</tr>
<tr>
<td>such as ATCC 6633, NCIMB 8054, CIP 52.62 or NBRC 3134</td>
<td>Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h</td>
<td>Soybean-Casein Digest Agar Medium and Fluid Soybean-Casein Digest Medium 3 ≤ 100 CFU 30 – 35°C 3 ≤ 3 days</td>
</tr>
<tr>
<td><strong>Candida albicans</strong></td>
<td>Sabouraud Glucose Agar Medium or Fluid Sabouraud Glucose Medium 20 – 25°C 2 – 3 days</td>
<td>Sabouraud Glucose Agar Medium 3 ≤ 100 CFU 20 – 25°C 5 ≤ 5 days</td>
</tr>
<tr>
<td>such as ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594</td>
<td>Sabouraud Glucose Agar Medium or Fluid Sabouraud Glucose Medium 20 – 25°C 2 – 3 days</td>
<td>Sabouraud Glucose Agar Medium 3 ≤ 100 CFU 20 – 25°C 5 ≤ 5 days</td>
</tr>
<tr>
<td><strong>Aspergillus brasilensis</strong></td>
<td>Sabouraud Glucose Agar Medium or Potato Dextrose Agar Medium 20 – 25°C 5 – 7 days, or until good sporulation is achieved</td>
<td>Sabouraud Glucose Agar Medium 3 ≤ 100 CFU 20 – 25°C 5 ≤ 5 days</td>
</tr>
<tr>
<td>such as ATCC 16404, IMI 149007, IP 1431.83 or NBRC 9455</td>
<td>Sabouraud Glucose Agar Medium or Potato Dextrose Agar Medium 20 – 25°C 5 – 7 days, or until good sporulation is achieved</td>
<td>Sabouraud Glucose Agar Medium 3 ≤ 100 CFU 20 – 25°C 5 ≤ 5 days</td>
</tr>
</tbody>
</table>

30 min.

3.4.2. Inoculation and dilution

Add to the sample prepared as described above (3.4.1.) and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than 100 CFU. The volume of the suspension of the inoculum should not exceed 1 per cent of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution factor of the prepared sample must be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols must be developed.

If inhibition of growth by the sample cannot otherwise be avoided, the aliquot of the microbial suspension may be added after neutralization, dilution or filtration.

3.4.3. Neutralization/removal of antimicrobial activity

The number of micro-organisms recovered from the prepared sample diluted as described in 3.4.2. and incubated following the procedure described in 3.4.4., is compared to
the number of micro-organisms recovered from the control preparation.

If growth is inhibited (reduction by a factor greater than 2), then modify the procedure for the particular enumeration test to ensure the validity of the results. Modification of the procedure may include, for example, (1) an increase in the volume of the diluent or culture medium, (2) incorporation of a specific or general neutralizing agents into the diluent, (3) membrane filtration or (4) a combination of the above measures. Neutralizing agents—Neutralizing agents may be used to neutralize the activity of antimicrobial agents (Table 4.05-I-2). They may be added to the chosen diluent or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for micro-organisms must be demonstrated by carrying out a blank with neutralizing agents, without product.

If no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This information serves to indicate that the article is not likely to be contaminated with the given species of the micro-organism. However, it is possible that the product only inhibits some of the micro-organisms specified herein, but does not inhibit others not included amongst the test strains or for which the latter are not representative. Then, perform the test with the highest dilution factor compatible with micro-organisms, or less if large numbers of CFU are expected.

to the membrane filter, filter immediately and rinse the membrane filter with an appropriate volume of diluent.

For the determination of total aerobic microbial count (TAMC), transfer the membrane filter to the surface of Soybean-Casein Digest Agar Medium. For the determination of total combined yeasts/moulds count (TYMC) transfer the membrane to the surface of Sabouraud Glucose Agar Medium. Incubate the plates as indicated in Table 4.05-I-1. Perform the counting.

### 3.4.4.2. Plate-count methods

Perform plate-count methods at least in duplicate for each medium and use the mean count of the result.

(i) Pour-plate method: For Petri dishes 9 cm in diameter, add to the dish 1 mL of the sample prepared as described under 3.4.1. to 3.4.3. and 15 – 20 mL of Soybean-Casein Digest Agar Medium or Sabouraud Glucose Agar Medium, both media being at not more than 45°C. If larger Petri dishes are used, the amount of agar medium is increased accordingly. For each of the micro-organisms listed in Table 4.05-I-1, at least 2 Petri dishes are used.

Incubate the plates as indicated in Table 4.05-I-1. Take the arithmetic mean of the counts per medium and calculate the number of CFU in the original inoculum.

(ii) Surface-spread method: For Petri dishes 9 cm in diameter, add 15 – 20 mL of Soybean-Casein Digest Agar Medium or Sabouraud Glucose Agar Medium at about 45°C to each Petri dish and allow to solidify. If larger Petri dishes are used, the volume of the agar is increased accordingly. Dry the plates, for example in a laminar-air-flow cabinet or in an incubator. For each of the micro-organisms listed in Table 4.05-I-1, at least 2 Petri dishes are used. Spread a measured volume of not less than 0.1 mL of the sample prepared as described under 3.4.1. to 3.4.3. over the surface of the medium. Incubate and count as prescribed under 3.4.4.2. (i).

### 3.4.4.3. Most-probable-number (MPN) method

The precision and accuracy of the MPN method is less than that of the membrane filtration method or the plate-count method. Unreliable results are obtained particularly for the enumeration of moulds. For these reasons the MPN method is reserved for the enumeration of TAMC in situations where no other method is available. If the use of the method is justified, proceed as follows.

<table>
<thead>
<tr>
<th>Interfering substance</th>
<th>Potential neutralizing agents/method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutaraldehyde, Mercurials</td>
<td>Sodium hydrogen sulfite (Sodium bisulfite)</td>
</tr>
<tr>
<td>Phenolics, Alcohol, Aldehydes, Sorbate</td>
<td>Dilution</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Glycine</td>
</tr>
<tr>
<td>Quaternary Ammonium Compounds (QACs), Parahydroxybenzoates (Parabens), Bis-biguaniodes</td>
<td>Lecithin</td>
</tr>
<tr>
<td>QAC, Parabens, Iodine</td>
<td>Polysorbate</td>
</tr>
<tr>
<td>Mercurials</td>
<td>Thioglycollate</td>
</tr>
<tr>
<td>Mercurials, Halogens, Aldehydes</td>
<td>Thiosulfate</td>
</tr>
<tr>
<td>EDTA (edetate)</td>
<td>Mg or Ca ions</td>
</tr>
</tbody>
</table>
Table 4.05-I-3  Most-probable-number values of micro-organisms

<table>
<thead>
<tr>
<th>Observed combinations of numbers of tubes showing growth in each set</th>
<th>Number of g or mL of product per tube</th>
<th>MPN per g or per mL of product</th>
<th>95 per cent confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>0 0 0</td>
<td>&lt;3</td>
<td>0 – 9.4</td>
<td></td>
</tr>
<tr>
<td>0 0 1</td>
<td>3</td>
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<td></td>
</tr>
<tr>
<td>0 1 0</td>
<td>3</td>
<td>0.1 – 10</td>
<td></td>
</tr>
<tr>
<td>0 1 1</td>
<td>6.1</td>
<td>1.2 – 17</td>
<td></td>
</tr>
<tr>
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<td>6.2</td>
<td>1.2 – 17</td>
<td></td>
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<td>3.5 – 35</td>
<td></td>
</tr>
<tr>
<td>1 0 0</td>
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<td>1.2 – 17</td>
<td></td>
</tr>
<tr>
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<td>11</td>
<td>4 – 35</td>
<td></td>
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<tr>
<td>1 1 0</td>
<td>7.4</td>
<td>1.3 – 20</td>
<td></td>
</tr>
<tr>
<td>1 1 1</td>
<td>11</td>
<td>4 – 35</td>
<td></td>
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<td>9 – 94</td>
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</tr>
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<td>9 – 94</td>
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</tr>
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<td>9 – 94</td>
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<td>5 – 94</td>
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<td>9 – 104</td>
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<td>64</td>
<td>16 – 181</td>
<td></td>
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<td>43</td>
<td>9 – 181</td>
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<td>17 – 199</td>
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<td>3 1 2</td>
<td>120</td>
<td>30 – 360</td>
<td></td>
</tr>
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<td>3 1 3</td>
<td>160</td>
<td>30 – 380</td>
<td></td>
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<td>3 2 0</td>
<td>93</td>
<td>18 – 360</td>
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<td>150</td>
<td>30 – 380</td>
<td></td>
</tr>
<tr>
<td>3 2 2</td>
<td>210</td>
<td>30 – 400</td>
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</tr>
<tr>
<td>3 2 3</td>
<td>290</td>
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</tr>
<tr>
<td>3 3 0</td>
<td>240</td>
<td>40 – 990</td>
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</tr>
<tr>
<td>3 3 1</td>
<td>460</td>
<td>90 – 1980</td>
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</tr>
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<td>1100</td>
<td>200 – 4000</td>
<td></td>
</tr>
<tr>
<td>3 3 3</td>
<td>&gt;1100</td>
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</tbody>
</table>

Prepare a series of at least 3 serial tenfold dilutions of the product as described under 3.4.1. to 3.4.3. From each level of dilution, 3 aliquots of 1 g or 1 mL are used to inoculate 3 tubes with 9 – 10 mL of Fluid Soybean-Casein Digest Medium. If necessary a surface-active agent such as polysorbate 80, or an inactivator of antimicrobial agents may be added.
to the medium. Thus, if 3 levels of dilution are prepared 9 tubes are inoculated.

Incubate all tubes at 30 – 35°C for not more than 3 days. If reading of the results is difficult or uncertain owing to the nature of the product to be examined, subculture in the same broth, or Soybean-Casein Digest Agar Medium, for 1 – 2 days at the same temperature and use these results. Determine the most probable number of micro-organisms per gram or millilitre of the product to be examined from Table 4.05-1.3.

3.5. Results and interpretation

When verifying the suitability of the membrane filtration method or the plate-count method, a mean count of any of the test organisms not differing by a factor greater than 2 from the value of the control defined in 3.4.2. in the absence of the product must be obtained. When verifying the suitability of the MPN method the calculated value from the inoculum must be within 95 per cent confidence limits of the results obtained with the control.

If the above criteria cannot be met for one or more of the organisms tested with any of the described methods, the method and test conditions that come closest to the criteria are used to test the product.

4. Testing of Products

4.1. Amount used for the test

Unless otherwise prescribed, use 10 g or 10 mL of the product to be examined taken with the precautions referred to above. For fluids or solids in aerosol form, sample 10 containers. For transdermal patches, sample 10 patches.

The amount to be tested may be reduced for Active Pharmaceutical Ingredients that will be formulated in the follow-

4.2. Examination of the product

4.2.1. Membrane filtration method

Use a filtration apparatus designed to allow the transfer of the filter to the medium. Prepare the sample using a method that has been shown suitable as described in section 3 and transfer the appropriate amount to each of 2 membrane filters and filter immediately. Wash each filter following the procedure shown to be suitable.

For the determination of TAMC, transfer one of the membrane filters to the surface of Sabouraud Glucose Agar Medium. For the determination of TYMC, transfer the other membrane to the surface of Sabouraud Glucose Agar Medium. Incubate the plate of Soybean-Casein Digest Agar Medium at 30 – 35°C for 3 – 5 days and the plate of Sabouraud Glucose Agar Medium at 20 – 25°C for 5 – 7 days. Calculate the number of CFU per gram or per millilitre of product.

When examining transdermal patches, filter 10 per cent of the volume of the preparation described under 3.4.1. separately through each of 2 sterile filter membranes. Transfer one membrane to Soybean-Casein Digest Agar Medium for TAMC and the other membrane to Sabouraud Glucose Agar Medium for TYMC.

4.2.2. Plate-count methods

(i) Pour-plate method: Prepare the sample using a method that has been shown to be suitable as described in section 3. Prepare for each medium at least 2 Petri dishes for each level of dilution. Incubate the plates of Soybean-Casein Digest Agar Medium at 30 – 35°C for 3 – 5 days and the plates of Sabouraud Glucose Agar Medium at 20 – 25°C for 5 – 7 days. Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC. Take the arithmetic mean per culture medium of the counts and calculate the number of CFU per gram or per millilitre of product.

(ii) Surface-spread method: Prepare the sample using a method that has been shown to be suitable as described in section 3. Prepare at least 2 Petri dishes for each medium and each level of dilution. For incubation and calculation of the number of CFU proceed as described for the pour-plate method.

4.2.3. Most-probable-number method

Prepare and dilute the sample using a method that has been shown to be suitable as described in section 1.3. Incubate all tubes for 3 – 5 days at 30 – 35°C. Subculture if necessary, using the procedure shown to be suitable. Record for each level of dilution the number of tubes showing microbial growth. Determine the most probable number of micro-organisms per gram or millilitre of the product to be examined from Table 4.05-1.3.

4.3. Interpretation of the results

The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU found using Soybean-Casein Digest Agar Medium; if colonies of fungi are detected on this medium, they are counted as part of TAMC. The total combined yeasts/mould count (TYMC) is considered to be equal to the number of CFU found using Sabouraud Glucose Agar Medium; if colonies of bacteria are detected on this medium, they are counted as part of TYMC. When the TYMC is expected to exceed the acceptance criterion due to the bacterial growth, Sabouraud Glucose Agar Medium containing antibiotics may be used. If the count is carried out by the MPN method the calculated value is the TAMC.

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

- $10^3$ CFU: maximum acceptable count = 20,
- $10^4$ CFU: maximum acceptable count = 200,
- $10^5$ CFU: maximum acceptable count = 2000, and so forth.

The recommended solutions and media are described in Tests for specified micro-organisms.

II. Microbiological Examination of Non-sterile Products: Tests for Specified Micro-organisms

These tests are harmonized with the European Phar-
Macropoënia and the U.S. Pharmacopoeia.

The tests described hereafter will allow determination of the absence of, or limited occurrence of specified microorganisms which may be detected under the conditions described.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results as stated below.

Alternative microbiological procedures, including automated methods may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

1. General Procedures

The preparation of samples is carried out as described in I. Total viable aerobic count.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized as described in I. Total viable aerobic count.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated as described in I. Total viable aerobic count.

2. Growth Promoting and Inhibitory Properties of the Media, Suitability of the Test and Negative Controls

The ability of the test to detect micro-organisms in the presence of the product to be tested must be established. Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

2.1. Preparation of test strains

Use standardised stable suspensions of test strains or prepare as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages previously obtained with a previously tested and approved batch.

2.1.1. Aerobic micro-organisms

Grow each of the bacterial test strains separately in containers containing Fluid Soybean-Casein Digest Medium or on Soybean-Casein Digest Agar Medium at 30 – 35°C for 18 – 24 hours. Grow the test strain for Candida albicans separately on Sabouraud Glucose Agar Medium or in Fluid Sabouraud Glucose Medium at 20 – 25°C for 2–3 days.

Staphylococcus aureus such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276,

Pseudomonas aeruginosa such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275,

Escherichia coli such as ATCC 8739, NCIMB 8545, CIP 53.126 or NBRC 3972,

Salmonella enterica subsp. enterica serovar Typhimurium such as ATCC 14028

or, as an alternative, Salmonella enterica subsp. enterica serovar Abony such as NBRC 100797, NCTC 6017 or CIP 80.39, Candida albicans such as ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594.

Use Buffered Sodium Chloride-Peptone Solution (pH 7.0) or Phosphate Buffer (pH 7.2) to make test suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2 – 8°C.

2.1.2. Clostridia

Use Clostridium sporogenes such as ATCC 11437 (NBRC 14293, NCIMB 12343, CIP 100651) or ATCC 19404 (NCTC 532 or CIP 79.3). Grow the clostridial test strain under anaerobic conditions in Reinforced Clostridial Medium at 30 – 35°C for 24 – 48 hours. As an alternative to preparing and then diluting down a fresh suspension of vegetative cells of Cl. sporogenes, a stable spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 – 8°C for a validated period.

2.2. Negative control

To verify testing conditions a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described under 3. A failed negative control required an investigation.

2.3. Growth promotion and inhibitory properties of the media

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients.

Verify suitable properties of relevant media as described in Table 4.05-II-1.

(i) Test for growth promoting properties, liquid media: inoculate a portion of the appropriate medium with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

(ii) Test for growth promoting properties, solid media: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

(iii) Test for inhibitory properties, liquid or solid media: inoculate the appropriate medium with at least 100 CFU of the appropriate micro-organism. Incubate at the specified temperature for not less than the longest period of time specified in the test. Growth of the micro-organism occurs.

(iv) Test for indicative properties: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

2.4. Suitability of the test method

For each product to be tested perform sample preparation as described in the relevant paragraph in section 3. Add each test strain at the time of mixing, in the prescribed growth medium. Inoculate the test strains individually. Use a number of micro-organisms equivalent to not more than 100 CFU in the inoculated test preparation.

Perform the test as described in the relevant paragraph in section 3 using the shortest incubation period prescribed.
**Table 4.05-II-1**  Growth promoting, inhibitory and indicative properties of media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Property</th>
<th>Test strains</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test for bile-tolerant gram-negative bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid Enterobacteria Enrichment Broth Mossel Medium</td>
<td>Growth promoting</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td></td>
<td>Inhibitory</td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>VRB (Violet/Red/Bile) Agar with glucose</td>
<td>Growth promoting +</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td></td>
<td>Indicative</td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td><strong>Test for Escherichia coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid MacConkey Broth Medium</td>
<td>Growth promoting</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td></td>
<td>Inhibitory</td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>MacConkey Agar Medium</td>
<td>Growth promoting +</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td></td>
<td>Indicative</td>
<td></td>
</tr>
<tr>
<td><strong>Test for Salmonella</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid Rappaport Vassiliadis Salmonella Enrichment Broth Medium</td>
<td>Growth promoting</td>
<td><em>Salmonella enterica</em> subsp. <em>enterica</em> serovar Typhimurium or <em>Salmonella enterica</em> subsp. <em>enterica</em> serovar Abony</td>
</tr>
<tr>
<td></td>
<td>Inhibitory</td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>XLD (Xylose-Lysine-Desoxycholate) Agar Medium</td>
<td>Growth promoting +</td>
<td><em>Salmonella enterica</em> subsp. <em>enterica</em> serovar Typhimurium or <em>Salmonella enterica</em> subsp. <em>enterica</em> serovar Abony</td>
</tr>
<tr>
<td></td>
<td>Indicative</td>
<td></td>
</tr>
<tr>
<td><strong>Test for Pseudomonas aeruginosa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cetrimide Agar Medium</td>
<td>Growth promoting</td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td></td>
<td>Inhibitory</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td><strong>Test for Staphylococcus aureus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannitol Salt Agar Medium</td>
<td>Growth promoting +</td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td></td>
<td>Indicative</td>
<td></td>
</tr>
<tr>
<td><strong>Test for Clostridia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reinforced Clostridial Medium</td>
<td>Growth promoting</td>
<td><em>Cl. sporogenes</em></td>
</tr>
<tr>
<td>Columbia Agar Medium</td>
<td>Growth promoting</td>
<td><em>Cl. sporogenes</em></td>
</tr>
<tr>
<td><strong>Test for Candida albicans</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid Sabouraud Glucose Medium</td>
<td>Growth promoting</td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td>Sabouraud Glucose Agar Medium</td>
<td>Growth promoting +</td>
<td><em>C. albicans</em></td>
</tr>
</tbody>
</table>

The specified micro-organisms must be detected with the indication reactions as described in section 3.

Any antimicrobial activity of the product necessitates a modification of the test procedure (see I. of Total viable aerobic count).

If for a given product the antimicrobial activity with respect to a micro-organism for which testing is prescribed cannot be neutralised, then it is to be assumed that the inhibited micro-organism will not be present in the product.
3. Testing of Products

3.1. Bile-tolerant gram-negative bacteria

3.1.1. Sample preparation and pre-incubation
Preparation a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. Total viable aerobic count, but using Fluid Soybean-Casein Digest Medium as the chosen diluent, mix and incubate at 20 – 25°C for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 hours but not more than 5 hours).

3.1.2. Test for absence
Unless otherwise prescribed use the volume corresponding to 1 g of the product, as prepared in 3.1.1. to inoculate Fluid Enterobacteria Enrichment Broth Mossel Medium. Incubate at 30 – 35°C for 24 – 48 hours. Subculture on plates of VRB (Violet/Red/Bile) Agar with glucose. Incubate at 30 – 35°C for 18 – 24 hours.

The product complies with the test if there is no growth of colonies.

3.1.3. Quantitative test

3.1.3.1. Selection and subculture
Inoculate suitable quantities of Fluid Enterobacteria Enrichment Broth Mossel Medium with the preparation as described under 3.1.1. and/or dilutions of it containing respectively 0.1 g, 0.01 g and 0.001 g (or 0.1 mL, 0.01 mL and 0.001 mL) of the product to be examined. Incubate at 30 – 35°C for 24 – 48 hours. Subculture each of the cultures on a plate of VRB (Violet/Red/Bile) Agar with glucose. Incubate at 30 – 35°C for 18 – 24 hours.

3.1.3.2. Interpretation
Growth of colonies constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result. Determine from Table 4.05-II-2 the probable number of bacteria.

3.2. Escherichia coli

3.2.1. Sample preparation and pre-incubation
Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. Microbial Enumeration Tests and use 10 mL of the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium, mix and incubate at 30 – 35°C for 18 – 24 hours.

3.2.2. Selection and subculture
Shake the container, transfer 1 mL of Fluid Soybean-Casein Digest Medium to 100 mL of Fluid MacConkey Broth Medium and incubate at 42 – 44°C for 24 – 48 hours. Subculture on a plate of MacConkey Agar Medium at 30 – 35°C for 18 – 72 hours.

3.2.3. Interpretation
Growth of colonies indicates the possible presence of E. coli. This is confirmed by identification tests.

The product complies with the test if no colonies are present or if the identification tests are negative.

3.3. Salmonella

3.3.1. Sample preparation and pre-incubation
Prepare the product to be examined as described in I. Total viable aerobic count and use the quantity corresponding to not less than 10 g or 10 mL to inoculate a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium, mix and incubate at 30 – 35°C for 18 – 24 hours.

3.3.2. Selection and subculture
Transfer 0.1 mL of Fluid Soybean-Casein Digest Medium to 10 mL of Fluid Rappaport Vassiliadis Salmonella Enrichment Broth Medium and incubate at 30 – 35°C for 18 – 24 hours. Subculture on plates of XLD (Xylose-Lysine-Desoxycholate) Agar Medium. Incubate at 30 – 35°C for 18 – 48 hours.

3.3.3. Interpretation
The possible presence of Salmonella is indicated by the growth of well-developed, red colonies, with or without black centres. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

3.4. Pseudomonas aeruginosa

3.4.1. Sample preparation and pre-incubation
Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. Total viable aerobic count and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium and mix. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in I. Total viable aerobic count (3.4.1.) through a sterile filter membrane and place in 100 mL of Fluid Soybean-Casein Digest Medium. Incubate at 30 – 35°C for 18 – 24 hours.

3.4.2. Selection and subculture
Subculture on a plate of Cetrimide Agar Medium and incubate at 30 – 35°C for 18 – 72 hours.

3.4.3. Interpretation
Growth of colonies indicates the possible presence of P. aeruginosa. This is confirmed by identification tests.

The product complies with the test if colonies are not present or if the confirmatory identification tests are negative.

### Table 4.05-II-2 Interpretation of results

<table>
<thead>
<tr>
<th>Results for each quantity of product</th>
<th>Probable number of bacteria per gram or mL of product</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 g or 0.1 mL</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>more than 10³</td>
</tr>
<tr>
<td>+</td>
<td>less than 10³ and more than 10²</td>
</tr>
<tr>
<td>+</td>
<td>less than 10² and more than 10</td>
</tr>
<tr>
<td>-</td>
<td>less than 10</td>
</tr>
<tr>
<td>0.01 g or 0.01 mL</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>more than 10³</td>
</tr>
<tr>
<td>+</td>
<td>less than 10³ and more than 10²</td>
</tr>
<tr>
<td>+</td>
<td>less than 10² and more than 10</td>
</tr>
<tr>
<td>-</td>
<td>less than 10</td>
</tr>
<tr>
<td>0.001 g or 0.001 mL</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>more than 10³</td>
</tr>
<tr>
<td>+</td>
<td>less than 10³ and more than 10²</td>
</tr>
<tr>
<td>+</td>
<td>less than 10² and more than 10</td>
</tr>
<tr>
<td>-</td>
<td>less than 10</td>
</tr>
</tbody>
</table>
3.5.  *Staphylococcus aureus*

3.5.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. Microbial Enumeration Tests and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium and homogenise. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in I. Total viable aerobic count (3.4.1.) through a sterile filter membrane and place in 100 mL of Fluid Soybean-Casein Digest Medium. Incubate at 30 – 35°C for 18 – 24 hours.

3.5.2. Selection and subculture

Subculture on a plate of Mannitol Salt Agar Medium and incubate at 30 – 35°C for 18 – 72 hours.

3.5.3. Interpretation

The possible presence of *S. aureus* is indicated by the growth of yellow/white colonies surrounded by a yellow zone. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

3.6.  *Clostridia*

3.6.1. Sample preparation and heat treatment

Prepare a sample using a 1 in 10 dilution (with a minimum total volume of 20 mL) of not less than 2 g or 2 mL of the product to be examined as described in I. Total viable aerobic count.

Divide the sample into two portions of at least 10 mL. Heat 1 portion at 80°C for 10 min and cool rapidly. Do not heat the other portion.

3.6.2. Selection and subculture

Use 10 mL or the quantity corresponding to 1 g or 1 mL of the product to be examined of both portions to inoculate suitable amounts (determined as described under 2.4.) of Reinforced Clostridial Medium. Incubate under anaerobic conditions at 30 – 35°C for 48 hours. After incubation, make subcultures from each tube on Columbia Agar Medium and incubate under anaerobic conditions at 30 – 35°C for 48 – 72 hours.

3.6.3. Interpretation

The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of *Clostridia*.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

3.7.  *Candida albicans*

3.7.1. Sample preparation and pre-incubation

Prepare the product to be examined as described in I. Microbial Enumeration Tests and use 10 mL or the quantity corresponding to not less than 1 g or 1 mL to inoculate 100 mL of Fluid Sabouraud Glucose Medium and mix. Incubate at 30 – 35°C for 3-5 days.

3.7.2. Selection and subculture

Subculture on a plate of Sabouraud Glucose Agar Medium and incubate at 30 – 35°C for 24 – 48 hours.

3.7.3. Interpretation

Growth of white colonies may indicate the presence of *C. albicans*. This is confirmed by identification tests.

The product complies with the test if such colonies are not present or if the confirmatory identification tests are negative.

The following section is given for information.

4.  Recommended Solutions and Culture Media

The following solutions and culture media have been found satisfactory for the purposes for which they are prescribed in the test for microbial contamination in the Pharmacopoeia. Other media may be used provided that their suitability can be demonstrated.

(i)  Phosphate Buffer (pH 7.2)

Prepare a mixture of water and stock buffer solution (800:1 V/V) and sterilize.

Stock buffer solution. Transfer 34 g of potassium dihydrogen phosphate to a 1000 mL volumetric flask, dissolve in 500 mL of purified water, adjust to pH 7.1 to 7.3 with sodium hydroxide, add purified water to volume and mix. Dispense in containers and sterilize. Store at a temperature of 2 – 8°C.

(ii) Buffered Sodium Chloride-Peptone Solution (pH 7.0)

Potassium dihydrogen phosphate 3.6 g

Disodium hydrogen phosphate dihydrate 7.2 g

(equivalent to 0.067 mol phosphate)

Sodium chloride 4.3 g

Peptone (meat or casein) 1.0 g

Water 1000 mL

Sterilize in an autoclave using a validated cycle.

(iii) Fluid Soybean-Casein Digest Medium

Casein peptone 17.0 g

Soybean peptone 3.0 g

Sodium chloride 5.0 g

Dipotassium hydrogen phosphate 2.5 g

Glucose 2.5 g

Water 1000 mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25°C. Sterilize in an autoclave using a validated cycle.

(iv) Soybean-Casein Digest Agar Medium

Casein peptone 15.0 g

Soybean peptone 5.0 g

Sodium chloride 5.0 g

Agar 15.0 g

Water 1000 mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25°C. Sterilize in an autoclave using a validated cycle.

(v) Sabouraud Glucose Agar Medium

Glucose 40.0 g

Peptones (animal tissue and casein 1:1) 10.0 g

Agar 15.0 g

Water 1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25°C. Sterilize in an autoclave using a validated cycle.

(vi) Potato Dextrose Agar Medium

Infusion from potatoes 200 g

Glucose 20.0 g

Agar 15.0 g

Water 1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25°C. Sterilize in an autoclave using a validated cycle.
### Fluid Sabouraud Glucose Medium
- **Glucose**: 20.0 g
- **Peptones (animal tissue and casein 1:1)**: 10.0 g
- **Water**: 1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25°C. Sterilize in an autoclave using a validated cycle.

### Fluid Enterobacteria Enrichment Broth Mossel Medium
- **Gelatin peptone**: 10.0 g
- **Glucose**: 5.0 g
- **Bile salts**: 20.0 g
- **Potassium dihydrogen phosphate**: 2.0 g
- **Disodium hydrogen phosphate dihydrate**: 8.0 g
- **Brilliant green**: 15 mg
- **Water**: 1000 mL

Adjust the pH so that after heating it is 7.0 – 7.4 at 25°C. Heat at 100°C for 30 min and cool immediately.

### VRB (Violet/Red/Bile) Agar with glucose
- **Yeast extract**: 3.0 g
- **Gelatin peptone**: 7.0 g
- **Bile salts**: 1.5 g
- **Sodium chloride**: 5.0 g
- **Neutral red**: 30 mg
- **Crystal violet**: 2 mg
- **Water**: 1000 mL

Adjust the pH so that after heating it is 7.2 – 7.6 at 25°C. Heat to boiling; do not heat in an autoclave.

### Fluid MacConkey Broth Medium
- **Gelatin peptone**: 20.0 g
- **Lactose monohydrate**: 10.0 g
- **Dehydrated ox bile**: 5.0 g
- **Bromocresol purple**: 10 mg
- **Water**: 1000 mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25°C. Sterilize in an autoclave using a validated cycle.

### MacConkey Agar Medium
- **Gelatin peptone**: 17.0 g
- **Peptones (meat and casein)**: 3.0 g
- **Lactose monohydrate**: 10.0 g
- **Sodium chloride**: 5.0 g
- **Bile salts**: 1.5 g
- **Agar**: 13.5 g
- **Neutral red**: 30 mg
- **Crystal violet**: 1 mg
- **Water**: 1000 mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25°C. Boil for 1 min with constant shaking then sterilize in an autoclave using a validated cycle.

### Fluid Rappaport Vassiliadis Salmonella Enrichment Medium
- **Soya peptone**: 4.5 g
- **Magnesium chloride hexahydrate**: 29.0 g
- **Sodium chloride**: 8.0 g
- **Potassium dihydrogen phosphate**: 0.4 g
- **Malachite green**: 36 mg
- **Water**: 1000 mL

Hydrate the agar, dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilization it is about 6.6 – 7.0 at 25°C. Sterilize in an autoclave using a validated cycle.
4.06 Sterility Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (ⅩⅦ). The test is applied to active pharmaceutical ingredients, preparations or articles which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating micro-organisms has been found in the sample examined in the conditions of the test.

1. Precautions against microbial contamination

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any micro-organisms which are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.

2. Culture media and incubation temperatures

Media for the test may be prepared as described below, or equivalent commercial media may be used provided that they comply with the growth promotion test. The following culture media have been found to be suitable for the test for sterility. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. Soybean casein digest medium is suitable for the culture of both fungi and aerobic bacteria.

(i) Fluid thioglycollate medium

Mix the l-cystine, agar, sodium chloride, glucose, watersoluble yeast extract and pancreatic digest of casein with water, and heat until solution is effectuated. Dissolve the sodium thioglycollate or thioglycolic acid in the solution and, if necessary, add sodium hydroxide TS so that, after sterilization, the solution will have a pH of 7.1 ± 0.2. If filtration is necessary, heat the solution again without boiling and filter while hot through moistened filter paper. Add the resazurin sodium solution (1 in 1000), mix and place the medium in suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a color change indicative of oxygen uptake at the end of the incubation period. Sterilize using a validated process. If the medium is stored, store at a temperature between 2°C and 25°C in a sterile, tight container. If more than the upper one-third of the medium has acquired a pink color, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink color disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container. Do not use the medium for a longer storage period than has been validated.

Fluid thioglycollate medium is to be incubated at 30–35°C. For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycollate medium incubated at 20–25°C may be used instead of soya-bean casein digest medium provided that it has been validated as described in growth promotion test.

Where prescribed or justified and authorized, the following alternative thioglycollate medium might be used. Prepare a mixture having the same composition as that of the fluid thioglycollate medium, but omitting the agar and the resazurin sodium solution (1 in 1000), sterilize as directed above. The pH after sterilization is 7.1 ± 0.2. Heat in a water bath prior to use and incubate at 30–35°C under anaerobic conditions.

(ii) Soybean casein digest medium

Mix the l-cystine, agar, sodium chloride, glucose, watersoluble yeast extract and pancreatic digest of casein with water, and heat until solution is effectuated. Dissolve the sodium thioglycollate or thioglycolic acid in the solution and, if necessary, add sodium hydroxide TS so that, after sterilization, the solution will have a pH of 7.3 ± 0.2. If filtration is necessary, heat the solution again without boiling and filter while hot through moistened filter paper. Add the resazurin sodium solution (1 in 1000), mix and place the medium in suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a color change indicative of oxygen uptake at the end of the incubation period. Sterilize using a validated process. If the medium is stored, store at a temperature between 2°C and 25°C in a sterile, tight container. If more than the upper one-third of the medium has acquired a pink color, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink color disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container. Do not use the medium for a longer storage period than has been validated.

Fluid thioglycollate medium is to be incubated at 30–35°C. For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycollate medium incubated at 20–25°C may be used instead of soya-bean casein digest medium provided that it has been validated as described in growth promotion test.

Where prescribed or justified and authorized, the following alternative thioglycollate medium might be used. Prepare a mixture having the same composition as that of the fluid thioglycollate medium, but omitting the agar and the resazurin sodium solution (1 in 1000), sterilize as directed above. The pH after sterilization is 7.1 ± 0.2. Heat in a water bath prior to use and incubate at 30–35°C under anaerobic conditions.

3. Suitability of the culture medium

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined.

3.1 Sterility

Incubate portions of the media for 14 days. No growth of micro-organisms occurs.
Table 4.06-1. Strains of the test micro-organisms suitable for use in the Growth Promotion Test and the Method suitability Test

<table>
<thead>
<tr>
<th>Aerobic bacteria</th>
<th>Strain</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275</td>
<td></td>
</tr>
<tr>
<td>Anaerobic bacterium</td>
<td><em>Clostridium sporogenes</em></td>
<td>ATCC 19404, CIP 79.3, NCTC 532 or ATCC 11437, NBRC 14293</td>
</tr>
<tr>
<td>Fungi</td>
<td><em>Candida albicans</em></td>
<td>ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus brasiliensis</em></td>
<td>ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455</td>
</tr>
</tbody>
</table>

3.2. Growth promotion test of aerobes, anaerobes and fungi

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Suitable strains of micro-organisms are indicated in Table 4.06-1.

Inoculate portions of fluid thioglycollate medium with a small number (not more than 100 CFU) of the following microorganisms, using a separate portion of medium for each of the following species of micro-organism:

- *Aspergillus brasiliensis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium sporogenes*, *Pseudomonas aeruginosa*, *Candida albicans*.

Inoculate portions of soya-bean casein digest medium with a small number (not more than 100 CFU) of the following microorganisms, using a separate portion of medium for each of the following species of micro-organism:

- *Aspergillus brasiliensis*, *Staphylococcus aureus*.

Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi.

Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than five passages removed from the original master seed-lot.

The media are suitable if a clearly visible growth of the micro-organisms occurs.

4. Method suitability test

Carry out a test as described below under 5. Test for sterility of the product to be examined using exactly the same methods except for the following modifications.

(i) Membrane filtration: After transferring the content of the container or containers to be tested to the membrane add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the final portion of sterile diluent used to rinse the filter.

(ii) Direct inoculation: After transferring the contents of the container or containers to be tested to the culture medium add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the medium.

In both cases use the same micro-organisms as those described above under 3.2. Growth promotion test of aerobes, anaerobes and fungi. Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 5 days.

If clearly visible growth of micro-organisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without further modification.

If clearly visible growth is not obtained in the presence of the product to be tested, visually comparable to that in the control vessels without product, the product possesses antimicrobial activity that has not been satisfactorily eliminated under the conditions of the test. Modify the conditions in order to eliminate the antimicrobial activity and repeat the method suitability test.

This method suitability is performed:

a) when the test for sterility has to be carried out on a new product;

b) whenever there is a change in the experimental conditions of the test.

The method suitability may be performed simultaneously with the Test for sterility of the product to be examined.

5. Test for sterility of the product to be examined

The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits, that is, for filterable aqueous preparations, for alcoholic or oily preparations and for preparations miscible with or soluble in aqueous or oily solvents provided these solvents do not have an antimicrobial effect in the conditions of the test.

5.1. Membrane filtration

Use membrane filters having a nominal pore size not greater than 0.45 μm whose effectiveness to retain microorganisms has been established. Cellulose nitrate filters, for example, are used for aqueous, oily and weakly alcoholic solutions and cellulose acetate filters, for example, for strongly alcoholic solutions. Specially adapted filters may be needed for certain products, e.g. for antibiotics.

The technique described below assumes that membranes about 50 mm in diameter will be used. If filters of a different diameter are used the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and membrane are sterilized by appropriate means. The apparatus is designed so that the solution to be examined can be introduced and filtered under aseptic conditions; it permits the aseptic removal of the membrane for transfer to the medium or it is suitable for carrying out the incubation after adding the medium to the apparatus itself.

(i) Aqueous solutions: If appropriate, transfer a small quantity of a suitable, sterile diluent such as a 1 g/L neutral solution of meat or casein peptone pH 7.1 ± 0.2 onto the membrane in the apparatus and filter. The diluent may contain suitable neutralizing substances and/or appropriate inactivating substances for example in the case of antibiotics.

Transfer the contents of the container or containers to be
tested to the membrane or membranes, if necessary after diluting to the volume used in the method suitability test with the chosen sterile diluent but in any case using not less than the quantities of the product to be examined prescribed in Table 4.06-2. Filter immediately. If the product has antimicrobial properties, wash the membrane not less than three times by filtering through it each time the volume of the chosen sterile diluent used in the method suitability test. Do not exceed a washing cycle of 5 times 100 mL per filter, even if during method suitability it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity. Transfer the whole membrane to the culture medium or cut it aseptically into two equal parts and transfer one half to each of two suitable media. Use the same volume of each medium as in the method suitability test. Alternatively, transfer the membrane onto the membrane in the apparatus. Incubate the media for not less than 14 days. If no evidence of microbial growth is found, the product complies with the test for sterility. If evidence of microbial growth is found examine the media for macroscopic evidence of microbial growth. If the material being tested renders the detection of anaerobic micro-organisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions. Incubate the media for not less than 14 days.

(ii) Soluble solids: Use for each medium not less than the quantity prescribed in Table 4.06-2 of the product dissolved in a suitable solvent such as the solvent provided with the preparation, water for injection, saline or a 1 g/L neutral solution of meat or casein peptone and proceed with the test as described above for aqueous solutions using a membrane appropriate to the chosen solvent.

(iii) Oils and oily solutions: Use for each medium not less than the quantity of the product prescribed in Table 4.06-2. Oils and oily solutions of sufficiently low viscosity may be filtered without dilution through a dry membrane. Viscous oils may be diluted as necessary with a suitable sterile diluent such as isopropyl myristate shown not to have antimicrobial activity in the conditions of the test. Allow the oil to penetrate the membrane by its own weight then filter, applying the pressure or suction gradually. Wash the membrane at least three times by filtering through it each time about 100 mL of a suitable sterile solution such as 1 g/L neutral meat or casein peptone containing a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability of the test, for example polysorbate 80 at a concentration of 10 g/L. Transfer the membrane or membranes to the culture medium or media or vice versa as described above for aqueous solutions, and incubate at the same temperatures and for the same times.

(iv) Ointments and creams: Use for each medium not less than the quantities of the product prescribed in Table 4.06-2. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1 per cent in isopropyl myristate as described above, by heating, if necessary, to not more than 40°C. In exceptional cases it may be necessary to heat to not more than 44°C. Filter as rapidly as possible and proceed as described above for oils and oily solutions.

5.2. Direct inoculation of the culture medium
Transfer the quantity of the preparation to be examined prescribed in Table 4.06-2 directly into the culture medium so that the volume of the product is not more than 10 per cent of the volume of the medium, unless otherwise prescribed. If the product to be examined has antimicrobial activity, carry out the test after neutralizing this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate the concentrated medium may be added directly to the product in its container.

(i) Oily liquids: Use media to which have been added a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability of the test, for example polysorbate 80 at a concentration of 10 g/L.

(ii) Ointments and creams: Prepare by diluting to about 1 in 10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluent such as a 1 g/L neutral solution of meat or casein peptone. Transfer the diluted product to a medium not containing an emulsifying agent. Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Shake cultures containing oily products gently each day. However when fluid thioglycollate medium is used for the detection of anaerobic micro-organisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

6. Observation and interpretation of results
At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1 mL) of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days.

If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found the product to be exa-
Table 4.06-3. Minimum number of items to be tested

<table>
<thead>
<tr>
<th>Number of items in the batch*</th>
<th>Minimum number of items to be tested for each medium, unless otherwise justified and authorized**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenteral preparations</td>
<td>10% or 4 containers whichever is the greater 10 containers</td>
</tr>
<tr>
<td>— Not more than 100 containers</td>
<td></td>
</tr>
<tr>
<td>— More than 100 but not more than 500 containers</td>
<td>2% or 20 containers <em>(10 containers for parenterals with a nominal volume of 100 mL or more)</em> whichever is the less</td>
</tr>
<tr>
<td>— More than 500 containers</td>
<td></td>
</tr>
<tr>
<td>Ophthalmic and other non-injectable preparations</td>
<td>5% or 2 containers whichever is the greater 10 containers</td>
</tr>
<tr>
<td>— Not more than 200 containers</td>
<td></td>
</tr>
<tr>
<td>— More than 200 containers</td>
<td></td>
</tr>
<tr>
<td>— If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral use</td>
<td></td>
</tr>
<tr>
<td>Bulk solid products</td>
<td>Each container 20% or 4 containers whichever is the greater 10 containers</td>
</tr>
<tr>
<td>— Up to 4 containers</td>
<td></td>
</tr>
<tr>
<td>— More than 4 containers but not more than 50 containers</td>
<td></td>
</tr>
<tr>
<td>— More than 50 containers</td>
<td>2% or 10 containers whichever is the greater</td>
</tr>
</tbody>
</table>

* If the batch size is not known, use the maximum number of items prescribed

** If the contents of one container are enough to inoculate the two media, this column gives the number of containers needed for both the media together.

examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. The test may be considered invalid only if one or more of the following conditions are fulfilled:

(i) the data of the microbiological monitoring of the sterility testing facility show a fault;
(ii) a review of the testing procedure used during the test in question reveals a fault;
(iii) microbial growth is found in the negative controls;
(iv) after determination of the identity of the microorganisms isolated from the test, the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and/or the technique used in conducting the sterility test procedure.

If the test is declared to be invalid it is repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test the product examined complies with the test for sterility. If microbial growth is found in the repeat test the product examined does not comply with the test for sterility.

7. Application of the test to parenteral preparations, ophthalmic and other non-injectable preparations required to comply with the test for sterility

When using the technique of membrane filtration, use, whenever possible, the whole contents of the container, but not less than the quantities indicated in Table 4.06-2, diluting where necessary to about 100 mL with a suitable sterile solution, such as 1 g/L neutral meat or casein peptone.

When using the technique of direct inoculation of media, use the quantities shown in Table 4.06-2, unless otherwise justified and authorized. The tests for bacterial and fungal sterility are carried out on the same sample of the product to be examined. When the volume or the quantity in a single container is insufficient to carry out the tests, the contents of two or more containers are used to inoculate the different media.

8. Minimum number of items to be tested

The minimum number of items to be tested in relation to the size of the batch is given in Table 4.06-3.

5. Tests for Crude Drugs

5.01 Crude Drugs Test

Crude Drugs Test is applied to the crude drugs mentioned in the General Rules for Crude Drugs.

1. Sampling

Unless otherwise specified, sample should be taken by the following methods. If necessary, preserve the samples in tight containers.

(i) When crude drugs to be sampled are small-sized, cut or powdered, 50 to 250 g of sample should be taken after mixing thoroughly.
(ii) When crude drugs to be sampled are large-sized, 250 to 500 g of sample should be taken after mixing thoroughly.
(iii) When the mass of each single piece of the crude drugs is not less than 100 g, not less than 5 pieces should be taken for a sample, or not less than 500 g of the sample should be taken after cutting to a suitable size and mixing thoroughly.

2. Preparation of the test sample for analysis

Preparations are to be made by mixing the sample well. Powdered drugs should be used as they are, and in the case of unpowdered drugs, unless otherwise specified, grind the sample into powder. If the sample cannot be ground into powder, reduce it as finely as possible, spread it out in a thin layer, and withdraw a typical portion for analysis. If necessary, preserve the test sample in a tight container.

3. Micropic examination

3.1. Apparatus

Use an optical microscope with objectives of 10 and 40 magnifications, and an ocular of 10 magnifications.

3.2. Preparation for microscopic examination

(i) Section: To a section on a slide glass add 1 to 2 drops
of a mounting agent, and put a cover glass on it, taking precaution against inclusion of bubbles. Usually use a section 10 to 20 μm in thickness.

(ii) Powder: Place about 1 mg of powdered sample on a slide glass, add 1 to 2 drops of a swelling agent, stir well with a small rod preventing inclusion of bubbles, and allow to stand for a while to swell the sample. Add 1 drop of the mounting agent, and put a cover glass on it so that the tissue sections spread evenly without overlapping each other, taking precaution against inclusion of bubbles. In the case where the tissue sections are opaque, place about 1 mg of powdered sample on a slide glass, add 1 to 2 drops of chloral hydrate TS, heat to make the tissues clear while stirring with a small glass rod to prevent boiling. After cooling, add 1 drop of mounting agent, and put a cover glass on it in the same manner as above.

Unless otherwise specified, use a mixture of glycerin and water (1:1) or a mixture of water, ethanol (95) and glycerin (1:1:1) as the mounting agent and swelling agent.

3.3. Observation of components in the Description

In each monograph, description is usually given of the outer portion and the inner portion of a section in this order, followed by a specification of cell contents. Observation should be made in the same order. In the case of a powdered sample, description is given of a characteristic component or a matter present in large amount, rarely existing matter, and cell contents in this order. Observation should be made in the same order.

4. Purity

4.1. Foreign matter

Unless otherwise specified, weigh 25 to 500 g of the sample, spread out in a thin layer, and separate the foreign matter by inspecting with the naked eye or with the use of a magnifying glass of 10 magnifications. Weigh, and determine the percentage of foreign matter.

4.2. Total BHC’s and total DDT’s

Sodium chloride, anhydrous sodium sulfate and synthetic magnesium silicate for column chromatography used in this procedure are used after drying by heating at about 130°C for more than 12 hours and cooling in a desiccator (silica gel). Chromatographic column is prepared as follows: Place 20 g of synthetic magnesium silicate for column chromatography in a 200-mL flask, add 50 mL of hexane for Purity of crude drug, shake vigorously, and immediately pour the mixture into a chromatographic tube about 2 cm in inside diameter and about 30 cm in length. Drip until the depth of hexane layer at the upper part is about 5 cm, introduce 8 g of anhydrous sodium sulfate from the top, and further drip until a small quantity of hexane is left at the upper part.

Weigh accurately about 5 g of pulverized sample, place in a glass-stoppered centrifuge tube, add 30 mL of a mixture of acetone for Purity of crude drug and water (5:2), stopper tightly, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the same procedure twice with the residue using two 30-mL portions of the mixture of acetone for Purity of crude drug and water (5:2). Combine all the supernatant liquids, and concentrate under reduced pressure at a temperature not higher than 40°C until the order of acetone is faint. Transfer the concentrated solution to a separator containing 50 mL of sodium chloride TS, and shake twice with two 50-mL portions of hexane for Purity of crude drug for 5 minutes each. Combine the hexane layers, transfer to a separator containing 50 mL of sodium chloride TS, and shake for 5 minutes. Take the hexane layer, dry with 30 g of anhydrous sodium sulfate, and filter. Wash the residue on the filter paper with 20 mL of hexane for Purity of crude drug. Combine the filtrate and the washings, and concentrate under reduced pressure at a temperature not higher than 40°C to about 5 mL. Transfer this solution to the chromatographic column and allow to pass with 300 mL of a mixture of hexane for Purity of crude drug and diethyl ether for Purity of crude drug (17:3) at a rate of not more than 5 mL per minute. After concentrating the eluate under reduced pressure at a temperature not higher than 40°C, add hexane for Purity of crude drug to make exactly 5 mL. Transfer this solution to a glass-stoppered test tube, add 1 mL of sulfuric acid, and shake carefully. Take 4 mL of the upper layer, transfer to a separate glass-stoppered test tube, add 2 mL of water, and shake gently. Usually take 3 mL of the upper layer so obtained, transfer to a glass-stoppered centrifuge tube, dry with 1 g of anhydrous sodium sulfate, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg each of α-BHC, β-BHC, γ-BHC, δ-BHC, o,p′-DDT, p,p′-DDT, p,p′-DDE and p,p′-DDE, dissolve in 5 mL of acetone for Purity of crude drug, and add hexane for Purity of crude drug to make exactly 100 mL. Pipet 10 mL of this solution, and add hexane for Purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, add hexane for Purity of crude drug to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution as directed under Gas Chromatography. Calculate the content of each of α-BHC, β-BHC, γ-BHC, δ-BHC, o,p′-DDT, p,p′-DDT, p,p′-DDE and p,p′-DDE by means of the following equations.

Content (ppm) of α-BHC

\[
\text{Content (ppm) of} \ \alpha-BHC = \frac{\text{amount (g) of } \alpha-BHC}{M} \times \frac{A_{\text{TA}}}{A_{\text{SA}}} \times 50
\]

Content (ppm) of β-BHC

\[
\text{Content (ppm) of} \ \beta-BHC = \frac{\text{amount (g) of } \beta-BHC}{M} \times \frac{A_{\text{TB}}}{A_{\text{SB}}} \times 50
\]

Content (ppm) of γ-BHC

\[
\text{Content (ppm) of} \ \gamma-BHC = \frac{\text{amount (g) of } \gamma-BHC}{M} \times \frac{A_{\text{TC}}}{A_{\text{SC}}} \times 50
\]

Content (ppm) of δ-BHC

\[
\text{Content (ppm) of} \ \delta-BHC = \frac{\text{amount (g) of } \delta-BHC}{M} \times \frac{A_{\text{TD}}}{A_{\text{SD}}} \times 50
\]

Content (ppm) of o,p′-DDT

\[
\text{Content (ppm) of} \ o,p′-DDT = \frac{\text{amount (g) of } o,p′-DDT}{M} \times \frac{A_{\text{TE}}}{A_{\text{SE}}} \times 50
\]

Content (ppm) of p,p′-DDT

\[
\text{Content (ppm) of} \ p,p′-DDT = \frac{\text{amount (g) of } p,p′-DDT}{M} \times \frac{A_{\text{TF}}}{A_{\text{SF}}} \times 50
\]
6. Total ash

Ignite previously a crucible of platinum, quartz or porcelain between 500°C and 550°C for 1 hour. Cool, and weigh accurately the crucible. Unless otherwise specified, weigh accurately 2 to 4 g of the test sample for analysis in this crucible, take off the lid or keep it open a little if necessary, heat the crucible at a low temperature at first, then gradually heat to a temperature between 500°C and 550°C, ignite to incinerate the residue for more than 4 hours until no carbonized substance remains in the ash, cool, and weigh accurately the ash. Incinerate repeatedly to constant mass, cool, weigh accurately, and determine the amount (%) of total ash. If a carbonized substance remains and a constant mass cannot be obtained in the above-mentioned method, extract the charred mass with hot water, collect the insoluble residue on filter paper for assay, and incinerate the residue and filter paper until no carbonized substance remains in the ash. Then add the filtrate, evaporate it to dryness, and incinerate. Cool, weigh accurately, and determine the mass (%) of the total ash. If a carbon-free ash cannot be obtained even in this way, moisten the ash with a small amount of ethanol (95), break up the ash with a glass rod, wash the rod with a small amount of ethanol (95), evaporate carefully, and determine the mass of the total ash as described above. A desiccator (silica gel) is used for cooling.

7. Acid-insoluble ash

Add carefully 25 mL of dilute hydrochloric acid to the total ash, boil gently for 5 minutes, collect the insoluble matter on filter paper for assay, and wash thoroughly with hot water. Dry the residue together with the filter paper, and ignite to incinerate in a tared crucible of platinum, quartz or porcelain for 3 hours. Cool in a desiccator (silica gel), weigh, and determine the amount (%) of acid-insoluble ash. When the amount determined exceeds the limit specified, incinerate repeatedly to a constant mass.

8. Extract content

The test for the extract content in crude drugs is performed as directed in the following methods:

8.1. Dilute ethanol-soluble extract

Unless otherwise specified, weigh accurately about 2.3 g of the sample for analysis, extract with 70 mL of dilute ethanol in a suitable flask with occasional shaking for 5 hours, and allow to stand for 16 to 20 hours. Filter, and wash the flask and residue with small portions of dilute ethanol until the filtrate measures 100 mL. Evaporate a 50 mL aliquot of the filtrate to dryness, dry at 105°C for 4 hours, and cool in a desiccator (silica gel). Weigh accurately the amount, multiply it by 2, and determine the amount of dilute ethanol-soluble extract. Calculate the extract content (%) with respect to the amount of the sample on the dried basis, obtained under the loss on drying.

8.2. Water-soluble extract

Proceed as directed in 8.1., using water instead of dilute ethanol, weigh accurately the amount, multiply by 2, and determine the amount of water-soluble extract. Calculate the extract content (%) with respect to the amount of the sample on the dried basis, obtained under the loss on drying.

8.3. Diethyl ether-soluble extract

Unless otherwise specified, dry the test sample for analysis in a desiccator (silica gel) for 48 hours, weigh accurately about 2 g of it, and place in a suitable flask. Add 70 mL of diethyl ether, attach a reflux condenser to the flask, and boil
gently on a water bath for 4 hours. Cool, filter, and wash the flask and the residue with small portions of diethyl ether until the filtrate measures 100 mL. Evaporate a 50 mL aliquot of the filtrate to dryness on a water bath, dry in a desiccator (silica gel) for 24 hours, weigh accurately the amount, multiply it by 2, determine the amount of diethyl ether-soluble extract, and calculate the extract content (\(z\)).

9. Essential oil content

The test of essential oil content in crude drugs is performed as directed in the following method.

9.1. Essential oil determination

Weigh the quantity of the test sample for analysis directed in the monograph in a 1-L hard glass-stoppered flask, and add from 5 to 10 times as much water as the drug. Set up an apparatus for essential oil determination (Fig. 5.01-1), inserting a reflux condenser (Fig. 5.01-2) in the upper mouth of it, and heat the content of the flask in an oil bath between 130°C and 150°C to boiling. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2.0 mL of xylene is added to the graduated tube. Unless otherwise specified, continue boiling for 5 hours, allow to stand for some time, and open the stopper of the apparatus. Draw off the water slowly until the surface of the oil layer corresponds to the preparation line, and allow it to stand for more than 1 hour at ordinary temperature. Then lower the surface of the oil layer to the zero line, and read the volume (mL) of the oil at ordinary temperature. Subtract the volume (mL) of xylene from the volume of the total oil.

5.02 Microbial Limit Test for Crude Drugs

This chapter provides tests for the qualitative and quantitative estimation of viable microorganisms present in crude drugs. It includes tests for total viable count (aerobic bacteria and fungi) and specified microbial species (Enterobacteria and other gram-negative bacteria, Escherichia coli, Salmonella, and Staphylococcus aureus). Microbial limit test must be carried out under conditions designed to avoid accidental microbial contamination of the preparation during the test. When test specimens have antimicrobial activity, or contain antimicrobial substances, any such antimicrobial properties must be eliminated by means of procedures such as dilution, filtration, neutralization or inactivation. For the test, use a mixture of several portions selected at random from the bulk or from the contents of a sufficient number of containers. If test specimens are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard.

1. Total viable aerobic count

This test determines mesophilic aerobic bacteria and fungi (molds and yeasts) which grow under aerobic conditions. Psychrophilic, thermophilic, basophilic, and anaerobic bacteria, and microorganisms which require specific ingredients for growth, may give a negative result, even if a significant number exists in the test specimens. The test may be carried out using one of the following 4 methods, i.e., pour plate method, spread plate method, serial dilution method (most probable number method) or membrane filtration method. Use an appropriate method depending on the purpose. An automated method may be used for the test presented here, provided it has been properly validated as giving equivalent or better results. Different culture media and temperature are required for the growth of bacteria and fungi (molds and yeasts). The serial dilution method is applicable only to the enumeration of bacteria.

1.1. Sampling and Preparation of the test specimens

Unless otherwise specified, samples should be taken by the following methods.

(i) When crude drugs to be sampled are small-sized, cut or powdered, 50 to 250 g of sample should be taken after mixing thoroughly.

(ii) When crude drugs to be sampled are large-sized, 250 to 500 g of sample should be taken after mixing thoroughly and cutting.

(iii) When the mass of each single piece of the crude drug is not less than 100 g, not less than 5 pieces should be taken for a sample, or not less than 500 g of the sample should be taken after cutting to a suitable size and mixing thoroughly. If necessary, cut more for use.

(iv) When crude drugs to be sampled are in the form of a solution or a preparation, the sample should be taken after mixing thoroughly.

(v) An insoluble solid should be taken after reducing the substance to a moderately fine powder.

1.2. Preparation of the test fluid

Phosphate Buffer, pH 7.2, Buffered Sodium Chloride-Peptone Solution, pH 7.0 or fluid medium used for the test is used to suspend or dilute the test specimen. Unless other-
wised specified, usually take 10 g or 10 mL of the test specimen, and suspend or dissolve it in 90 mL of the buffer or fluid medium specified. A test specimen as a suspension must be shaken for 10 minutes. If necessary, for crude drugs to which microorganisms might adhere, repeat the same method and use this as the test fluid. A different quantity or volume may be used if the nature of the test specimen requires it. The pH of the test fluid is adjusted to between 6 and 8. The test fluid must be used within an hour after preparation.

(i) Fluid specimen: Take 10 mL of the test specimen, and suspend or dissolve it in 90 mL of the buffer or fluid medium specified. A different quantity or volume may be used if the nature of the test specimen requires it.

(ii) Insoluble solids: Pulverize the substance to a moderately fine powder, take 10 g of the test specimen, and suspend it in 90 mL of the buffer or fluid medium specified. A different quantity or a larger volume of buffer and fluid medium than indicated may be used for the suspension, if the nature of the test specimen requires it. The suspension may be dispersed well using, if necessary, a mechanical blender. A suitable surface active agent (such as 0.1 w/v % Polysorbate 80) may be added to aid dissolution.

1.3. Test procedures

1.3.1. Pour Plate Method

Use petri dishes 9 to 10 cm in diameter. Use at least two petri dishes for each dilution. Pipet 1 mL of the test fluid or its diluted solution onto each petri dish aseptically. Promptly add to each dish 15 to 20 mL of sterilized agar medium that has previously been melted and kept below 45°C, and mix. Primarily for the detection of aerobic microbes, use Casein Soya Bean Digest Agar Medium. For specimens that consist of fragments of crude drugs, or to control the growth of fungi, TTC TS for aerobic bacterial strains and amphotericin B TS as an antimycotic may be added to the agar. Just prior to use, add 2.5–5 mL of TTC TS or 2 mL of amphotericin B TS per liter of sterile medium and mix. Primarily for the detection of fungi, use one of Sabouraud-Dextrose Agar Medium with Antibiotics, Potato Dextrose Agar Medium with Antibiotics, and GP Agar Medium with Antibiotics. For an agar medium that is suffused with fungi, Rose Bengal TS may be added to the agar. Add 5 mL of Rose Bengal TS per liter of agar medium, mix and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. After the agar solidifies, incubate the plates for at least 5 days at between 30°C and 35°C for aerobic bacteria, and between 20°C and 25°C for fungi. If too many colonies are observed, dilute the fluid as described above so that a colony count of not more than 300 per plate may be expected in the case of aerobic bacteria, and not more than 100 per plate in the case of fungi. If a reliable count is obtained in a shorter incubation time than 5 days, this may be adopted.

1.3.2. Spread Plate Method

On the solidified and dried surface of the agar medium, pipet 0.05 to 0.2 mL of the test fluid and spread it on the surface with a spreader. The diameter of petri dishes, the kind and volume of the medium to be used, TS to be added, temperature and time of incubation, and the method for calculation of total viable count are the same as described in the Pour Plate Method section.

1.3.3. Serial Dilution Method (Most Probable Number Method)

Prepare tubes each containing 9 to 10 mL of Fluid Casein Soya Bean Digest Medium. To each of the first three tubes add 1 mL of the test fluid (containing 0.1 g or 0.1 mL of specimen), resulting in 1 in 10 dilution. To the next three tubes add 1 mL of a 1 in 10 dilution of the fluid, resulting in 1 in 100 dilution. To the next three tubes add 1 mL of a 1 in 100 dilution of the fluid, resulting in 1 in 1000 dilution. If necessary, dilute further. To the last three tubes add 1 mL of the diluent as a control. Incubate the tubes between 30°C and 35°C for not less than 5 days. The control tubes should show no microbial growth. If the reading of the results is difficult or uncertain, transfer about 0.1 mL to a liquid or solid medium and read the results after a further period of incubation between 30°C and 35°C for 24 to 72 hours. Determine the most probable number of microorganisms per g or per mL of the specimen from Table 5.02-1.

If, for the first column (0.1 g or 0.1 mL of specimen), the number of tubes showing microbial growth is two or less, the most probable number of microorganisms per g or per mL is likely to be less than 100.

1.3.4. Membrane Filtration Method

This method employs membrane filters of appropriate materials, having a normal pore size not greater than 0.45 μm. Filter discs about 50 mm in diameter are recommended, but filters of a different diameter may also be used. Filters, the filtration apparatus, media, etc., should be well sterilized. Usually, take 20 mL of the test fluid (containing 2 g of test specimen), transfer 10 mL of the solution to each of two membrane filters, and filter. If necessary, dilute the pretreated preparation so that a colony count of 10 to 100 may be expected. After the filtration of the test fluid, wash each membrane by filtering through it three or more times with a suitable liquid such as Buffered Sodium Chloride-Peptone Solution, pH 7.0, Phosphate Buffer, pH 7.2, or the fluid medium to be used. The volume of the washing to be used is approximately 100 mL each time, but if the filter disc is not about 50 mm in diameter, the volume may be adjusted according to the size of the filter. For fatty substances, the

<table>
<thead>
<tr>
<th>Number of tubes in which microbial growth is observed for each quantity of the specimen</th>
<th>Most probable number of microorganisms per g or per mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 g or 0.1 mL per tube</td>
<td>0.01 g or 0.01 mL per tube</td>
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</table>
washings may contain a suitable surface-active agent such as Polysorbate 80. Put one of the membrane filters, intended primarily for the enumeration of aerobic bacteria, on the surface of a plate of Casein Soya Bean Digest Agar Medium and the other, intended primarily for the enumeration of fungi, on the surface of a plate of one of Sabouraud-Dextrose Agar Medium with Antibiotics, Potato Dextrose Agar Medium with Antibiotics, and GP Agar Medium with Antibiotics. After incubation of the plates for at least 5 days, at between 30°C and 35°C in the test for the detection of aerobic bacteria and between 20°C and 25°C in the test for fungi, count the number of colonies that are formed. If a reliable count is obtained in a shorter incubation time than 5 days, this may be adopted.

1.4. Effectiveness of culture media and confirmation of antimicrobial substances

Use microorganisms of the following strains or their equivalent. Grow them in Casein Soya Bean Digest Agar Medium between 30°C and 35°C for aerobic bacteria and between 20°C and 25°C for Candida albicans.

Escherichia coli, NBRC 3972, ATCC 8739, NCIMB 8545, etc.

Bacillus subtilis, NBRC 3134, ATCC 6633, NCIMB 8054, etc.

Staphylococcus aureus, NBRC 13276, ATCC 6538, NCIMB 9518, etc.

Candida albicans, NBRC 1393, NBRC 1594, ATCC 2091, ATCC 10231, etc.

Dilute portions of each of the cultures using Buffered Sodium Chloride-Peptone Solution, pH 7.0, or Phosphate Buffer, pH 7.2 to prepare test suspensions containing 50 to 200 cfu per mL. Growth-promoting qualities are tested by inoculating 1 mL of each microorganism into each medium. The test media are satisfactory if clear evidence of growth appears in all inoculated media after incubation at the indicated temperature for 5 days. When a count of test organisms with a test specimen is less than 1/5 of that without the test specimen, any such effect must be eliminated by dilution, filtration, neutralization or inactivation. To confirm the sterility of the medium and of the diluent and the aseptic performance of the test, carry out the total viable count method using sterile Buffered Sodium Chloride-Peptone Solution, pH 7.0 or Phosphate Buffer, pH 7.2 as the control.

2. Test for the detection of specified microorganisms

Enterobacteria and certain other gram-negative bacteria, Escherichia coli, Salmonella and Staphylococcus aureus, are included as target strains of the test.

2.1. Sampling and Preparation of the test specimens

Refer to the paragraph on 1.1. Sampling and Preparation of the test specimens.

2.2. Preparation of the test fluid

If necessary, refer to the paragraph on 1.2. Preparation of the test fluid. When test specimens are prepared, use the medium designated in each test, unless otherwise specified. If necessary, eliminate antimicrobial substances so as to permit growth of the inocula, and adjust the quantity of test specimen or increase the volume of medium to suitable values.

2.3. Test Procedure

2.3.1. Enterobacteria and certain other gram-negative bacteria

2.3.1.1. Detection of bacteria

To 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to form a suspension or solution. Transfer 10 mL to 90 mL of Fluid Enterobacteria Enrichment Broth-Mossel Medium and incubate at between 35°C and 37°C for 18 to 24 hours. Mix by gently shaking the container, take a portion by means of an inoculating loop, and streak it on the surface of Violet Red Bile Glucose Agar. Incubate at between 35°C and 37°C for 18 to 24 hours. If red or reddish colonies are found, the specimen may contain Enterobacteria and certain other gram-negative bacteria.

2.3.1.2. Quantitative evaluation

If Enterobacteria and certain other gram-negative bacteria are found, to 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to form a suspension or solution. Transfer 1 mL of the test fluid (containing 0.1 g or 0.1 mL of specimen) to a tube containing 9 mL of Fluid Enterobacteria Enrichment Broth-Mossel Medium, and mix. Next, transfer 1 mL of the test fluid (containing 0.01 g or 0.01 mL of specimen) to a tube containing 9 mL of Fluid Enterobacteria Enrichment Broth-Mossel Medium, and mix. Furthermore, transfer 1 mL of the test fluid (containing 1 mg or 1 μL of specimen) to a tube containing 9 mL of Fluid Enterobacteria Enrichment Broth-Mossel Medium, and mix. Incubate the tubes at between 35°C and 37°C for 18 to 24 hours, take a portion by means of an inoculating loop, and streak it on the surface of Violet Red Bile Glucose Agar. Incubate at between 35°C and 37°C for 18 to 24 hours. If red or reddish colonies are found, this constitutes a positive result. Note the smallest quantity of the product which gives a positive result and the largest quantity that gives a negative result. Determine from Table 5.02-2 the probable number of microorganisms.

2.3.2. Escherichia coli

2.3.2.1. Detection of bacteria

To 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to make a suspension or solution. Transfer 1 mL to a fermentation tube containing 9 to 10 mL of Fluid EC Medium and incubate the tube at 44.5 ± 0.2°C for 24 ± 2 hours in a water bath. If gas bubbles are not found, the specimen meets the requirements of the test for absence of Escherichia coli. If gas bubbles are found, take a portion by means of an inoculating loop, and streak it on the surface of EMB Agar Medium. Incubate at between 30°C and 35°C for 18 to 24 hours. Upon examination, if none of the colonies exhibits both a characteristic metallic sheen and a blue-black appearance under transmitted light, the specimen meets the requirements of the test for absence of Escherichia coli. Confirm any suspect colonies on the plate by means of the IMViC test. (Indole production test, Methyl red reaction test, Voges-Proskauer test, and Citrate utilization test); colonies which exhibit the pattern of either [+ + + −] or [− + + −] are judged as Escherichia coli. Rapid detection kits for Escherichia coli may also be used.

2.3.2.2. Quantitative evaluation

If Escherichia coli is found, prepare tubes each containing 9 to 10 mL of Fluid EC Medium. Use three tubes for each dilution. To 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium and suspend or dissolve. To each of the first three fermentation tubes add 1 mL of the test fluid (containing 0.1 g or 0.1 mL of specimen), resulting in 1 in 10 dilution. To the next three fermentation tubes add 1 mL of 1 in 10 dilution of the fluid, resulting in 1 in 100 dilution. To the next three fermentation tubes add 1 mL of a 1 in 100 dilution of the fluid, resulting in 1 in 1000 dilution. To the last
three fermentation tubes add 1 mL of the diluent as a control. Incubate the tubes at 44.5 ± 0.2°C for 24 ± 2 hours in a water bath. If gas bubbles are found, take a portion by means of an inoculating loop, and streak it on the surface of EMB Agar Medium. Incubate at between 30°C to 35°C for 18 to 24 hours. Upon examination, colonies of Gram-negative organisms show both a characteristic metallic sheen and a blue-black appearance under transmitted light. Determine the most probable number of microorganisms per g or per mL of the specimen from Table 5.02-1.

2.3.3. Salmonella

As in the case of the detection of *Escherichia coli*, to 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to form a suspension or solution. Incubate at between 30°C to 35°C for 24 to 72 hours. Examine the medium for growth, and if growth is apparent, mix by gentle shaking, then pipet 1 mL portions, respectively, into 10 mL of Fluid Selenite-Cystine Medium and Fluid Tetrathionate Medium, and incubate for 12 to 24 hours. 10 mL of Fluid Selenite-Cystine Medium may be replaced by the same volume of Fluid Rappaport Medium. After the incubation, streak portions from both the fluid media on the surface of at least two of Brilliant Green Agar Medium, XLD Agar Medium, and Bismuth Sulfite Agar Medium, and incubate at between 30°C and 35°C for 24 to 48 hours. Upon examination, if none of the colonies conforms to the description given in Table 5.02-3, the specimen meets the requirements of the test for the absence of the genus *Salmonella*. If colonies of Gram-negative rods matching the description in Table 5.02-3 are found, transfer suspect colonies individually, by means of an inoculating wire, to a slant of TSI Agar Medium using both surface and deep inoculation. Incubate at between 35°C and 37°C for 18 to 24 hours. The presence of genus *Salmonella* is confirmed if, in the deep culture but not in the surface culture, there is a change of color from red to yellow and usually formation of gas with or without production of hydrogen sulfide. Precise identification and typing of genus *Salmonella* may be carried out by using appropriate biochemical and serological tests additionally, including an identification kit.

2.3.4. *Staphylococcus aureus*

To 10 g or 10 mL of the test specimen add 90 mL of Fluid Casein Soya Bean Digest Medium, or another suitable fluid medium without antimicrobial activity, to form a suspension or solution. Incubate the fluid containing the specimen at between 30°C and 35°C for 24 to 48 hours, and pipet 1 mL into 9 mL of Fluid Casein Soya Bean Digest Medium with 7.5% sodium chloride. If, upon examination, growth is apparent, use an inoculating loop to streak a portion of the medium on the surface of one of Vogel-Johnson Agar Medium, Baird-Parker Agar Medium, or Mannitol-Salt Agar Medium, and incubate at between 30°C and 35°C for 24 to 48 hours. Upon examination, if no colonies of Gram-positive rods having the characteristics listed in Table 5.02-4 are found, the specimen meets the requirements of the test for the absence of *Staphylococcus aureus*. Confirm any suspect colonies as *Staphylococcus aureus* by means of the coagulate test. With the aid of an inoculating loop, transfer suspect colonies to individual tubes, each containing 0.5 mL of mammalian, preferably rabbit or horse, plasma with or without suitable additives. Incubate in a thermostat at 37 ± 1°C. Examine the coagulation after 3 hours and subsequently at suitable intervals up to 24 hours. Test positive and negative controls simultaneously. If no coagulation is observed, the specimen meets the requirements of the test for the absence of *Staphylococcus aureus*.

2.4. Effectiveness of culture media and confirmation of antimicrobial substances

Grow the test strains listed in Table 5.02-5 in the media indicated at between 30°C and 35°C for 18 to 24 hours. Dilute portions of each of the cultures using Buffered Sodium

<table>
<thead>
<tr>
<th>Table 5.02-2</th>
<th>Most probable number of microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results for each quantity of product</td>
<td>Probable number of microorganisms (CFU per g or per mL)</td>
</tr>
<tr>
<td>0.1 g or 0.1 mL</td>
<td>0.01 g or 0.01 mL</td>
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<tr>
<td>+</td>
<td>+</td>
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<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5.02-3</th>
<th>Morphologic characteristics of <em>Salmonella</em> species on selective agar media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>Description of colony</td>
</tr>
<tr>
<td>Brilliant Green Agar Medium</td>
<td>Small, transparent and colorless, or opaque, pink or white (often surrounded by a pink to red zone)</td>
</tr>
<tr>
<td>XLD Agar Medium</td>
<td>Red, with or without a black center</td>
</tr>
<tr>
<td>Bismuth Sulfite Agar Medium</td>
<td>Black or green</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5.02-4</th>
<th>Morphologic characteristics of <em>Staphylococcus aureus</em> on selective agar media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>Colonial characteristics</td>
</tr>
<tr>
<td>Vogel-Johnson Agar Medium</td>
<td>Black surrounded by a yellow zone</td>
</tr>
<tr>
<td>Baird-Parker Agar Medium</td>
<td>Black, shiny, surrounded by a clear zone</td>
</tr>
<tr>
<td>Mannitol-Salt Agar Medium</td>
<td>Yellow colonies surrounded by a yellow zone</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5.02-5</th>
<th>Bacteria strains and media used for confirmation of the effectiveness of culture medium and validity of the test for specified microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microorganism</td>
<td>Strain number</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>NBRC 3972, ATCC 8739, NCIMB 8545 or equivalent strains</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>No strain number is recommended*</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>NBRC 15276, ATCC 6538, NCIMB 9518 or equivalent strains</td>
</tr>
</tbody>
</table>

*Salmonella* strains of weak or no pathogenicity may be used. *Salmonella* typhi may not be used.
Chloride-Peptone Solution, pH 7.0, Phosphate Buffer, pH 7.2, or medium indicated for each bacterial strain to make
test suspensions containing about 1000 CFU per mL. As occa-
sion demands, using a mixture of 0.1 mL of each suspen-
sion of Escherichia coli, Salmonella and Staphylococcus aureus
containing about 1000 CFU, test the validity of the medium
and the presence of antimicrobial substances in the
presence or absence of the specimen.

2.5. Retest
For the purpose of confirming a doubtful result, a retest is
conducted using a test specimen 2.5 times the first test speci-
men. Proceed as directed under Test procedure, but make
allowance for the larger specimen size, for example by ad-
justing the volume of the medium.

3. Buffer solution, media and test solution (TS)
Buffer solution, media and TS used for the microbial limit
test are described below. Other media may be used if they
have similar nutritive ingredients, and selective and growth-
promoting properties for the microorganisms to be tested.

3.1. Buffer solution
(i) Phosphate Buffer, pH 7.2
For use, dilute the Stock Solution with water in the ratio
of 1 to 800, and sterilize at 121°C for 15 to 20 minutes.
Stock Solution: Dissolve 34 g of potassium dihydrogen
phosphate in about 500 mL of water. Adjust to pH 7.1 to 7.3
by the addition of 175 mL of sodium hydroxide TS, add
water to make 1000 mL, and use this solution as the stock
solution. After sterilization by heating in an autoclave, store
under refrigeration.

(ii) Buffered Sodium Chloride-Peptone Solution, pH 7.0
Potassium dihydrogen phosphate 3.6 g
Disodium hydrogen phosphate dihy-
drate (equivalent to 0.067 mol
phosphate) 7.2 g
Sodium chloride 4.3 g
Peptone (animal tissue
or casein) 1.0 g
Water 1000 mL
Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization:
6.9 – 7.1. Polysorbate 20 or 80 (0.1 to 1.0 w/v%)
may be added.

3.2. Media
(i) Casein Soya Bean Digest Agar Medium
Pancreatic digest of casein 15.0 g
Soybean peptone 5.0 g
Sodium chloride 5.0 g
Agar 15.0 g
Water 1000 mL
Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization:
7.1 – 7.5.

(ii) Fluid Casein Soya Bean Digest Medium
Pancreatic digest of casein 17.0 g
Soybean peptone 3.0 g
Sodium chloride 5.0 g
Dipotassium hydrogen phosphate 2.5 g
Glucose monohydrate 2.5 g
Water 1000 mL
Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization:
7.1 – 7.5.

(iii) Sabouraud-Dextrose Agar Medium with Antibiotics
Glucose 40.0 g
Peptone (animal tissue and casein, 1:1) 10.0 g
Agar 15.0 g
Water 1000 mL
Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, pH after sterilization:
5.4 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin
potassium and 0.10 g of tetracycline per liter of medium as
sterile solutions or, alternatively, add 50 mg of chloram-
phenicol per liter of medium.

(iv) Potato Dextrose Agar Medium with Antibiotics
Potato extract 4.0 g
Glucose 20.0 g
Agar 15.0 g
Water 1000 mL
Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization:
5.4 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin
potassium and 0.10 g of tetracycline per liter of medium as
sterile solutions or, alternatively, add 50 mg of chloram-
phenicol per liter of medium.

(v) GP (Glucose-peptone) Agar Medium with Antibio-
tics
Glucose 20.0 g
Yeast extract 2.0 g
Magnesium sulfate heptahydrate 0.5 g
Peptone 5.0 g
Potassium dihydrogen phosphate 1.0 g
Agar 15.0 g
Water 1000 mL
Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization:
5.6 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin
potassium and 0.10 g of tetracycline per liter of medium as
sterile solutions or, alternatively, add 50 mg of chloram-
phenicol per liter of medium.

(vi) Fluid Lactose Medium
Meat extract 3.0 g
Gelatin peptone 5.0 g
Lactose monohydrate 5.0 g
Water 1000 mL
Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization:
6.7 – 7.1. After sterilization, cool immediately.

(vii) Fluid EC Medium
Peptone 20.0 g
Lactose monohydrate 5.0 g
Bile salts 1.5 g
Dipotassium hydrogen phosphate 4.0 g
Potassium dihydrogen phosphate 1.5 g
Sodium chloride 5.0 g
Water 1000 mL
Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization:
6.8 – 7.0. After sterilization cool immediately. If gas remains
in durham tube after cool, do not use the tube.

(viii) EMB (Eosin-Methylene Blue) Agar Medium
Gelatin peptone 10.0 g
Dipotassium hydrogen phosphate 2.0 g
Lactose monohydrate 10.0 g
Agar 15.0 g
(x) Fluid Enterobacteria Enrichment Broth-Mossel Medium

- Gelatin peptone: 10.0 g
- Glucose monohydrate: 5.0 g
- Bile salts: 20.0 g
- Potassium dihydrogen phosphate: 2.0 g
- Disodium hydrogen phosphate dihydrate: 8.0 g
- Brilliant green: 15 mg
- Water: 1000 mL

Mix all the components, boil at 100°C for 30 minutes, and cool immediately. pH after boiling: 7.0 – 7.4. Do not sterilize in an autoclave.

(xi) Fluid Selenite-Cystine Medium

- Gelatin peptone: 5.0 g
- Lactose monohydrate: 4.0 g
- Trisodium phosphate dodecahydrate: 10.0 g
- Sodium selenite: 4.0 g
- L-Cystine: 10 mg
- Water: 1000 mL

Mix all the components, heat to dissolve. Final pH: 6.8 – 7.2. Do not sterilize.

(xii) Fluid Tetrathionate Medium

- Pancreatic digest of casein: 2.5 g
- Animal tissue peptone: 2.5 g
- Sodium deoxycholate: 1.0 g
- Calcium carbonate: 10.0 g
- Sodium thiosulfate pentahydrate: 30.0 g
- Water: 1000 mL

Heat the solution of solids to boiling. On the day of use, add a solution prepared by dissolving 5 g of potassium iodide and 6 g of iodine in 20 mL of water. Then add 10 mL of a solution of brilliant green (1 in 1000), and mix. Do not heat the medium after adding the brilliant green solution.

(xiii) Fluid Rappaport Medium

- Soybean peptone: 5.0 g
- Sodium chloride: 8.0 g
- Potassium dihydrogen phosphate: 1.6 g
- Malachite green oxalate: 0.12 g
- Magnesium chloride hexahydrate: 40.0 g
- Water: 1000 mL

Dissolve malachite green oxalate and magnesium chloride hexahydrate, and the remaining solids separately in the water, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. For the use, mix the both solutions after sterilization. Final pH: 5.4 – 5.8.
nium iron (II) sulfate hexahydrate may be used.

(xviii) Fluid Casein Soya Bean Digest Medium with 7.5% Sodium Chloride

- Pancreatic digest of casein: 17.0 g
- Soybean peptone: 3.0 g
- Sodium chloride: 75.0 g
- Dipotassium hydrogen phosphate: 2.5 g
- Glucose monohydrate: 2.5 g
- Water: 1000 mL

Add the sodium chloride 70.0 g for (ii) Fluid Casein Soya Bean Digest Medium (containing 5 g of sodium chloride), mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.5.

(xix) Vogel-Johnson Agar Medium

- Pancreatic digest of casein: 10.0 g
- Yeast extract: 5.0 g
- D-Mannitol: 10.0 g
- Dipotassium hydrogen phosphate: 5.0 g
- Lithium chloride: 5.0 g
- Glycine: 10.0 g
- Phenol red: 25 mg
- Agar: 16.0 g
- Water: 1000 mL

Mix all the components, and boil for 1 minute to make solution. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 7.0 – 7.4. To this solution add 20 mL of sterile potassium tellurite solution (1 in 100), and mix.

(xx) Baird-Parker Agar Medium

- Pancreatic digest of casein: 10.0 g
- Meat extract: 5.0 g
- Yeast extract: 1.0 g
- Lithium chloride: 5.0 g
- Glycine: 12.0 g
- Sodium pyruvate: 10.0 g
- Agar: 20.0 g
- Water: 950 mL

Mix all the components. Heat the mixture with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 6.6 – 7.0. To this solution add 10 mL of sterile potassium tellurite solution (1 in 100) and 50 mL of egg-yolk emulsion. Mix gently, and pour into petri dishes. Prepare the egg-yolk emulsion by mixing egg-yolk and sterile saline with the ratio of about 30% to 70%.

(xxi) Mannitol-Salt Agar Medium

- Pancreatic digest of casein: 5.0 g
- Animal tissue peptone: 5.0 g
- Bovine meat extract: 1.0 g
- D-Mannitol: 10.0 g
- Sodium chloride: 75.0 g
- Phenol red: 25 mg
- Agar: 15.0 g
- Water: 1000 mL

Mix all the components. Heat with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.2 – 7.6.

3.3. Reagents/Testing solutions

(i) Amphotericin B powder: Amphotericin B added sodium deoxycholic acid, sterilized by γ-ray. Amphotericin B TS Dissolve 22.5 mg of amphotericin B powder in 9 mL of sterile purified water.

(ii) Bile salts: Yellow-brown powder made from dried bile of animal, consist of sodium taurocholic acid and sodium glycocholic acid, and containing not less than 45% of cholic acid. pH of 5% solution: 5.5 – 7.5.

(iii) Rose Bengal TS: Dissolve 1 g of rose bengal in water to make 100 mL. Rose bengal C₂₅H₃₈Cl₂I₄Na₂O₅. [Special class] Red-brown powder, purple-red solution in water.

(iv) TTC TS: Dissolve 0.8 g of 2,3,5-triphenyl-2H-tetrazolium chloride in water to make 100 mL, distribute in small tubes, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. Store in light-resistant containers.

6. Tests for Preparations

6.01 Test for Metal Particles in Ophthalmic Ointments

Test of Metal Particles in Ophthalmic Ointments is a method to test the existence of foreign metal particles in the ophthalmic ointments described in General Rules for Preparations.

1. Preparation of Test Sample

The test should be carried out in a clean place. Take 10 ophthalmic ointments to be tested, and extrude 5 g each of their contents into separate flat-bottomed petri dishes 60 mm in diameter. Cover the dishes, and heat between 85°C and 110°C for 2 hours to dissolve bases completely. Allow the samples to cool to room temperature without agitation to solidify the contents. When the amount of the content is less than 5 g, extrude the contents as completely as practicable, and proceed in the same manner as described above.

2. Procedure

Invert each dish on the stage of a suitable microscope previously adjusted to provide not less than 40 times magnifications and equipped with an eyepiece micrometer disk. Each dish is illuminated from above 45° relative to the plane of the dish. Examine the entire bottom of each dish for metal particles, and record the total number of particles, measuring 50 μm or more in any dimension.

Note: Use petri dishes with a clean bottom and free from foams and scratches, and if possible, the walls are at right angles with the bottom.

3. Evaluation

The preparation complies with the test if the total number...
of metal particles of a size equal to or greater than 50 μm found in 10 units tested, is not more than 50, and also the number of dishes containing more than 8 particles is not more than 1. If this requirement is not met, repeat the test with a further 20 units in the same manner, and if the total number of the particles found in the total of 30 units is not more than 150, and also the number of dishes containing more than 8 particles is not more than 3, the preparation complies with the test.

6.02 Uniformity of Dosage Units

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (••). The term “Uniformity of dosage unit” is defined as the degree of uniformity in the amount of the drug substance among dosage units. Therefore, the requirements of this chapter apply to each drug substance being comprised in dosage units containing one or more drug substances, unless otherwise specified elsewhere in this Pharmacopoeia.

To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage units are defined as dosage forms containing a single dose or a part of a dose of a drug substance in each dosage unit. The Uniformity of Dosage Units specification is not intended to apply to suspensions, emulsions, or gels in unit-dose containers intended for external, cutaneous administration.

The uniformity of dosage units can be demonstrated by either of two methods, Content Uniformity or Mass variation (see Table 6.02-1.). The test for Content Uniformity of preparations presented in dosage units is based on the assay of the individual contents of drug substance(s) of a number of dosage units to determine whether the individual contents are within the limits set. The Content Uniformity method may be applied in all cases.

The test for Mass Variation is applicable for the following dosage forms:

(i) solutions enclosed in unit-dose containers and into soft capsules •in which all components are perfectly dissolved•;

(ii) solids (including powders, granules and sterile solids) that are packaged in single-unit containers and contain no active or inactive added substances;

(iii) solids (including sterile solids) that are packaged in single-unit containers, with or without active or inactive added substances, that have been prepared from true solutions •in which all components are perfectly dissolved•, and freeze-dried in the final containers and are labeled to indicate this method of preparation; and

(iv) hard capsules, uncoated tablets, or film-coated tablets, containing 25 mg or more of a drug substance comprising 25% or more, by weight, of the dosage unit or, in the case of hard capsules, the capsule contents, •or in the case of film-coated tablets, the pre-coated tablets•, except that uniformity of other drug substances present in lesser proportions is demonstrated by meeting Content Uniformity requirements.

The test for Content Uniformity is required for all dosage forms not meeting the above conditions for the Mass Variation test. Alternatively, products listed in item (4) above that do not meet the 25 mg/25% threshold limit may be tested for uniformity of dosage units by Mass Variation instead of the Content Uniformity test if the concentration relative standard deviation (RSD) of the drug substance in the final dosage units is not more than 2%, based on process validation data and development data, and if there has been regulatory approval of such a change. The concentration RSD is the RSD of the concentration per dosage unit (w/w

---

### Table 6.02-1 Application of Content Uniformity (CU) and Mass Variation (MV) Test for Dosage Forms

<table>
<thead>
<tr>
<th>Dosage Forms</th>
<th>Type</th>
<th>Sub-type</th>
<th>Dose and ratio of drug substance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥25 mg &amp; ≥25%</td>
</tr>
<tr>
<td>Tablets</td>
<td>uncoated</td>
<td>Film</td>
<td>MV</td>
</tr>
<tr>
<td></td>
<td>coated</td>
<td>others</td>
<td>MV</td>
</tr>
<tr>
<td>Capsules</td>
<td>hard</td>
<td>MV</td>
<td>CU</td>
</tr>
<tr>
<td></td>
<td>soft</td>
<td>Sus., eml., gel</td>
<td>CU</td>
</tr>
<tr>
<td></td>
<td>solutions</td>
<td>MV</td>
<td>MV</td>
</tr>
<tr>
<td>Solids in single unit</td>
<td>Single component</td>
<td>MV</td>
<td>MV</td>
</tr>
<tr>
<td>containers *(divided forms, lyophilized forms, et al.)•</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multiple components</td>
<td>Solution freeze-dried in final container</td>
<td>MV</td>
</tr>
<tr>
<td></td>
<td>others</td>
<td>MV</td>
<td>CU</td>
</tr>
<tr>
<td>Solutions *(perfectly</td>
<td>MV</td>
<td></td>
<td>MV</td>
</tr>
<tr>
<td>dissolved•, enclosed in</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unit-dose containers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td>CU</td>
</tr>
</tbody>
</table>

Sus.: suspension; eml.: emulsion;
Table 6.02-2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Conditions</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}$</td>
<td>mean of individual contents $(x_1, x_2, \ldots, x_n)$ expressed as a percentage of the label claim</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$x_1, x_2, \ldots, x_n$</td>
<td>individual contents of the dosage units tested, expressed as a percentage of the label claim</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>sample size (number of dosage units in a sample)</td>
<td>If $n = 10$, then</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If $n = 30$, then</td>
<td>2.0</td>
</tr>
<tr>
<td>$k$</td>
<td>acceptability constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$s$</td>
<td>sample standard deviation</td>
<td>$s = \sqrt{\frac{\sum(x_i-\bar{x})^2}{n-1}}$</td>
<td></td>
</tr>
<tr>
<td>$RSD$</td>
<td>relative standard deviation (the sample standard deviation expressed as a percentage of the mean)</td>
<td>$100s/\bar{x}$</td>
<td></td>
</tr>
<tr>
<td>$M$ (case 1)</td>
<td>reference value</td>
<td>If $98.5% \leq \bar{x} \leq 101.5%$, then</td>
<td>$M = \bar{x}$ $(AV = ks)$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If $\bar{x} &lt; 98.5%$, then</td>
<td>$M = 98.5%$ $(AV = 98.5 - \bar{x} + ks)$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If $\bar{x} &gt; 101.5%$, then</td>
<td>$M = 101.5%$ $(AV = \bar{x} - 101.5 + ks)$</td>
</tr>
<tr>
<td>$M$ (case 2)</td>
<td>reference value</td>
<td>If $98.5% \leq \bar{x} \leq T$, then</td>
<td>$M = \bar{x}$ $(AV = ks)$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If $\bar{x} &lt; 98.5%$, then</td>
<td>$M = 98.5%$ $(AV = 98.5 - \bar{x} + ks)$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If $\bar{x} &gt; T$, then</td>
<td>$M = T%$ $(AV = \bar{x} - T + ks)$</td>
</tr>
<tr>
<td>Acceptance Value $(AV)$</td>
<td></td>
<td>general formula: $</td>
<td>M - \bar{x}</td>
</tr>
<tr>
<td>$L_1$</td>
<td>maximum allowed acceptance value</td>
<td></td>
<td>$L_1 = 15.0$ unless otherwise specified.</td>
</tr>
<tr>
<td>$L_2$</td>
<td>maximum allowed range for deviation of each dosage unit tested from the calculated value of $M$</td>
<td>On the low side, no dosage unit result can be less than 0.75M while on the high side, no dosage unit result can be greater than 1.25M (This is based on an $L_2$ value of 25.0.)</td>
<td>$L_2 = 25.0$ unless otherwise specified.</td>
</tr>
<tr>
<td>$T$</td>
<td>target test sample amount at time of manufacture. Unless otherwise specified in the individual monograph, $T$ is 100.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
or w/v), where concentration per dosage unit equals the assay result per dosage unit divided by the individual dosage unit weight. See the RSD formula in Table 6.02-2.

1. Content Uniformity

Select not less than 30 units, and proceed as follows for the dosage form designated.

Where different procedures are used for assay of the preparation and for the content uniformity test, it may be necessary to establish a correction factor to be applied to the results of the latter.

(i) Solid dosage forms: Assay 10 units individually using an appropriate analytical method. Calculate the acceptance value (see Table 6.02-2).
(ii) Liquid dosage forms: Assay 10 units individually using an appropriate analytical method. Carry out the assay on the amount of well-mixed material that is removed from an individual container in conditions of normal use and express the results as delivered dose. Calculate the acceptance value (see Table 6.02-2).

1.1. Calculation of Acceptance Value

Calculate the acceptance value by the formula:

\[ |M - X| + ks \]

in which the terms are as defined in Table 6.02-2.

2. Mass Variation

Mass Variation is carried out based on the assumption that the concentration (mass of drug substance per mass of dosage unit) is uniform in a lot.

Carry out an assay for the drug substance(s) on a representative sample of the batch using an appropriate analytical method. This value is result A, expressed as % of label claim (see Calculation of the Acceptance Value). Select not less than 30 dosage units, and proceed as follows for the dosage form designated.

(i) Uncoated or film-coated Tablets: Accurately weigh 10 tablets individually. Calculate the content, expressed as % of label claim, of each tablet from the individual masses of the dosage units tested, and the result of the assay. Calculate the acceptance value.
(ii) Hard Capsules: Accurately weigh 10 capsules individually, taking care to preserve the identity of each capsule. Remove the contents of each capsule by suitable means. Accurately weigh the emptied shells individually, and calculate for each capsule the net mass of its contents by subtracting the mass of the shell from the respective gross mass. Calculate the drug substance content of each capsule from the mass of the individual capsules and the result of the assay. Calculate the acceptance value.
(iii) Soft Capsules: Accurately weigh the 10 intact capsules individually to obtain their gross masses, taking care to preserve the identity of each capsule. Then cut open the capsules by means of a suitable clean, dry cutting instrument such as scissors or a sharp open blade, and remove the contents by washing with a suitable solvent. Allow the occluded solvent to evaporate from the shells at room temperature over a period of about 30 min, taking precautions to avoid uptake or loss of moisture. Weigh the individual shells, and calculate the net contents. Calculate the drug substance content in each capsule from the mass of product removed from the individual capsules and the result of the assay. Calculate the acceptance value.
(iv) Solid dosage forms other than tablets and capsules: Proceed as directed for Hard Capsules, treating each dosage unit as described therein. Calculate the acceptance value.
(v) Liquid dosage forms: Accurately weigh the amount of liquid that is removed from each of 10 individual containers in conditions of normal use. Calculate the drug substance content in each container from the mass of product removed from the individual containers and the result of the assay. Calculate the acceptance value.

2.1. Calculation of Acceptance Value

Calculate the acceptance value as shown in Content Uniformity, except that \( X \) is replaced with \( A \), and that the individual contents of the dosage units are replaced with the individual estimated contents defined below.

\[ x_1, x_2, \ldots, x_n \] individual estimated contents of the dosage units tested, where

\[ x_i = w_i \times \frac{A}{W} \]

\( w_1, w_2, \ldots, w_n \) individual masses of the dosage units tested, \( A \) = content of drug substance (% of label claim) obtained using an appropriate analytical method.
\( W \) = mean of individual masses (\( w_1, w_2, \ldots, w_n \)).

3. Criteria

Apply the following criteria, unless otherwise specified.
(i) Solid and Liquid dosage forms: The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to 1%, and that the individual contents of the dosage units are replaced with the individual estimated contents defined below.
(ii) Solid and Liquid dosage forms: The requirements are met if the final acceptance value of the 30 dosage units is less than or equal to 1% and no individual content of the dosage unit is less than \((1 - L_2 \times 0.01)M\) nor more than 
\((1 + L_2 \times 0.01)M\) in Calculation of Acceptance Value under Content Uniformity or under Mass Variation. Unless otherwise specified, \( L_1 \) is 15.0 and \( L_2 \) is 25.0.

### 6.03 Particle Size Distribution

#### Test for Preparations

Particle Size Distribution Test for Preparations is a method to determine the particle size distribution of preparations described in General Rules for Preparations.

1. Procedure

The test is performed employing No. 18 (850 μm) and No. 30 (500 μm) sieves with the inside diameter of 75 mm.

Weigh accurately 10.0 g of sample to be tested, and place on the uppermost sieve which is placed on the other sieves described above and a close-fitting receiving pan and is covered with a lid. Shake the sieves in a horizontal direction for 3 minutes, and tap slightly at intervals. Weigh the amount remaining on each sieve and in the receiving pan.

### 6.04 Test for Acid-neutralizing Capacity of Gastrointestinal Medicines

Test for Acid-neutralizing Capacity of Gastrointestinal Medicines is a test to determine the acid-neutralizing capaci-
ty of a medicine, as a crude material or preparation, which reacts with the stomach acid and exercises an acid control action in the stomach. When performing the test according to the following procedure, the acid-neutralizing capacity of a crude material is expressed in terms of the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per g of the material, and that of a preparation is expressed by the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per dose per day (when the daily dose varies, the minimum dose is used).

1. Preparation of sample
   A crude material and a solid preparation which conforms to Powders in the General Rules for Preparations: may be used, without any treatment, as the sample. Preparations in dose-unit packages: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, mix uniformly, and use the mixture as the sample. Granules in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, powder it, and use as the sample. Granules not in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: take not less than 20 doses, powder it, and use as the sample. Capsules and tablets: take not less than 20 doses, weigh accurately, calculate the average mass for a daily dose, powder it, and use as the sample. Liquid preparations: shake well, and use as the sample.

2. Procedure
   Take an amount of the sample so that \( a \) in the equation falls between 20 mL and 30 mL, and perform the test.

   Accurately weigh the sample of the crude material or preparation, and place it in a glass-stoppered, 200-mL flask. Add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper tightly, shake at 37 ± 9°C for 1 hour, and filter. Take precaution against gas to be generated on the addition of 0.1 mol/L hydrochloric acid VS, and stopper tightly. After cooling, filter the solution again, if necessary. Pipet 50 mL of the filtrate, and titrate \( \Delta \) the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination \( \Delta \), end point: pH 3.5). Perform a blank determination.

   For liquid preparations, pipet the sample in a 100-mL volumetric flask, add water to make 45 mL, then add exactly 50 mL of 0.1 mol/L hydrochloric acid VS while shaking. Add water again to make the solution 100 mL. Transfer the solution to a glass-stoppered, 200-mL flask, wash the residue with 20.0 mL of water, stopper tightly, shake at 37 ± 2°C for 1 hour, and filter. Pipet 60 mL of the filtrate, and titrate \( \Delta \) the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination \( \Delta \), end point: pH 3.5). Perform a blank determination.

   Acid-neutralizing capacity (amount of 0.1 mol/L hydrochloric acid VS consumed per g or daily dose) (mL)
   \[
   t = (b - a) / f \times 2 \times (l/s)
   \]
   \( a \): Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed
   \( b \): Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed in the blank determination
   \( f \): The molarity coefficient of 0.1 mol/L sodium hydroxide VS

6.05 Test for Extractable Volume of Parenteral Preparations

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (*, **).

*Test for Extractable Volume of Parenteral Preparations is performed to confirm that a slightly excess volume is filled for the nominal volume to be withdrawn. Injections may be supplied in single-dose containers such as ampoules or plastic bags, or in multi-dose containers filled with a volume of injection which is sufficient to permit administration of the nominal volume declared on the label. The excess volume is determined by the characteristics of the product.

Suspensions and emulsions must be shaken before withdrawal of the contents and before the determination of the density. Oily and viscous preparations may be warmed according to the instructions on the label, if necessary, and thoroughly shaken immediately before removing the contents. The contents are then cooled to 20–25°C before measuring the volume.

1. Single-dose containers
   Select one container if the volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. Take up individually the total contents of each container selected into a dry syringe of a capacity not exceeding three times the volume to be measured, and fitted with a 21-gauge needle not less than 2.5 cm in length. Expel any air bubbles from the syringe and needle, then discharge the contents of the syringe without reinserting the needle into a standardized dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40% of its graduated volume. Alternatively, the volume of the contents in milliliters may be calculated as the mass in grams divided by the density.

   For containers with a nominal volume of 2 mL or less the contents of a sufficient number of containers may be pooled to obtain the volume required for the measurement provided that a separate, dry syringe assembly is used for each container. The contents of containers holding 10 mL or more may be determined by opening them and emptying the contents directly into the graduated cylinder or tared beaker.

   The volume is not less than the nominal volume in case of containers examined individually, or, in case of containers with a nominal volume of 2 mL or less, is not less than the sum of the nominal volumes of the containers taken collectively.

2. Multi-dose containers
   For injections in multidose containers labeled to yield a specific number of doses of a stated volume, select one container and proceed as directed for single-dose containers using the same number of separate syringe assemblies as the
number of doses specified. The volume is such that each syringe delivers not less than the stated dose.

3. Cartridges and pre-filled syringes
   Select one container if the volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. If necessary, fit the containers with the accessories required for their use (needle, piston, syringe) and transfer the entire contents of each container without emptying the needle into a dry tared beaker by slowly and constantly depressing the piston. Determine the volume in milliliters calculated as the mass in grams divided by the density. The volume measured for each of the containers is not less than the nominal volume.

4. Parenteral infusions
   Select one container. Transfer the contents into a dry measuring cylinder of such a capacity that the volume to be determined occupies at least 40% of the nominal volume of the cylinder. Measure the volume transferred. The volume is not less than the nominal volume.

6.06 Foreign Insoluble Matter Test for Injections

Foreign Insoluble Matter Test for Injections is a test method to examine foreign insoluble matters in injections.

1. Method 1.
   This method is applied to either injections in solutions, or vehicles for solid injections to be dissolved before use.
   Clean the exterior of containers, and inspect with the unaided eyes at a position of light intensity of approximately 1000 lx under an incandescent lamp. Injections or vehicles must be clear and free from readily detectable foreign insoluble matters. As to Injections in plastic containers for aqueous injections, the inspection should be performed with the unaided eyes at a position of light intensity of approximately 8000 to 10,000 lx, with an incandescent lamp at appropriate distances above and below the container.

   This method is applied to solid injections to be dissolved before use.
   Clean the exterior of containers, and dissolve the contents with vehicles or with Water for Injection carefully, avoiding any contamination with extraneous foreign substances. The solution thus constituted must be clear and free from foreign insoluble matters that is clearly detectable when inspected with the unaided eyes at a position of light intensity of approximately 1000 lx, right under an incandescent lamp.

6.07 Insoluble Particulate Matter Test for Injections

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols ( *  •).

Insoluble particulate matters in injections and parenteral infusions consist of extraneous, mobile undissolved particles, other than gas bubbles, that are unintentionally present in the solutions.

For the determination of particulate contamination, 2 procedures, Method 1 (Light Obscuration Particle Count Test) and Method 2 (Microscopic Particle Count Test), are specified hereinafter. When examining injections and parenteral infusions for sub-visible particles, Method 1 is preferably applied. However, it may be necessary to test some preparations by Method 1 followed by Method 2 to reach a conclusion on conformance to the requirements.

Not all parenteral preparations can be examined for sub-visible particles by one or both of these methods. When Method 1 is not applicable, e.g. in case of preparations having reduced clarity or increased viscosity, the test should be carried out according to Method 2. Emulsions, colloids, and liposomal preparations are examples. Similarly, products that produce air or gas bubbles when drawn into the sensor may also require microscopic particle count testing. If the viscosity of the preparation to be tested is sufficiently high so as to preclude its examination by either test method, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

The results obtained in examining a discrete unit or group of units for particulate contamination cannot be extrapolated with certainty to other units that remain untested. Thus, statistically sound sampling plans must be developed if valid inferences are to be drawn from observed data to characterise the level of particulate contamination in a large group of units.

1. Method 1. Light Obscuration Particle Count Test

1.1. Apparatus
   Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of the size of particles and the number of particles according to size. *It is necessary to perform calibration, as well as to demonstrate the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution, and counting accuracy, at least once a year. •

   *1.1.1. Calibration
   Particles to be used for calibration should be subject to particle size sensitivity measurement, using spherical polystyrene particles having at least 5, 10 and 25 μm in diameter (PSL particles) in mono-dispersed suspension. The PSL particles should have either a domestic or international traceability in terms of length, with a level of uncertainty at not greater than 3%. The particles to be used for calibration should be dispersed in particle-free water.

   1.1.1.1. Manual method
   The particle size response of the system to be applied should be determined using at least 3 channels for threshold-voltage setting, according to the half counting method of window moving type. The threshold-voltage window should be ±20% of the measuring particle size. After measuring the sensitivity of response for the designated particle size, the size response curve is prepared by the method indicated by the manufacturer from particle-response measuring point, and threshold-voltage of 5, 10 and 25 μm of the apparatus is obtained.

   1.1.1.2. Electronic method
   In the use of multichannel peak height analyzer, the parti-
The particle size response curve of the apparatus may be obtained by using the software developed by the user or supplied by the instrument manufacturer, whereas the manufacturer or the user should validate the obtainability of the same result as that of the manual method.

1.1.3. Automated method

The particle size resolution of curve obtained should be 763 to 1155 particles per 1 mL.

1.1.2. Sample volume accuracy

Sample volume accuracy should fall within 5% of the measuring value in case the decrease of test solution is measured by the mass method after measuring the test solution of 10 mL.

1.1.3. Sample flow rate

The flow rate of the sample indicated into the sensor should be the observed sample volume and time, and should be confirmed within the range of the manufacturer’s specification for sensor used.

1.1.4. Sensor

There is a possibility of changes of particle size resolution and counting rate of particle-detecting sensor in each sensor by assembling accuracy and parts accuracy even in the same type sensor. The threshold accuracy also needs to be confirmed. Testing should accordingly be performed for each of the particle size resolution, accuracy in counting and threshold setting, using Particle Count Reference Standard Suspension (PSL spheres having mean diameter of approximately 10 μm, of a concentration at 1000 particles/mL ± 10%, not more than 5% of CV value).

During measurement, stirring should be made for assuring the uniformity in sample concentration.

1.1.4.1. Sensor resolution (Particle size resolution of apparatus)

Measurement should be made by either one of the following methods. The difference between the threshold of particle size counting 16% and 84% of the total counts and the test-particle size should be within 10%, whereas, electronic method and automated method should be both validated for obtaining the same result as that of the manual method.

(i) Manual method to obtain the spread of histogram prepared from the counting value of the apparatus.

(ii) Electronic method to obtain the spread of histogram of the classification of system-responding signal by using the multichannel peak height analyzer.

(iii) Automated method to obtain the spread of histogram of responsive signal of the test-particle by using the software prepared by the manufacturer or the user.

1.1.4.2. Particle counting accuracy

Data obtained by counting particles of 5 μm and greater should be 763 to 1155 particles per 1 mL.

1.1.4.3. Threshold accuracy

Particle size calculated from a threshold corresponding to 50% counts for particles of 5 μm and greater should fall within ±5% of the mean diameter of the test particles.

1.2. General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet.

Very carefully wash the glassware and filtration equipment used, except for the membrane filters, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse the equipment from top to bottom, outside and then inside, with particle-free water.

Take care not to introduce air bubbles into the preparation to be examined, especially when fractions of the preparation are being transferred to the container in which the determination is to be carried out.

In order to check that the environment is suitable for the test, that the glassware is properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of 5 samples of particle-free water, each of 5 mL, according to the method described below. If the number of particles of 10 μm or greater size exceeds 25 for the combined 25 mL, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware and water are suitable for the test.

1.3. Method

Mix the contents of the sample by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of particle-free water and remove the closure, avoiding any contamination of the contents. Eliminate gas bubbles by appropriate measures such as allowing to stand for 2 minutes or sonicating.

For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container to obtain a volume of not less than 25 mL; where justified and authorised, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with particle-free water or with an appropriate solvent without contamination of particles when particle-free water is not suitable.

Powders for parenteral use are reconstituted with particle-free water or with an appropriate solvent without contamination of particles when particle-free water is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Remove 4 portions, each of not less than 5 mL, and count the number of particles equal to or greater than 10 μm and 25 μm. Disregard the result obtained for the first portion, and calculate the mean number of particles for the preparation to be examined.

1.4. Evaluation

If the average number of particles exceeds the limits, test the preparation by Method 2 (Microscopic Particle Count Test).

Test 1.A—Solutions for injection supplied in containers with a nominal content of equal to or more than 100 mL.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 25 per milliliter equal to or greater than 10 μm and does not exceed 3 per milliliter equal to or greater than 25 μm.

Test 1.B—Solutions for injection supplied in containers with a nominal content of less than 100 mL.
The preparation complies with the test if the average number of particles present in the units tested does not exceed 6000 per container equal to or greater than 10 \( \mu m \) and does not exceed 600 per container equal to or greater than 25 \( \mu m \).

2. Method 2. Microscopic Particle Count Test

2.1. Apparatus
Use a suitable binocular microscope, filter assembly for retaining particulate contamination and membrane filter for examination.

The microscope is equipped with an ocular micrometer calibrated with an objective micrometer, a mechanical stage capable of holding and traversing the entire filtration area of the membrane filter, two suitable illuminators to provide episcopic illumination in addition to oblique illumination, and is adjusted to 100 ± 10 magnifications. The ocular micrometer is a circular diameter graticule (see Fig. 6.07-1) and consists of a large circle divided by crosshairs into quadrants, transparent and black reference circles 10 \( \mu m \) and 25 \( \mu m \) in diameter at 100 magnifications, and a linear scale graduated in 10 \( \mu m \) increments. It is calibrated using a stage micrometer that is certified by either a domestic or international standard institution. A relative error of the linear scale of the graticule within ± 2 per cent is acceptable. The large circle is designated the graticule field of view (GFOV).

Two illuminators are required. One is an episcopic bright-field illuminator internal to the microscope, the other is an external, focussable auxiliary illuminator adjustable to give reflected oblique illumination at an angle of 10° to 20°.

The filter assembly for retaining particulate contamination consists of a filter holder made of glass or other suitable material, and is equipped with a vacuum source and a suitable membrane filter.

The membrane filter is of suitable size, black or dark gray in color, non-gridded or gridded, and 1.0 \( \mu m \) or finer in nominal pore size.

2.2. General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet.

Very carefully wash the glassware and filter assembly used, except for the membrane filter, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse both sides of the membrane filter and the equipment from top to bottom, outside and then inside, with particle-free water.

In order to check that the environment is suitable for the test, that the glassware and the membrane filter are properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of a 50 mL volume of particle-free water according to the method described below. If more than 20 particles 10 \( \mu m \) or larger in size or if more than 5 particles 25 \( \mu m \) or larger in size are present within the filtration area, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, membrane filter and water are suitable for the test.

2.3. Method

Mix the contents of the samples by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of particle-free water and remove the closure, avoiding any contamination of the contents.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units is combined in a cleaned container; where justified and authorised, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with particle-free water or with an appropriate solvent without contamination of particles when particle-free water is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are constituted with particle-free water or with an appropriate solvent without contamination of particles when particle-free water is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several milliliters of particle-free water. Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply vacuum. If needed add stepwise a portion of the solution until the entire volume is filtered. After the last addition of solution, begin rinsing the inner walls of the filter holder by using a jet of particle-free water. Maintain the vacuum until the surface of the membrane filter is free from liquid. Place the filter in a petri dish and allow the filter to air-dry with the cover slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, scan the entire membrane filter under the reflected light from the illuminating device, and count the number of particles that are equal to or greater than 10 \( \mu m \) and the number of particles that are equal to or greater than 25 \( \mu m \). Alternatively, partial filter count and determination of the total filter count by calculation is allowed. Calculate the mean number of particles for the preparation to be examined.

The particle sizing process with the use of the circular diameter graticule is carried out by transforming mentally the image of each particle into a circle and then comparing it to the 10 \( \mu m \) and 25 \( \mu m \) graticule reference circles. Thereby the
particles are not moved from their initial locations within the graticule field of view and are not superimposed on the reference circles for comparison. The inner diameter of the transparent graticule reference circles is used to size white and transparent particles, while dark particles are sized by using the outer diameter of the black opaque graticule reference circles.

In performing the microscopic particle count test (Method 2) do not attempt to size or enumerate amorphous, semiliquid, or otherwise morphologically indistinct materials that have the appearance of a stain or discoloration on the membrane filter. These materials show little or no surface relief and present a gelatinous or film-like appearance. In such cases the interpretation of enumeration may be aided by testing a sample of the solution by Method 1.

2.4. Evaluation
Test 2.4.1—Solutions for injection supplied in containers with a nominal content of *equal to or* more than 100 mL
The preparation complies with the test if the average number of particles present in the units tested does not exceed 12 per milliliter equal to or greater than 10 μm and does not exceed 2 per milliliter equal to or greater than 25 μm.
Test 2.4.2—Solutions for injection supplied in containers with a nominal content of less than 100 mL
The preparation complies with the test if the average number of particles present in the units tested does not exceed 3000 per container equal to or greater than 10 μm and does not exceed 300 per container equal to or greater than 25 μm.

3. Reagents
Particle-free water: The filtered water through a membrane filter with a pore size not exceeding 0.45 μm, containing not more than 5 particles of 10 μm or greater size, and not more than 2 particles of 25 μm or greater size in 10 mL of the insoluble particle number measured by the light obscuration particle counter.

6.08 Insoluble Particulate Matter Test for Ophthalmic Solutions

Insoluble Particulate Matter Test for Ophthalmic Solutions is to examine for the size and the number of insoluble particulate matter in Ophthalmic Solutions.

1. Apparatus
Use a microscope, filter assembly for retaining insoluble particulate matter and membrane filter for determination.
(i) Microscope: The microscope is equipped with a micrometer system, a mobile stage and an illuminator, and is adjusted to 100 magnifications.
(ii) Filter assembly for retaining insoluble particulate matter: The filter assembly for retaining insoluble particulate matter consists of a filter holder made of glass or a proper material incapable of causing any trouble in testing, and a clip. The unit is capable of fitting with a membrane filter 25 mm or 13 mm in diameter and can be used under reduced pressure.
(iii) Membrane filter for testing: The membrane filter is white in color, 25 mm or 13 mm in diameter, not more than 10 μm in nominal pore size and is imprinted with about 3 mm grid marks. Upon preliminary testing, the insoluble particulate matter equal to or greater than 25 μm in size should not be found on the filter. When necessary, wash the filter with water for particulate matter test.

2. Reagents
(i) Water for particulate matter test: Water prepared before use by filtering through a membrane filter with a pore size not exceeding 0.45 μm. It contains not more than 10 particles of 10 μm or grater size in 100 mL.

3. Procedure

3.1. Aqueous ophthalmic solutions
Carry out all operations carefully in clean equipment and facilities which are low in dust. Fit the membrane filter onto the membrane filter holder, and fix them with the clip. Thoroughly rinse the holder inside with water for particulate matter test, and filter under reduced pressure with 200 mL of water for particulate matter test at a rate of 20 to 30 mL per minute. Apply the vacuum until the surface of the membrane filter is free from water, and remove the membrane filter. Place the filter in a flat-bottomed petri dish with the cover slightly ajar, and dry the filter fully at a temperature not exceeding 50°C. After the filter has been dried, place the petri dish on the stage of the microscope. Under a downlight from an illuminating device, adjust the grid of the membrane filter to the coordinate axes of the microscope, adjust the microscope so as to get the best view of the insoluble particulate matter, then count the number of particles that are equal to or greater than 150 μm within the effective filtering area of the filter, moving the mobile stage, and ascertain that the number is not more than 1. In this case the particle is sized on the longest axis.

Fit another membrane filter to the filtration device, and fix them with the clip, then wet the inside of the filter holder with several mL of water for particulate matter test. Clean the outer surface of the container, and mix the sample solution gently by inverting the container several times. Remove the cap, clean the outer surface of the nozzle, and pour the sample solution into a measuring cylinder which has been rinsed well with water for particulate matter test. Repeat the process to prepare 25 mL of the test solution. Pour the test solution into the filter holder along the inner wall of the holder. Apply the vacuum and filter mildly so as to keep the solution always on the filter. As for viscous sample solution, dilute suitably with water for particulate matter test or suitable diluent and then filter as described above. When the amount of the solution on the filter becomes small, add 30 mL of water for particulate matter test or suitable diluent in such manner as to wash the inner wall of the filter holder. Repeat the process 3 times with 30 mL of the water. Apply the vacuum gently until the surface of the membrane filter is free from water. Place the filter in a petri dish, and dry the filter at a temperature below 50°C with the cover slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, and count the number of particles which are equal to or larger than 300 μm within the effective filtering area of the filter according to the same procedure of the microscope as described above. In this case the particle is sized on the longest axis.

3.2. Ophthalmic solutions which are dissolved before use
Proceed as directed in Aqueous Ophthalmic Solutions after dissolving the sample with the constituted solution.

3.3. Suspension type ophthalmic solutions
Proceed as directed in Aqueous Ophthalmic Solutions.
Take 25 mL of the sample in a vessel, which has been rinsed well with water for particulate matter test, add a suitable amount of a suspension-solubilizing solvent or an adequate solvent, shake to dissolve the suspending particles, and use this solution as the sample solution. Use a membrane filter which is not affected by the solvent to be used.

3.4. Ophthalmic solutions contained in a single-dose container

Proceed as directed in Aqueous Ophthalmic Solutions, using 10 samples for the test. A 13-mm diameter membrane filter and a 4-mm diameter filter holder for retaining insoluble particulate matter are used.

4. Evaluation

The preparation complies with the test if the calculated number per mL of insoluble particles of a size equal to or greater than 300 μm is not more than 1.

6.09 Disintegration Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (* •).

Disintegration Test is provided to determine whether tablets, capsules, granules, dry syrups or pills, disintegrate within the prescribed time when placed in a liquid medium at the experimental conditions presented below.

For the purposes of this test, disintegration does not imply complete solution of the unit or even of its active constituent.

1. Apparatus

The apparatus consists of a basket-rack assembly, a 1000-mL, low-form beaker, 138 to 160 mm in height and having an inside diameter of 97 to 115 mm for the immersion fluid, a thermostatic arrangement for heating the fluid between 35° and 39°, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles per minute through a distance of not less than 53 mm and not more than 57 mm. The volume of the fluid in the vessel is such that at the highest point of the upward stroke the wire mesh remains at least 15 mm below the surface of the fluid and descends to not less than 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke, and the change in stroke direction is a smooth transition, rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.

(i) Basket-rack assembly: The basket-rack assembly consists of six open-ended transparent tubes, each 77.5 ± 2.5 mm long and having an inside diameter of 20.7 ± 2.8 mm and a wall 1.0 ± 2.8 mm thick; the tubes are held in a vertical position by two plates, each 88 ± 2.6 mm in diameter and 5 to 8.5 mm in thickness, with six holes, each 22.5 to 26 mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached to the under surface of the lower plate is a woven stainless steel wire cloth, which has a plain square weave with 1.8- to 2.2-mm apertures and with a wire diameter of 0.57 to 0.66 mm. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plates. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device using a point on its axis. The basket-rack assembly conforms to the dimensions found in Fig. 6.09-1. The design of the basket-rack assembly may be varied somewhat provided the specifications for the glass tubes and the screen mesh size are maintained: * for example, in order to secure the glass tubes and the upper and the lower plastic plates in position at the top or the bottom, an acid-resistant metal plate, 88 – 92 mm in diameter and 0.5 – 1 mm in thickness, having 6 perforations, each about 22 to 26 mm in diameter, may be used which coincide with those of the upper plastic plate and upper open ends of the glass tubes. •

(ii) Disks: The use of disks is permitted only where specified or allowed. Each tube is provided with a cylindrical disk 9.5 ± 0.15 mm thick and 20.7 ± 0.15 mm in diameter. The disk is made of a suitable, transparent plastic material having a specific gravity of between 1.18 and 1.20. Five parallel 2 ± 0.1 mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are centered 6 ± 0.2 mm from the axis on imaginary lines perpendicular to the axis and parallel to each other. Four identical trapezoidal-shaped planes are cut into the wall of the cylinder, nearly perpendicular to the ends of the cylinder. The trapezoidal shape is symmetrical; its parallel sides coincide with the ends of the cylinder and are parallel to an imaginary line connecting the centers of two adjacent holes 6 mm from the cylindrical axis. The parallel side of the trapezoid on the bottom of the cylinder has a length of 1.6 ± 0.1 mm, and its bottom edges lie at a depth of 1.5 – 1.8 mm from the cylinder’s circumference. The parallel side of the trapezoid on the top of the cylinder has a length of 9.4 ± 0.2 mm, and its center lies at a depth of 2.6 ± 0.1 mm from the cylinder’s circumference. All surfaces of the disk are smooth. If the use of disks is specified, add a disk to each tube, and operate the apparatus as directed under Proce-
dure. The disks conform to dimensions found in Fig. 6.09-1. The use of automatic detection employing modified disks is permitted where the use of disks is specified or allowed. Such disks must comply with the requirements for density and dimension given in this chapter.

* (iii) Auxiliary tube: The auxiliary tube, as illustrated in Fig. 6.09-2, consists of a plastic tube D, 12 ± 0.2 mm in inside diameter, 17 ± 0.2 mm in outside diameter, 20 ± 1 mm in length, having both outside ends screw-cut, and two plastic rings A, each 12 ± 0.2 mm in inside diameter, 17 ± 0.2 mm in outside diameter, 2.5 - 4.5 mm in length, having one inside end screw-cut. Acid-resistant woven wire gauze having 0.42-mm openings and 0.29-mm wire diameter is placed in each plastic ring and the rings are attached by screws to each end of the plastic tube. The distance between two wire gauses is 20 ± 1 mm. A handle of an acid-resistant wire, 1 mm in diameter and 80 ± 5 mm in length, is attached to the mid portion of the plastic tube. The auxiliary tube is used for the test of granules and capsules containing enteric coated granules.

2. Procedure

2.1. Immediate-release preparations

In case of tablets, capsules * and pills (except for pills containing crude drugs), place 1 dosage unit in each of the six tubes of the basket, and if prescribed add a disk. * Unless otherwise specified, operate the apparatus, using water as the immersion fluid, maintained at 37 ± 2°C as the immersion fluid. * Unless otherwise specified, carry out the test for 20 minutes for capsules, 30 minutes for plain tablets, and 60 minutes for coated tablets and pills. * Lift the basket from the fluid, and observe the dosage units. * Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the disks, if used, is a soft mass having no palpably firm core. * The test is met if all of the dosage units have disintegrated completely. If 1 or 2 dosage units fail to disintegrate, repeat the test on 12 additional dosage units. The test is met if not less than 16 of the total of 18 samples tested are disintegrated.

* For pills containing crude drugs, carry out the test for 60 minutes in the same manner, using 1st fluid for disintegration test as the immersion fluid. When any residue of the unit is observed, carry out the test successively for 60 minutes, using 2nd fluid for disintegration test.

* In case of granules and dry syrups, shake preparations on a No. 30 (500 µm) sieve as directed in Particle Size Distribution Test for Preparations <6.03>, transfer 0.10 g of the residue on the sieve to each of the 6 auxiliary tubes, secure the 6 tubes to the bottom of the basket tightly, and operate the apparatus, using water as the immersion fluid, maintained at 37 ± 2°C as the immersion fluid, unless otherwise specified. Observe the samples after 30 minutes of operation for plain granules and after 60 minutes for coated granules, unless otherwise specified. Complete disintegration is defined as that state in which any residue of the granules, except fragments of insoluble coating in the auxiliary tube, is a soft mass having no palpably firm core. The test is met if all of 6 samples in the auxiliary tubes have disintegrated completely. If 1 or 2 samples fail to disintegrate, repeat the test on 12 additional samples. The test is met if not less than 16 of the total of 18 samples tested are disintegrated.

2.2. Enteric coated preparations

Unless otherwise specified, perform the following two tests, (a) the test with 1st fluid for disintegration test and (b) the test with the 2nd fluid for disintegration test, separately.

2.2.1. Enteric coated tablets and capsules

(i) The test with 1st fluid for disintegration test: Carry out the test for 120 minutes, using 1st fluid for disintegration test according to the procedure described in immediate release preparations. Disintegration is defined as that state in which the tablet or capsule is broken or the enteric coating film is ruptured or broken. The test is met if none of six dosage units is disintegrated. If 1 or 2 dosage units are disintegrated, repeat the test on additional 12 dosage units. The test is met if not less than 16 of the total of 18 dosage units tested are not disintegrated.

(ii) The test with 2nd fluid for disintegration test: According to the procedure described in immediate-release preparations, carry out the test with new dosage units for 60 minutes, using 2nd fluid for disintegration test and determine if the test is met or not.

2.2.2. Enteric coated granules and capsules containing the enteric coated granules

Shake granules or contents taken out from capsules on a No. 30 (500 µm) sieve as directed in Particle Size Distribution Test for Preparations <6.03>, transfer 0.10 g of the residue on the sieve to each of the 6 auxiliary tubes, secure the 6 tubes to the bottom of the basket tightly, and operate the apparatus, using the 1st and 2nd fluids for disintegration test.

(i) The test with 1st fluid for disintegration test: According to the procedure described in immediate-release preparations, carry out the test for 60 minutes, using 1st fluid for disintegration test. The test is met if particles fallen from the openings of the wire gauze number not more than 15.

(ii) The test with 2nd fluid for disintegration test: According to the procedure described in immediate-release preparations, carry out the test with new samples for 30 minutes, using 2nd fluid for disintegration test and determine if test is met or not.
6.10 Dissolution Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (*) •.

Dissolution Test is provided to determine compliance with the dissolution requirements for dosage forms administered orally. *This test also aims at preventing significant bioinequivalence. • In this test, a dosage unit is defined as 1 tablet or 1 capsule or the amount specified equivalent to minimum dose.

1. Apparatus

1.1. Apparatus for Basket Method (Apparatus 1)

The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material*; a motor; a drive shaft; and a cylindrical basket. The vessel is partially immersed in a suitable water bath of any convenient size or heated by a suitable device such as a heating jacket. The water bath or heating device permits holding the temperature inside the vessel at $37 \pm 0.5^\circ C$ during the test and keeping the bath fluid in constant, smooth motion. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smoothly rotating stirring element. Make the apparatus to permit observation of the specimen and stirring element during the test. The vessel is cylindrical, with a hemispherical bottom and a capacity of 1 liter. Its height is 160 mm to 210 mm and its inside diameter is 98 mm to 106 mm. Its sides are flanged at the top. Use a fitted cover to prevent escape of undissolved particles from the top of the vessel and rotates smoothly and without significant wobble that could affect the results. Adjust a speed-regulating device to maintain the shaft rotation speed at a specified rate, within $\pm 4\%$.

Shaft and basket components of the stirring element shown in Fig. 6.10-1 are fabricated of stainless steel (SUS316) or other inert material. A basket having a gold coating of about $0.0001$ inch (2.5 µm) thick may be used. The dosage unit is placed in a dry basket at the beginning of each test. The distance between the inside bottom of the vessel and the bottom of the basket is maintained at $25 \pm 2$ mm during the test.

1.2. Apparatus for Paddle Method (Apparatus 2)

Use the assembly from Apparatus 1, except that a paddle formed from a blade and a shaft is used as the stirring element. The paddle blade and shaft component of the stirring element is not more than 2 mm from the vertical axis of the vessel, at any point, and rotates smoothly without significant wobble that could affect the results. The vertical center line of the blade passes through the axis of the shaft so that the bottom of the blade is flush with the bottom of the shaft. The paddle conforms to the specifications shown in Fig. 6.10-2. The distance of $25 \pm 2$ mm between the bottom of the blade and the inside bottom of the vessel is maintained during the test. The metallic or suitably inert, rigid blade and shaft comprise a single entity. A suitable two-part detachable design may be used provided the assembly remains firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating so as to make them inert. The dosage unit is usually allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of non-reactive material, such as not more than a few turns of wire helix or such one shown in Fig. 6.10-2a, may be attached to the dosage unit that would otherwise float. Other validated sinker devices may also be used. •If the use of sinker is specified, unless otherwise specified, use the sinker device shown in Fig. 6.10-2a.

1.3. Apparatus for Flow-Through Cell Method (Apparatus 3)

The assembly consists of a reservoir and a pump for the dissolution medium; a flow-through cell; a water bath that maintains the dissolution medium at $37 \pm 0.5^\circ C$. Use the cell size specified in the individual monograph.

The pump forces the dissolution medium upwards through the flow-through cell. The pump has a delivery range between 4 and 16 mL per minute, with standard flow rates of 4, 8, and 16 mL per minute. It must deliver a constant flow ($\pm 5$ per cent of the nominal flow rate); the flow profile should be sinusoidal with a pulsation of 120 $\pm 10$ pulses per minute. A pump without the pulsation may also be used. Dissolution test procedure using the flow-through cell must be characterized with respect to flow rate and any pulsation.

Fig. 6.10-1 Apparatus 1, Basket stirring element
cone is usually filled with small glass beads of about 1-mm diameter with one bead of about 5-mm positioned at the apex to protect the fluid entry tube; a tablet holder (see Figures 6.10-3 and 6.10-4) is available for positioning of special dosage forms. The cell is immersed in a water bath, and the temperature is maintained at 37 ± 0.5°C.

The apparatus uses a clamp mechanism of two O-rings to assemble the cell. The pump is separated from the dissolution unit in order to shield the latter against any vibrations originating from the pump. The position of the pump should not be on a level higher than the reservoir flasks. Tube connections are as short as possible. Use suitably inert tubing, such as polytet, with about 1.6-mm inner diameter and inert flanged-end connections.

Notes:
(1) A and B dimensions are not to vary more than 0.5 mm when part is rotated on centering axis.
(2) Tolerances are ±1.0 mm unless otherwise stated.

Fig. 6.10-2 Apparatus 2, Paddle stirring element

Fig. 6.10-2a Alternative sinker

Fig. 6.10-3 Apparatus 3
Large cell for tablets and capsules (top); tablet holder for the large cell (bottom)
(All dimensions are expressed in mm unless otherwise noted.)

2. Apparatus Suitability
The determination of suitability of a test assembly to perform dissolution testing must include conformance to the dimensions and tolerances of the apparatus as given above. In addition, critical test parameters that have to be monitored periodically during use include volume and temperature of the dissolution medium, rotation speed (Basket Method and Paddle Method), and flow rate of medium (Flow-Through Cell Method).

Determine the acceptable performance of the dissolution test assembly periodically.

3. Procedure
3.1. Basket Method or Paddle Method
3.1.1. Immediate-release Dosage Forms
(i) Procedure: Place the stated volume of the dissolution medium (±1%) in the vessel of the specified apparatus, assemble the apparatus, equilibrate the dissolution medium to
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37 ± 0.5°C, and remove the thermometer. Place 1 dosage unit in the apparatus, taking care to exclude air bubbles from the surface of the dosage unit, and immediately operate the apparatus at the specified rate. Within the time interval specified, or at each of the times stated, withdraw a specimen from a zone midway between the surface of the Dissolution Medium and the top of the rotating basket or blade, not less than 10 mm from the vessel wall.

[NOTE—Where multiple sampling times are specified, replace the aliquots withdrawn for analysis with equal volumes of fresh Dissolution Medium at 37°C or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test, and verify the temperature of the mixture under test at suitable times.] Perform the analysis using an indicated assay method.*3 Repeat the test with additional dosage units.

If automated equipment is used for sampling or the apparatus is otherwise modified, verification that the modified apparatus will produce results equivalent to those obtained with the standard apparatus described in this chapter, is necessary.

(iii) Time: Where a single time specification is given, the test may be concluded in a shorter period if the requirement for minimum amount dissolved is met. Specimens are to be withdrawn only at the stated times, within a tolerance of ±2%.

3.1.2. Extended-release Dosage Forms

(i) Procedure: Proceed as described for Immediate-Release Dosage Forms.

(ii) Dissolution Medium: Proceed as directed under Immediate-Release Dosage Forms.

(iii) Time: The test-time points, generally three, are expressed in hours.

3.1.3. Delayed-release Dosage Forms

(i) Procedure: Unless otherwise specified, proceed the acid stage test and buffer stage test separately as described for Immediate-Release Dosage Forms.

(ii) Dissolution Medium: Acid stage: Unless 1st fluid for dissolution test is used, proceed as directed under Immediate-Release Dosage Forms. Buffer stage: Unless 2nd fluid for dissolution test is used, proceed as directed under Immediate-Release Dosage Forms.

(iii) Time: Acid stage: Generally, test time is 2 hours for tablets and capsules, and 1 hour for granules. Buffer stage: The same as directed under Immediate-Release Dosage Forms. All test times stated are to be observed within a tolerance of ±2%, unless otherwise specified.

3.2. Flow-Through Cell Method

3.2.1. Immediate-release Dosage Forms

(i) Procedure: Place the glass beads into the cell specified in the individual monograph. Place 1 dosage unit on top of the beads or, if specified, on a wire carrier. Assemble the filter head and fix the parts together by means of a suitable clamping device. Introduce by the pump the dissolution medium warmed to 37 ± 0.5°C through the bottom of the cell to obtain the flow rate specified and measured with an accuracy of 5%. Collect the eluate by fractions at each of the times stated. Perform the analysis as directed. Repeat the test with additional dosage units.

(ii) Dissolution Medium: Proceed as directed under Immediate-Release Dosage Forms under Basket Method and Paddle Method.

(iii) Time: Proceed as directed under Immediate-Release Dosage Forms under Basket Method and Paddle Method.

3.2.2. Extended-release Dosage Forms

(i) Procedure: Proceed as described for Immediate-Release Dosage Forms under Flow-Through Cell Method.

(ii) Dissolution Medium: Proceed as described for Immediate-Release under Flow-Through Cell Method.

(iii) Time: The test-time points, generally three, are expressed in hours.

4. Interpretation

4.1. Immediate-release Dosage Forms

*Follow Interpretation 1 when the value Q is specified in
the individual monograph, otherwise follow Interpretation 2.

4.1.1. Interpretation 1

Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to Acceptance Table 6.10-1. Continue testing through the three stages unless the results conform at either S1 or S2. The quantity, expressed as a percentage of the labeled content, of active ingredient dissolved from the units tested conform to Acceptance Table 6.10-1. Continue testing through the three stages unless the results conform at A2.

4.1.2. Interpretation 2

Unless otherwise specified, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on another 6 dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test.

4.2. Extended-release Dosage Forms

4.2.1. Interpretation 1

Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to Acceptance Table 6.10-2. Continue testing through the three stages unless the results conform at either L1 or L2. Limits on the amounts of active ingredient dissolved are expressed in terms of the percentage of labeled content. The limits embrace each value of 

\[ Q \\]

\[ z \]

\[ 15 \]

\[ Q \]

\[ -15 \]

\[ z \]

\[ 25 \]

\[ z \]

\[ Q \]

\[ -25 \]

4.2.2. Interpretation 2

Unless otherwise specified, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on another 6 dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test. Where more than one range is specified, the acceptance criteria apply individually to each range.

4.3. Delayed-release Dosage Forms

4.3.1. Interpretation 1

(i) Test using 1st fluid for dissolution test: Unless otherwise specified, the requirements of this portion of the test are met if the quantities, based on the percentage of the labeled content, of active ingredient dissolved from the units tested conform to Acceptance Table 6.10-3. Continue testing through the three levels unless the results conform at A2.

(ii) Test using 2nd fluid for dissolution test: Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the units tested con-
form to Acceptance Table 6.10-4. Continue testing through the three levels unless the results of both stages conform at an earlier level. The value of $Q$ in Acceptance Table 6.10-4 is the amount $^\ast$specified in monograph $^\ast$ of active ingredient dissolved, expressed as a percentage of the labeled content. The 5% and 15% and 25% values in Acceptance Table 6.10-4 are percentages of the labeled contest so that these values and $Q$ are in the same terms.

4.3.2. $^\ast$Interpretation 2

Unless otherwise specified, both the tests using 1st fluid for dissolution test and 2nd fluid for dissolution test in acid and buffer stages, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on another 6 dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test.

$^\ast$The materials should not sorb, react, or interfere with the specimen being tested.

$^\ast$If a cover is used, it provides sufficient openings to allow ready insertion of the thermometer and withdrawal of specimens.

$^\ast$Test specimens are filtered immediately upon sampling unless filtration is demonstrated to be unnecessary. Use an inert filter that does not cause adsorption of the ingredient or contain extractable substances that would interfere with the analysis.

$^\ast$One method of deaeration is as follows: Heat the medium, while stirring gently, to about 41°C, immediately filter under vacuum using a filter having a porosity of 0.45 $\mu$m or less, with vigorous stirring, and continue stirring under vacuum for about 5 minutes. Other validated deaeration techniques for removal of dissolved gases may be used.

6.11 Foreign Insoluble Matter Test for Ophthalmic Solutions

Foreign Insoluble Matter Test for Ophthalmic Solutions is a test method to examine foreign insoluble matters in ophthalmic solutions.

When inspect with the unaided eyes at a position of luminous intensity of 3000 – 5000 lx under an incandescent lamp after cleaning the exterior of containers, Ophthalmic Solutions must be clear and free from readily detectable foreign insoluble matters.

7. Tests for Containers and Packing Materials

7.01 Test for Glass Containers for Injections

The glass containers for injections do not interact physically or chemically with the contained medicament to alter any property or quality, can protect the contained medicament from the invasion of microbes by means of perfect sealing or other suitable process, and meet the following requirements. The surface-treated container for aqueous infusion is made from glass which meets the requirements for the soluble alkali test for a container not to be fused under method 1.

(1) The containers are colorless or light brown and transparent, and have no bubbles which interfere the test of the Foreign Insoluble Matter Test for Injections $<6.06>$.

(2) Multiple-dose containers are closed by rubber stoppers or any other suitable stoppers. The stoppers permit penetration of an injection needle without detachment of fragments, and upon withdrawal of the needle, they reclose the containers immediately to prevent external contamination, and also do not interact physically or chemically with the contained medicaments.

Containers intended for aqueous infusions are closed by rubber stoppers meeting the requirements of the test for Rubber Closure for Aqueous Infusions $<7.07>$.

(3) Soluble alkali test—The testing methods may be divided into the following two methods according to the type of container or the dosage form of the medicament.

(i) Method 1: This method is applied to containers to be fused, or containers not to be fused except containers for aqueous infusions with a capacity exceeding 100 mL.

Rinse thoroughly the inside and outside of the containers to be tested with water, dry, and roughly crush, if necessary. Transfer 30 to 40 g of the glass to a steel mortar, and crush. Sieve the crushed glass through a No. 12 (1400 $\mu$m) sieve. Transfer the portion retained on the sieve to the steel mortar, and repeat this crushing procedure until 2/3 of the amount of powdered glass has passed through a No. 12 (1400 $\mu$m) sieve. Combine all portions of the glass powder passed through a No. 12 (1400 $\mu$m) sieve, shake the sieve in a horizontal direction for 5 minutes with slight tapping at intervals using No. 18 (850 $\mu$m) and No. 50 (300 $\mu$m) sieves. Transfer 7 g of the powder, which has passed through a No. 18 (850 $\mu$m) sieve but not through a No. 50 (300 $\mu$m) sieve to a No. 50 (300 $\mu$m) sieve, immerse it in a suitable container filled with water, and wash the contents with gentle shaking for 1 minute. Rinse again with ethanol (95%) for 1 minute, dry the washed glass powder at 100°C for 30 minutes, and allow to cool in a desiccator (silica gel). Transfer exactly 5.0 g of the powder thus prepared to a 200-mL conical flask of hard glass, add 50 mL of water, and gently shake the flask so that the powder disperses on the bottom of the flask evenly. Cover the flask with a small beaker of hard glass or a watch glass of hard glass, then heat it in boiling water for 2 hours, and immediately cool to room temperature. Decant the water from the flask into a 250-mL conical flask of hard glass, wash well the residual powdered glass with three 20-mL portions of water, and add the washings to the decanted water. Add 5 drops of bromocresol green-methyl red TS and titrate $<2.30>$ with 0.01 mol/L sulfuric acid VS until the color of the solution changes from green through slightly grayish blue to slightly grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

The quantity of 0.01 mol/L sulfuric acid VS consumed does not exceed the following quantity, according to the type of containers.

Containers to be fused 0.30 mL
Containers not to be fused (including injection
(ii) Method 2: This method is applied to containers not to be fused for aqueous infusions with a capacity exceeding 100 mL.

Rinse thoroughly the inside and outside of the containers to be tested with water, and dry. Add a volume of water equivalent to 90% of the overflow capacity of the container, cover it with a small beaker of hard glass or close tightly with a suitable stopper, heat in an autoclave at 121°C for 1 hour, and allow to stand until the temperature falls to room temperature, measure exactly 100 mL of the this solution, and transfer to a 250-mL conical flask of hard glass. Add 5 drops of bromocresol green-methyl red TS, and titrate ≤2.50 mL with 0.01 mol/L sulfuric acid VS until the color of the solution changes from green through slightly grayish blue to slightly grayish red-purple. Measure accurately 100 mL of water, transfer to a 250-mL conical flask of hard glass, perform a blank determination in the same manner, and make any necessary correction. The quantity of 0.01 mol/L sulfuric acid VS consumed does not exceed 0.10 mL.

(4) Soluble iron test for light-resistant containers—Rinse thoroughly five or more light-resistant containers to be tested with water, and dry at 105°C for 30 minutes. Pour a volume of 0.01 mol/L hydrochloric acid VS corresponding to the labeled volume of the container into individual containers, and fuse them. In the case of containers not to be fused, cover them with small beakers of hard glass or watch glasses of hard glass. Heat them at 105°C for 1 hour. After cooling, prepare the test solution with 40 mL of this solution according to Method 1 of the Iron Limit Test <1.10>, and perform the test according to Method B. Prepare the control solution with 2.0 mL of the Standard Iron Solution.

(5) Light transmission test for light-resistant containers—Cut five light-resistant containers to be tested, prepare test pieces with surfaces as flat as possible, and clean the surfaces. Fix a test piece in a cell-holder of a spectrophotometer to allow the light pass through the center of the test piece perpendicularly to its surface. Measure the light transmittance of the test piece with reference to air between 290 nm and 610 nm at intervals of 20 nm each. The percent transmissions obtained between 590 nm and 610 nm are not less than 45%, and that between 290 nm and 450 nm are not more than 50%.

7.02 Test Methods for Plastic Containers

Test methods for plastic containers may be used for designing and assuring quality of plastic containers. Not all tests described here will be necessary in any phases for any containers. On the other hand, the set does not include sufficient numbers and kinds of tests needed for any design verification and quality assurance of any containers. Additional tests may be considered if necessary.

It is not allowable for plastic containers for the aqueous injections to interact with the pharmaceutical contained therein resulting in the deterioration of its efficacy, safety or stability, and to contaminate with microorganisms. They should meet the requirements prescribed in 2. Requirements for Plastic Containers for Aqueous Injections.

1. Test methods

1.1. Combustion tests

1.1.1. Residue on ignition

Weigh accurately about 5 g of cut pieces of the container and perform the test according to Residue on Ignition <2.44>.

1.1.2. Heavy metals

Place an appropriate amount of cut pieces of the container in a porcelain crucible, and perform the test according to Method 2 of Heavy Metals Limit Test <1.07>. Prepare the control solution with 2.0 mL of Standard Lead Solution.

1.1.3. Lead

1.1.3.1. Method 1

Place 2.0 g of cut pieces of a container in a crucible of platinum or quartz, moisten with 2 mL of sulfuric acid, heat slowly to dryness, then heat to combustion at between 450°C and 500°C. Repeat this procedure, if necessary. After cooling, moisten the residue with water, add 2 to 4 mL of hydrochloric acid, evaporate to dryness on a water bath, then add 1 to 5 mL of hydrochloric acid, and warm to dissolve. Then add 0.5 to 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrosulphuric acid (1:1), and add 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). Filter through a glass filter (G3) if insoluble matter remains. To the obtained filtrate add 10 mL of a solution of diammonium hydrogen citrate (1 in 4), 2 drops of bromothymol blue TS and ammonia TS until the color of the solution changes from yellow to green. Then add 10 mL of a solution of ammonium sulfate (2 in 5) and water to make 100 mL. Add 20 mL of a solution of sodium N,N-diethyl-dithiocarbamate trihydrate (1 in 20) to this solution, mix, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter if necessary, and use the layer as the sample solution. Separately, to 2.0 mL of Standard Lead Solution add water to make exactly 10 mL. To 1.0 mL of this solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, then proceed in the same manner as for the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and standard solution according to Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the concentration of lead in the sample solution.

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: Lead hollow-cathode lamp.
Wavelength: 283.3 nm.

1.1.3.2. Method 2

Cut a container into pieces smaller than 5-mm square, take 2.0 g of the pieces into a glass beaker, add 50 mL of 2-butanol and 0.1 mL of nitric acid, and warm to dissolve. To this solution add 96 mL of methanol gradually to precipitate a resinous substance, and filter by suction. Wash the beaker and the resinous substance with 12 mL of methanol followed by 12 mL of water, combine the washings and the filtrate, and concentrate to about 10 mL under reduced pressure. Transfer into a separator, add 10 mL of ethyl acetate
and 10 mL of water, shake vigorously, and allow to stand to separate the water layer. Evaporate the water layer to dryness, add 5 mL of hydrochloric acid to the residue, and warm to dissolve. Then add 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1), and add 1 mL of a warmed solution of ammonium acetate gently heating in a 500 mL beaker (3). Filter through a glass filter (G3) if insoluble matter remains. To the solution so obtained add 10 mL of a solution of diaminonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and then add ammonia TS until the color of the solution changes from yellow to green. Further add 10 mL of a solution of ammonium sulfate (2 in 5) and water to make 100 mL. Add 20 mL of a solution of sodium N,N-diethylthiophosphate trihydrate (1 in 20) to this solution, mix, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter the layer if necessary, and use the layer as the sample solution. Separately, pipet 5 mL of Standard Lead Solution, add water to make exactly 50 mL, and add 2.0 mL of this solution add 10 mL of a solution of diaminonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, then proceed in the same manner as for the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and standard solution according to Atomic Adsorption Spectrophotometry \( \text{C2.2.25} \) under the conditions described in Method 1, and determine the concentration of lead in the sample solution.

### 1.1.4. Cadmium

#### 1.1.4.1. Method 1

To 2.0 mL of Standard Cadmium Solution add 10 mL of a solution of diaminonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the sample solution in “1.1.3.1. Method 1”, and use the solution so obtained as the standard solution. Perform the test with the sample solution obtained in “1.1.3.1. Method 1” and the standard solution according to Atomic Adsorption Spectrophotometry \( \text{C2.2.25} \) under the following conditions, and determine the concentration of cadmium in the sample solution.

Gas: Combustible gas—Acetylene or hydrogen.

Supporting gas—Air.

Lamp: Cadmium hollow-cathode lamp.

Wavelength: 228.8 nm.

#### 1.1.4.2. Method 2

To 2.0 mL of Standard Cadmium Solution add 10 mL of a solution of diaminonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the sample solution in “1.1.3.2. Method 2”, and use the solution so obtained as the standard solution. Perform the test with the sample solution obtained in “1.1.3.2. Method 2” and the standard solution according to Atomic Adsorption Spectrophotometry \( \text{C2.2.25} \) under the conditions described in “1.1.4.1. Method 1”, and determine the concentration of cadmium in the sample solution.

### 1.1.5. Tin

Cut a container into pieces smaller than 5-mm square, place 5.0 g of pieces in a Kjeldahl flask, add 30 mL of a mixture of sulfuric acid and nitric acid (1:1), and decompose by gentle heating in a muffle furnace, occasionally adding dropwise a mixture of sulfuric acid and nitric acid (1:1) until the content changes to a clear, light brown solution. Then heat until the color of the solution changes to a clear, light yellow, and heat to slowly concentrate to practical dryness. After cooling, dissolve the residue in 5 mL of hydrochloric acid by warming, and after cooling, add water to make exactly 10 mL. Pipet 5 mL of this solution into a 25-mL volumetric flask (A). Transfer the remaining solution to a 25-mL beaker (B) by washing out with 10 mL of water, add 2 drops of bromocresol green TS, neutralize with diluted ammonia solution (28) (1 in 2), and measure the volume consumed for neutralization as a mL. To the volumetric flask, A, add potassium permanganate TS dropwise until a slight pale red color develops, and add a small amount of l-ascorbic acid to decolorize. Add 1.5 mL of 1 mol/L hydrochloric acid TS, 5 mL of a solution of citric acid monohydrate (1 in 10), a mL of diluted ammonia solution (28) (1 in 2), 2.5 mL of polyvinyl alcohol TS, 5.0 mL of phenylfluorone-ethanol TS and water to make 25 mL. Shake well, then allow to stand for about 20 minutes, and use this solution as the sample solution. Separately, pipet 1.0 mL of Standard Tin Solution, add 5 mL of water, add potassium permanganate TS dropwise until a slight pale red color develops, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and the standard solution according to Ultraviolet-visible Spectrophotometry \( \text{C2.2.4} \) at 510 nm, using water as the blank.

### 1.2. Extractable substances

Cut the container at homogeneous regions of low curvature and preferably the same thickness, gather pieces to make a total surface area of about 1200 cm² when the thickness is 0.5 mm or less, or about 600 cm² when the thickness is greater than 0.5 mm, and subdivide in general into strips approximately 0.5 cm in width and 5 cm in length. Wash them with water, and dry at room temperature. Place these strips in a 300-mL hard glass vessel, add exactly 200 mL of water, and seal the opening with a suitable stopper. After heating the vessel in an autoclave at 121°C for 1 hour, take out the vessel, allow to stand until the temperature falls to room temperature, and use the content as the test solution.

For containers made of composite plastics, the extraction may be performed by filling a labeled volume of water in the container. In this case, it is necessary to record the volume of water used and the inside area of the container.

When containers are deformed at 121°C, the extraction may be performed at the highest temperature which does not cause deformation among the following conditions: at 100 ± 2°C for 2 ± 0.2 hours, at 70 ± 2°C for 24 ± 2 hours, at 50 ± 2°C for 72 ± 2 hours or at 37 ± 1°C for 72 ± 2 hours.

Prepare the blank solution with water in the same manner. For containers made of composite plastics, water is used as the blank solution.

Perform the following tests with the test solution and the blank solution:

(i) Foaming test: Place 5 mL of the test solution in a glass-stoppered test tube about 15 mm in inside diameter and about 200 mm in length, shake vigorously for 3 minutes, and measure the time needed for almost complete disappearance of the foam thus generated.

(ii) pH \( \text{C2.5.4} \): To 20 mL each of the test solution and the blank solution add 1.0 mL of a solution of potassium chloride (1 in 1000), and obtain the difference in the reading of pH between these solutions.

(iii) Potassium permanganate-reducing substances: Place
20.0 mL of the test solution in a glass-stoppered, conical flask, add 20.0 mL of 0.002 mol/L potassium permanganate VS and 1 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper tightly, shake, then allow to stand for 10 minutes, and titrate \(<?50\)` with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the test in the same manner, using 20.0 mL of the blank solution, and obtain the difference of the consumption of 0.002 mol/L potassium permanganate VS between these solutions.

(iv) UV spectrum: Read the maximum absorbances between 220 nm and 240 nm and between 241 nm and 350 nm of the test solution against the blank solution as directed under Ultraviolet-visible Spectrophotometry \(<?2.24\).

(v) Residue on evaporation: Evaporate 20 mL of the test solution on a water bath to dryness, and weigh the residue after drying at 105°C for 1 hour.

1.3. Test for fine particles
1.3.1. Test procedure
Rinse thoroughly the inside and outside of containers to be tested with water for particle matter test, fill the container with the labeled volume of water for particulate matter test or 0.9 w/v% sodium chloride solution, adjust the amount of air in the container to about 50 mL per 500 mL of the labeled volume, put tight stopper to the container, and heat it at 121°C for 25 minutes in an autoclave. After allowing to cool for 2 hours, take out the container from the autoclave, and then allow to stand at ordinary temperature for about 24 hours. If the containers are deformed at 121°C, employ a suitable temperature-time combination as directed under 1.2. Extractable substances. Clean the outside of the container, mix by turning upside-down 5 or 6 times, insert immediately a clean needle of filterless infusion set into the container through the rubber closure of the container, take the effluent from the container while mixing gently in a clean container for measurement, and use it as the test solution.

Counting of the fine particles must be performed in dustless, clean facilities or apparatus, using a light-shielded automatic fine particle counter. The sensor of the counter to be used must be able to count fine particles of 1.5 μm or more in diameter. The volume to be measured is 10 mL. Adjust the counter before measurement. For calibration of the diameter and number of particles, the standard particles for calibration of the light-shielded automatic fine particle counter should be used in suspension in water for particulate matter test or 0.9 w/v% sodium chloride solution.

Count five times the numbers of particles with diameters of 5 – 10 μm, 10 – 25 μm and more than 25 μm while stirring the test solution, and calculate the average particle numbers of four counts, excluding the first, as the number of particles in 1.0 mL of the test solution.

1.3.2. Reagent
Water for particulate matter test and 0.9 w/v% sodium chloride solution to be used for the tests should not contain more than 0.5 particles of 5 – 10 μm in size per 1.0 mL.

1.4. Transparency test
1.4.1. Method 1
This method can only be applied to containers which have a smooth and not embossed surface and rather low curvature. Cut the container at homogeneous regions of low curvature and preferably the same thickness to make 5 pieces of about 0.9 × 4 cm in size, immerse each piece in water filled in a cell for determination of the ultraviolet spectrum, and determine the transmittance at 450 nm as directed under Ultraviolet-visible Spectrophotometry \(<?2.24\) using a cell filled with water as a blank.

1.4.2. Method 2
Sensory test—This method can be applied to containers which have a rough or embossed surface. It can also be applied to testing of the transparency of containers in case where the turbidity of their pharmaceutical contents must be checked.

1.4.2.1. Test solutions
(i) Formadin standard suspension: To 15 mL of the formadin stock suspension add water to make 1000 mL. Use within 24 hours of preparation. Shake thoroughly before use.

(ii) Reference suspension: To 50 mL of Formadin standard suspension add water to make 100 mL.

1.4.2.2. Test procedures
(i) Method 2A (with control): Take two of containers to be tested, and fill one of them with the labeled volume of the reference suspension and the other with the same volume of water. Show these two containers to five subjects, separately, ask which one seems to be more turbid, and calculate the rate of correct answers.

(ii) Method 2B (without control): Take six of containers to be tested, put number to each of them, and fill three of them with the labeled volume of the reference suspension and the others with the same volume of water. Show each one of these containers at random order to five subjects, separately, ask if it is turbid or not, and calculate the percentage of the answer judged as “turbid” (100 X/15, X: number of containers judged as “turbid”) for reference suspension-filled containers group and water-filled containers group, respectively.

1.5. Water vapor permeability test
1.5.1. Method 1
This test method is applicable to containers for aqueous injection. Fill the container with the labeled volume of water. After closing it hermetically, accurately weigh the container and record the value. Store the container at 65 ± 5% relative humidity and a temperature of 20 ± 2°C for 14 days, and then accurately weigh the container again and record the value. Calculate the mass loss during storage.

1.5.2. Method 2
This test method is provided for evaluating moisture permeability of containers for hygroscopic drugs. Unless otherwise specified, perform the test according to the following procedure.

1.5.2.1. Desiccant
Place a quantity of calcium chloride for water determination in a shallow container, taking care to exclude any fine powder, then dry at 110°C for 1 hour, and cool in a desiccator.

1.5.2.2. Procedure
Select 12 containers, clean their surfaces with a dry cloth, and close and open each container 30 times in the same manner. Ten among the 12 containers are used as “test containers” and the remaining two, as “control containers”. A torque for closing screw-capped containers is specified in Table 7.02-1. Add desiccant to 10 of the containers, designated test containers, filling each to within 13 mm of the closure if the container volume is 20 mL or more, or filling each to two-thirds of capacity if the container volume is less than 20 mL. If the interior of the container is more than 63


mm in depth, an inert filler or spacer may be placed in the bottom to minimize the total mass of the container and desiccant; the layer of desiccant in such a container shall be not less than 5 cm in depth. Close each container immediately after adding desiccant, applying the torque designated in the table. To each of the control containers, add a sufficient number of glass beads to attain a mass approximately equal to that of each of the test containers, and close, applying the torque designated in the table. Record the mass of the individual containers so prepared to the nearest 0.1 mg if the container volume is less than 20 mL, to the nearest 1 mg if the container volume is 20 mL or more but less than 200 mL, or to the nearest 10 mg if the container volume is 200 mL or more, and store the containers at 75 ± 3% relative humidity and a temperature of 20 ± 2°C. After 14 days, record the mass of the individual containers in the same manner. Completely fill 5 empty containers with water or a non-compressible, free-flowing solid such as fine glass beads, to the level indicated by the closure surface when in place. Transfer the contents of each to a graduated cylinder, and determine the average container volume, in mL. Calculate the rate of moisture permeability, in mg per day per liter, by use of the formula:

\[
\frac{(1000/14 \cdot V) \cdot (T_i - T_f) - (C_i - C_f)}{C_i - C_f}
\]

\(V\): average volume (mL)

\(T_i - T_f\): difference between the final and initial masses of each test container (mg)

\(C_i - C_f\): average of the differences between the final and initial masses of the two controls (mg)

### 1.6. Leakage test

Fill a container with a solution of fluorescein sodium (1 in 1000), stopper tightly, place filter papers on and under the container, and apply a pressure of 6.9 N (0.7 kg)/cm² at 20°C for 10 minutes. Judge the leakiness by observing the color of the paper.

### 1.7. Cytotoxicity test

The following test methods are designed to detect cytotoxic substances in plastic materials by evaluating the cytotoxicity of the culture medium extracts from plastic containers for pharmaceutical products. Other appropriate standard methods of cytotoxicity testing may be used for the evaluation, if appropriate. However, the final decision shall be made based upon the test methods given here, if the test results obtained according to the other methods are questionable.

#### 1.7.1. Cell lines

The recommended cell lines are L929 (American Type Culture Collection-ATCC CCL1) and V79 (Health Science Research Resources Bank-JCRB0603). In addition, other established cell lines may be used when it is confirmed that they form well-defined colonies reproducibly, with characteristics comparable to those of L929 and V79 cells.

#### 1.7.2. Culture medium

Eagle’s minimum essential medium prepared as follows shall be used. Dissolve the chemicals listed below in 1000 mL of water. Sterilize the solution by autoclaving at 121°C for 20 minutes. Cool the solution to room temperature, and add 22 mL of sterilized sodium hydrogen carbonate TS and 10 mL of sterilized glutamine TS. To the resultant solution add fetal calf serum (FCS) to make 10 vol% FCS in the medium.

- sodium chloride 6.80 g
- potassium chloride 400 mg
- sodium dihydrogen phosphate (anhydrous) 115 mg
- magnesium sulfate (anhydrous) 93.5 mg
- calcium chloride (anhydrous) 200 mg
- glucose 1.00 g
- L-arginine hydrochloride 126 mg
- L-cysteine hydrochloride monohydrate 31.4 mg
- L-tyrosine 36.0 mg
- L-histidine hydrochloride monohydrate 42.0 mg
- L-isoleucine 52.0 mg
- L-leucine 52.0 mg
- L-lysine hydrochloride 73.0 mg
- L-methionine 15.0 mg
- L-phenylalanine 32.0 mg
- L-threonine 48.0 mg
- L-tryptophan 10.0 mg
- L-valine 46.0 mg
- succinic acid 75.0 mg
- succinic acid hexahydrate 100 mg
- choline bitartrate 1.8 mg
- folic acid 1.0 mg
- myo-inositol 2.0 mg
- nicotinamide 1.0 mg
- calcium D-pantothenate 1.0 mg
- pyridoxal hydrochloride 1.0 mg
- riboflavin 0.1 mg
- thiamine hydrochloride 1.0 mg
- biotin 0.02 mg
- phenol red 6.0 mg

#### 1.7.3. Reagents

1. Sodium hydroxide carbonate TS: Dissolve 10 g of sodium hydroxide carbonate in water to make 100 mL. Sterilize the solution either by autoclaving in a well-sealed container at 121°C for 20 minutes or by filtration through a membrane filter with a nominal pore diameter of 0.22 μm or less.
1.7.5. Control materials and substances

(i) Glutamine TS: Dissolve 2.92 g of L-glutamine in water to make 100 mL. Sterilize the solution by passing it through a membrane filter of pore size equal to or less than 0.22 μm.

(ii) Phosphate buffer solution (PBS): Dissolve 0.20 g of potassium chloride, 0.20 g of potassium dihydrogenphosphate, 8.00 g of sodium chloride, and 1.15 g of disodium hydrogenphosphate (anhydrous) in water to make 1000 mL. Sterilize the solution by autoclaving at 121°C for 20 minutes.

(iii) Trypsin TS: Dissolve 0.5 g of trypsin and 0.2 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in phosphate buffer solution to make 100 mL. Sterilize the solution by passing it through a membrane filter of pore size equal to or less than 0.22 μm.

(iv) Formaldehyde solution: Dilute formaldehyde solution with water by a factor of ten.

(v) Giemsa’s stain solution: Dilute a commercially available Giemsa’s test solution with the diluent by a factor of fifty. Prepare before use.

(vi) Diluent: Dissolve 4.54 g of potassium dihydrogenphosphate and 4.75 g of disodium hydrogen phosphate (anhydrous) in water to make 1000 mL.

1.7.4. Devices and instruments

(i) Pipets: Pasteur pipet, pipet for partial delivery, measuring pipet for partial delivery, and dispenser with micropipet.

(ii) Screw-capped glass bottles: 50 – 1000 mL volume.

(iii) Sterile disposable centrifuge tubes: 15 and 50 mL volume.

(iv) Sterile disposable tissue culture flasks with a flat bottom surface of the well. Discard the supernatant, resuspend the cells in an appropriate volume of fresh culture medium and gently pipet the cells completely out of the flask by using a Pasteur pipet. Transfer the pipetted cell suspension into a sterile disposable centrifuge tube and centrifuge the tube at 800 – 1000 revolutions per minute for 2 – 5 minutes. Discard the supernatant, resuspend the cells in an appropriate volume of fresh culture medium to the flask. Resuspend the cells by pipetting and make a single cell suspension. Determine the cell concentration using a hemocytometer.

(v) Cytotoxicity testing: Dilute the cell suspension prepared according to procedure (iii) with culture medium to 100 cells/mL. Place a 0.5 mL aliquot of the diluted cell suspension on each well of a sterile disposable multiple well plate. Incubate the plate in the humidified incubator for 4 – 6 hours to attach the cells to the bottom surface of the well. Discard the medium from each well, and add a 0.5 mL aliquot of the test solution or fresh medium to quadruplicate wells. Place the plate immediately in the humidified incubator and incubate the plate for the appropriate period: 7 – 9 days for L929 cells; 6 – 7 days for V79 cells. After the incubation, discard the medium from the plate, and add an appropriate volume of dilute formaldehyde TS to each well and allow the plate to stand for 30 minutes to fix the cells. Discard the dilute formaldehyde TS from each well and add an appropriate volume of dilute Giemsa’s TS to each well. After ensuring good staining of the colonies, discard the stain solution from the wells and count the number of colonies in each well. Calculate a mean number of colonies for each concentration of the test solution, and divide the mean by the mean number of colonies for the fresh medium to obtain the colony formation rate (%) for each extract concentration of the test solution. Plot the extract concentration (%) of the test solution on a logarithmic scale and the colony formation rate on an ordinary scale on semilogarithmic graph paper to obtain a colony formation inhibition curve of the container. Read the % extract concentration which inhibits colony formation to 50%, IC50 (%), from the inhibition curve. It is recommended to check the sensitivity and the reproducibility of the test system by the use of suitable control materials or substances in the test system, if necessary.
2. Requirements for Plastic Containers for Aqueous Injections

2.1. Polyethylene or polypropylene containers for aqueous injections

The containers are made of polyethylene or polypropylene and free from any adhesive.

(1) Transparency—The containers have a transmittance of not less than 55%, when tested as directed in “1.4.1. Method 1”. When “1.4.1. Method 1” can not be applied, test according to “1.4.2.2. (ii) Method 2B”. In this case, the rate that the water-containing container is judged as “being turbid” is not more than 20%, and the rate that the reference suspension-containing container is judged as “being turbid” is not less than 80%.

(2) Appearance—The containers do not have strips, cracks, bubbles, or other faults which cause difficulties in practical use.

(3) Water vapor permeability—Proceed as directed in “1.5.1. Method 1”. The loss of mass is not more than 0.20%.

(4) Heavy metals $\langle 0.07 \rangle$—The turbidity of the test solution is not greater than that of the control solution when the amount of the sample taken is 1.0 g.

(5) Lead—Perform the test as directed in “1.1.3.1. Method 1”. The absorbance of the sample solution is not more than that of the standard solution.

(6) Cadmium—Perform the test as directed in “1.1.4.1. Method 1”. The absorbance of the sample solution is not more than that of the standard solution.

(7) Residue on ignition $\langle 2.40 \rangle$—Not more than 0.1% (5 g).

(8) Extractable substances—
   (i) Foaming test: the foam formed almost disappears within 3 minutes.
   (ii) pH: the difference in the reading of pH between the test solution and the blank solution is not more than 1.5.
   (iii) Potassium permanganate-reducing substances: The difference in the consumption of 0.002 mol/L potassium permanganate VS between the test solution and the blank solution is not more than 1.0 mL.
   (iv) UV spectrum: The maximum absorbance between 220 nm and 240 nm is not more than 0.08, and that between 241 nm and 350 nm is not more than 0.05.
   (v) Residue on evaporation: Not more than 1.0 mg.

(9) Cytotoxicity—IC$_{50}$ (%) is not less than 90%. The result obtained by the other standard methods is negative.

2.2. Polyvinyl chloride containers for aqueous injections

The containers are composed of homopolymer of vinyl chloride, free from any adhesive, and the plasticizer added to the material should be di(2-ethylhexyl)phthalate. The containers may be covered with easily removable material to prevent the permeation of water vapor. In this case, perform the water vapor permeability test on the covered containers.

(1) Thickness—Measure the thickness of a container at five different locations. The difference between the maximum and minimum values of thickness is 0.05 mm or less.

(2) Transparency—Proceed as directed in (1) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(3) Appearance—Proceed as directed in (2) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(4) Leakage—Proceed with the test according to “1.6. Leakage test”. The solution contained does not leak.

(5) Flexibility—Insert the spike needle for infusion through a rubber closure of the container used in (4) Leakage. The contained solution is almost completely discharged without displacement by air.

(6) Water vapor permeability—Proceed as directed in (3) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(7) Heavy metals $\langle 1.07 \rangle$—The turbidity of the test solution is not greater than that of the control solution when the amount of the sample taken is 1.0 g.

(8) Lead—Perform the test as directed in “1.1.3.2. Method 2”. The absorbance of the sample solution is not more than that of the standard solution.

(9) Cadmium—Perform the test as directed in “1.1.4.2. Method 2”. The absorbance of the sample solution is not more than that of the standard solution.

(10) Tin—The absorbance of the sample solution is not more than that of the standard solution.

(11) Vinyl chloride—Wash cut pieces of a container with water, wipe them thoroughly with a filter paper, subdivide them into pieces smaller than 5 mm square, and put 0.5 g of them into a 20-mL vial. Add 2.5 mL of N,N-dimethylacetamide to the vial to dissolve the sample pieces, put a tight stopper on the vial, and use the solution in the vial as the sample solution. If the sample is hardly soluble, allow to stand the vial at room temperature for a night, put a tight stopper on the vial, and use the liquid part in the vial as the sample solution. Separately, to a 20-mL vial add 2.5 mL of N,N-dimethylacetamide, add 50 μL of Standard Vinyl Chloride Solution, previously cooled with dry ice-methanol, put a tight stopper on the vial, and use the solution in the vial as the sample solution.

After heating the vials containing sample solution and standard solution at 90°C for 1 hour, perform the test with 0.5 mL of each of vapor phases in these vials as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions: the peak area of vinyl chloride obtained from the sample solution is not larger than that from the standard solution.

Operating conditions—

   Detector: A hydrogen flame-ionization detector.
   Column: A fused silica column 0.25 mm in inside diameter and 25 m in length, coated the inside surface in 3 μm thickness with porous styrene-divinylbenzene copolymer for gas chromatography.
   Column temperature: Maintain at 50°C for 2 minutes after injection, then rise to 120°C in the rate of 10°C per minute, then rise to 250°C in the rate of 20°C per minute, and keep at 250°C for 10 minutes.
   Injection port temperature: A constant temperature of about 200°C.
   Detector temperature: A constant temperature of about 250°C.
   Carrier gas: Nitrogen or helium.
   Flow rate: Adjust the flow rate so that the retention time of vinyl chloride is about 7 minutes.
   Split ratio: 1:5.

System suitability—

System performance: When the procedure is run under the above operating conditions with 0.5 mL of the vapor phase of the standard solution heated at 90°C for 1 hour, vinyl chloride and ethanol are eluted in this order with the resolu-
7.03 Test for Rubber Closure for Aqueous Infusions / General Tests

The rubber closure for aqueous infusions means a rubber closure (containing material coated or laminated with the stuff like plastics) used for a container for aqueous infusion having a capacity of 100 mL or more, and is in direct contact with the contained aqueous infusion. The rubber closure when in use does not interact physically or chemically with the contained medication to alter any property or quality, does not permit the invasion of microbes, does not disturb the use of the contained infusion, and meets the following requirements.

1. Cadmium

Wash the rubber closures with water, dry at room temperature, cut into minute pieces, mix well, place 2.0 g of them in 50 mL of sulfuric acid, heat gradually to dryness, and ignite between 450°C and 500°C until the residue is incinerated. When incineration was insufficient, moisten the residue with 1 mL of sulfuric acid, heat to dryness, and ignite again. Repeat the above-mentioned procedure if necessary. Cool the crucible, moisten the residue with water, add 2 to 4 mL of hydrochloric acid, heat on a water bath to dryness, add 1 to 5 mL of hydrochloric acid, and dissolve by heating. Then add 0.5 to 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1) and 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). When any insoluble residue remains, filter through a glass filter. To the solution thus obtained add 10 mL of a solution of diammonium hydrogen citrate (1 in 4), 2 drops of bromothymol blue TS and ammonium TS until the color of the solution changes from yellow to green. Then add 10 mL of ammonium sulfate solution (2 in 5) and water to make 100 mL. Next, add 20 mL of a solution of sodium N,N-diethyldithiocarbamate trihydrate (1 in 20), mix, allow to stand for a few minutes, add 20.0 mL of 4-methyl-2-pentanone, and mix by vigorous shaking. Allow to stand to separate the 4-methyl-2-pentanone layer from the solution, filter if necessary, and use as the sample solution. On the other hand, to 10.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.23> under the following conditions, using the sample solution and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: Cadmium hollow-cathode lamp.
Wavelength: 228.8 nm.

2. Lead

To 1.0 mL of the Standard Lead Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed as directed for the sample solution under (1), and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.23> under the following conditions, using the sample solution obtained in (1) and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: Lead hollow-cathode lamp.
Wavelength: 283.3 nm.

3. Extractable substances

Wash the rubber closures with water, and dry at room temperature. Place them in a glass container, add water exactly 10 times the mass of the test material, close with a suitable stopper, heat at 121°C for 1 hour in an autoclave, take out the glass container, allow to cool to room temperature, then take out immediately the rubber closures, and use the remaining solution as the test solution. Prepare the blank solution with water in the same manner. Perform the following tests with the test solution and the blank solution.

3.1. Description

The test solution is clear and colorless. Read the transparency of the test solution at 430 nm and 650 nm (10 mm), using the blank solution as the blank. Both of them are not less than 99.0%.

3.2. Foam test

Place 5 mL of the test solution in a glass-stoppered test tube of about 15 mm in inner diameter and about 200 mm in...
length, and shake vigorously for 3 minutes. The foam arisen disappears almost completely within 3 minutes.

3.3. pH <2.5>  
To 20 mL each of the test solution and the blank solution add 1.0 mL each of potassium chloride solution, prepared by dissolving 1.0 g of potassium chloride in water to make 1000 mL. The difference of pH between the two solutions is not more than 1.0.

3.4. Zinc  
To 10.0 mL of the test solution add diluted dilute nitric acid (1 in 3) to make 20 mL, and use this solution as the sample solution. Further, to 1.0 mL of Standard Zinc Solution for atomic absorption spectrophotometry add diluted nitric acid (1 in 3) to make exactly 20 mL, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.23>, using these solutions, under the following conditions. The absorbance of the sample solution is not more than that of the standard solution.

- Gas: Combustible gas—Acetylene.
- Supporting gas—Air.
- Lamp: Zinc hollow-cathode lamp.
- Wavelength: 213.9 nm.
- Standard Zinc Solution for atomic absorption spectrophotometry: Measure exactly 10 mL of the Standard Zinc Stock Solution, and add water to make exactly 1000 mL. Prepare before use. One mL of this solution contains 0.01 mg of zinc (Zn).

3.5. Potassium Permanganate-reducing substances  
Measure 100 mL of the test solution in a glass-stoppered, Erlenmeyer flask, add 10.0 mL of 0.002 mol/L potassium permanganate VS and 5 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper, mix by shaking, then allow to stand for 10 minutes, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the blank test in the same manner, using 100 mL of the blank solution. The difference in mL of 0.002 mol/L potassium permanganate VS required between the tests is not more than 2.0 mL.

3.6. Residue on evaporation  
Measure 100 mL of the test solution, evaporate on a water bath to dryness, and dry the residue at 105°C for 1 hour. The mass of the residue is not more than 2.0 mg.

3.7. UV spectrum  
Read the absorbance of the test solution between 220 nm and 350 nm against the blank solution as directed under Ultraviolet-visible Spectrophotometry <2.5>: it is not more than 0.20.

4. Acute systemic toxicity  
The test solution meets the requirements, when examined under the following conditions against the blank solution.

4.1. Preparation of the test solution and the blank solution  
Wash the rubber closures with water and Water for Injection successively, and dry under clean conditions at room temperature. Transfer the rubber closures to a glass container. Add isotonic sodium chloride solution 10 times the mass of the test material, stopper adequately, heat in an autoclave at 121°C for 1 hour, take out the glass container, and allow to cool to room temperature. The solution thus obtained is used as the test solution. The blank solution is prepared in the same manner.

4.2. Test procedures  

(i) Test animals: Use healthy male mice of inbred strain or from a closed colony, weighing 17 to 23 g.

(ii) Procedure: Separate the animals into two groups of 10 mice, and inject intravenously 50 mL each of the solutions per kg body mass.

4.3. Interpretation  
Observe the animals for 5 days after injection: During the observation period, none of the animals treated with the test solution show any abnormality or death.

5. Pyrogen test  
The test solution specified in 4.1. meets the requirements of the Pyrogen Test <4.04> as does the blank solution.

6. Hemolysis test  
When 0.1 mL of defibrinated blood of rabbit is added to 10 mL of the test solution specified in 4.1. and the mixture is allowed to stand at 37°C for 24 hours, hemolysis is not observed. Perform the blank test in the same manner, using 10 mL of the blank solution.

8. Other Methods

8.01 Sterilization and Aseptic Manipulation

1. Sterilization  
Sterilization means a process whereby the killing or removal of all living microorganisms is accomplished. Generally, the sterilization process requires the choice of appropriate procedure and accurately controlled operation and conditions depending on the kind of microorganism, the conditions of contamination and the quality and nature of the substance to be sterilized.

The adequacy of sterilization is decided by means of the Sterility Test <4.06>.

The procedure for sterilization should be carried out after confirming that the temperature, pressure, etc. are adequate for the desired sterilization.

For the choice of the conditions for sterilization or verification of the integrity of sterilization, biological indicators suitable for individual conditions of sterilization may be used.

2. Aseptic manipulation  
Aseptic manipulation is a technique used for processing the sterile drug products which are not terminally sterilized in their final containers, and applied to a series of aseptic processing of the sterile products which are prepared by filtration sterilization and/or with sterile raw materials.

Generally, aseptic manipulation requires the presterilization of all equipments and materials used for processing the sterile products, and then the products are processed in a way to give a defined sterility assurance level in the aseptic processing facilities where microbial and particulate levels are adequately maintained.
9. Reference Standards; Standard Solutions; Reagents, Test Solutions; Measuring Instruments, Appliances, etc.

Reference Standards

9.01 Reference Standards

Reference Standards are the reference substances prepared to a specified quality necessary with regard to their intended use as prescribed in monographs of the Pharmacopoeia. The Japanese Pharmacopoeia Reference Standards are as follows:

1) The reference standards which are prepared by those who have been registered to prepare them by the Minister of Health, Labour and Welfare, according to the Ministerial ordinance established by the Minister separately.

- Aceglutamide RS
- Acetaminophen RS
- Aciclovir RS
- Adrenaline Bitartrate RS
- Alendronate Sodium RS
- Alprostadil RS
- p-Aminobenzoyl Glutamic Acid RS
- Amitriptyline Hydrochloride RS
- Amlexanox RS
- Amlodipine Besilate RS
- Anhydrous Lactose RS
- Ascorbic Acid RS
- Aspirin RS
- Atorvastatin Calcium RS
- Atropine Sulfate RS
- Azathioprine RS
- Baclofen RS
- Baicalin RS
- Beclometasone Dipropionate RS
- Berberine Chloride RS
- Betamethasone RS
- Betamethasone Sodium Phosphate RS
- Betamethasone Valerate RS
- Bisacodyl RS
- Caffeine RS
- Calcitonin (Salmon) RS
- Calcium Folinate RS
- Calcium Oxalate Monohydrate RS
- Camostat Mesilate RS
- d-Camphor RS
- dl-Camphor RS
- Carbide RS
- Cellacefate RS
- Chlordiazepoxide RS
- Chlormadinone Acetate RS
- Chlormpheniramime Maleate RS
- Cholecalciferol RS
- Ciclosporin RS
- Cilostazol RS
- Cisplatin RS
- Clopenterol Propionate RS
- Clofibrate RS
- Clomifene Citrate RS
- Cortisone Acetate RS
- Cyanocobalamin RS
- Danazol RS
- Deferoxamine Mesilate RS
- Desflanoside RS
- Dexamethasone RS
- Diclofenamide RS
- Diethylcarbamazine Citrate RS
- Diflucortolone Valerate RS
- Digitoxin RS
- Doxazosin Mesilate RS
- Dihydroergotoxine Mesilate RS
- Doxycycline Hydrochloride RS
- Donepezil Hydrochloride RS
- Edrophonium Chloride RS
- ELCETONIN RS
- Enalapril Maleate RS
- Endotoxin RS
- Epitiostanol RS
- Ergocalciferol RS
- Ergometrine Maleate RS
- Estradiol Benzoate RS
- Estriol RS
- Ethanamidine RS
- Ethinylestradiol RS
- Ethyl Aminobenzoate RS
- Etoposide RS
- Fexofenadine Hydrochloride RS
- Fludrocortisone Acetate RS
- Fluocinolone Acetonide RS
- Fluocinonide RS
- Fluorometholone RS
- Flumethasone RS
- Fluorometholone RS
- Flumethasone RS
- Flusulomelone Hydrochloride RS
- Gabexate Mesilate RS
- Gefarnate RS
- Ginsenoside Rb1 RS
- Ginsenoside Rg1 RS
- Ginkgolide A RS
- Glycyrrhizic Acid RS
- Gonadorelin Acetate RS
- Guanfacine RS
- Heparin Sodium RS
- Heparin Sodium for Physical and Chemical Test RS
- High-molecular Mass Urokinase RS
- Human Chorionic Gonadotrophin RS
- Human Insulin RS
- Human Menopausal Gonadotrophin RS
- Hydrochlorothiazide RS
- Hydrocortisone RS
- Hydrocortisone Acetate RS
Hydrocortisone Sodium Phosphate RS
Hydrocortisone Succinate RS
Idoxuridine RS
Imipramine Hydrochloride RS
Indapamide RS
Indomethacin RS
Interleukin-2 RS
Ipriflavone RS
Isoflurane RS
Kallidinogenase RS
Lactose RS
Lactulose RS
Lanatoside C RS
Limaprost RS
Losartan Potassium RS
Low-molecular Mass Heparin RS
Loxoprofen RS
Lysozyme RS
Maltose RS
Manidipine Hydrochloride RS
Mecobalamin RS
Melting Point Standard-Acetanilide RS
Melting Point Standard-Acetophenetidine RS
Melting Point Standard-Caffeine RS
Melting Point Standard-Sulfanilamide RS
Melting Point Standard-Sulfapyridine RS
Melting Point Standard-Vanillin RS
Menatetrenone RS
Mestranol RS
Methotrexate RS
Methoxsalen RS
Methyldopa RS
Methylerygometrine Maleate RS
Methyldiprisone Succinate RS
Methyltestosterone RS
Metildigoxin RS
Mexiletine Hydrochloride RS
Mizoribine RS
Nabumetone RS
Nategline RS
Neostigmine Methylsulfate RS
Nicotinamide RS
Nicotinic Acid RS
Nilvadipine RS
Nizatidine RS
Noradrenaline Bitartrate RS
Norgestrel RS
Over-sulfated Chondroitin Sulfate RS
Oxytocin RS
Ozagrel Sodium RS
Paeoniflorin RS
Pentobarbital RS
Pemirolast Potassium RS
Perphenazine RS
Phytonadione RS
Plioglitazone Hydrochloride RS
Potassium Sucrose Octasulfate RS
Povidone RS
Pravastatin 1,1,3,3-tetramethylbutylammonium RS
Prazosin Hydrochloride RS
Prednisolone RS
Prednisolone Acetate RS
Prednisolone Succinate RS

(2) The reference standards which are prepared by National Institute of Infectious Diseases.

Aclarubicin RS
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Standard Solutions

9.21 Standard Solutions for Volumetric Analysis

Standard Solutions for Volumetric Analysis are the solutions of reagent with an accurately known concentration, mainly used for the volumetric analysis. They are prepared to a specified molar concentration. A 1 molar solution is a solution which contains exactly 1 mole of a specified substance in each 1000 mL of the solution and is designated as 1 mol/L. If necessary, these solutions are diluted to other specified molar concentrations and the diluted solutions are also used as standard solutions. For example, 0.1 mol/L solution is obtained by diluting 1 mol/L solution 10 times by volume.

Unless otherwise directed, standard solutions for volumetric analysis should be stored in colorless or light-resistant, glass-stoppered bottles.

Preparation and Standardization

A volumetric standard solution is prepared according to one of the following methods. The degree of difference from a specified concentration \( n \) (mol/L) is expressed as a factor (molar concentration coefficient) \( f \). Usually, standard solutions are prepared so that the factor is in the range of 0.970 – 1.030. The determination procedure of the factor is called standardization of the standard solution.

1. Weigh accurately a quantity equivalent to about 1 mole or its multiple or a fractional mole number of the pure substance, and dissolve it in the specified solvent to make exactly 1000 mL to prepare a standard solution having a concentration close to exactly 1 mole or its multiple or a fractional mole number of the specified molar concentration and the diluted solutions are also used as standard solutions. For example, 0.1 mol/L solution is obtained by diluting 1 mol/L solution 10 times by volume.

When a pure substance is not obtainable, it is permissible to use a highly purified substance whose purity has been exactly determined and certified.

2. In the case where a pure substance or a highly purified substance is not obtainable, weigh a quantity equivalent to about 1 mole or its multiple or a fractional mole number of the substance specified for each standard solution and dissolve it in the specified solvent to make about 1000 mL to prepare a standard solution having a concentration close to the specified molar concentration of the substance (g) and the specified molar concentration number \( n \).

When a pure substance is not obtainable, it is permissible to use a highly purified substance whose purity has been exactly determined and certified.

Standardization

The degree of difference from a specified molar concentration \( n \) (mol/L) is obtained by applying the following equation.

\[
f = \frac{1000m}{VMn}
\]

Where:

- \( m \): Mass of the standard reagent or the specified substance (g)
- \( V \): Volume of the prepared standard solution consumed for the titration (mL)
- \( n \): Arithmetical mole number of the specified molar concentration of the solution to be standardized (e.g. \( n = 0.02 \) for 0.02 mol/L standard solution)
- \( f \): Factor of the titrating standard solution having a known factor
- \( f_1 \): Factor of the titrating standard solution having a known factor
- \( V_1 \): Volume of the titrating standard solution consumed (mL)
- \( V_2 \): Volume of the prepared standard solution taken (mL)

(3) Standard solutions may be prepared by diluting exactly an accurately measured volume of a standard solution having a known factor, according to the specified dilution procedure. During this dilution procedure, the original factor of the standard solution is assumed to remain constant.

Ammonium Iron (II) Sulfate, 0.1 mol/L

1000 mL of this solution contains 39.214 g of ammonium iron (II) sulfate hexahydrate \([\text{Fe}(\text{NH}_4)\text{SO}_4\cdot6\text{H}_2\text{O}: 392.14]\).

Preparation—Dissolve 49 g of ammonium iron (II) sulfate hexahydrate in a cooled mixture of 30 mL of sulfuric acid and 300 mL of water, dilute with water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared ammonium iron (II) sulfate solution, and add 25 mL of water and 5 mL of phosphoric acid. Titrate \( <2.50 \) the solution with 0.02 mol/L potassium permanganate VS. Calculate the molarity factor.

Note: Prepare before use.

Ammonium Iron (II) Sulfate, 0.02 mol/L

1000 mL of this solution contains 7.843 g of ammonium iron (II) sulfate hexahydrate \([\text{Fe}(\text{NH}_4)\text{SO}_4\cdot6\text{H}_2\text{O}: 392.14]\).

Preparation—Before use, dilute 0.1 mol/L ammonium iron (II) sulfate VS with diluted sulfuric acid (3 in 100) to make exactly 5 times the initial volume.

Ammonium Iron (III) Sulfate, 0.1 mol/L

1000 mL of this solution contains 48.22 g of ammonium iron (III) sulfate dodecahydrate \([\text{FeNH}_4\text{SO}_4\cdot12\text{H}_2\text{O}: 482.19]\).

Preparation—Dissolve 49 g of ammonium iron (III) sulfate dodecahydrate in a cooled mixture of 6 mL of sulfuric acid and 300 mL of water, add water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared ammonium iron (III) sulfate solution into an iodine flask, add 5 mL of hydrochloric acid, and shake the mixture. Dis-
solve 2 g of potassium iodide, and stopper the flask. After allowing the mixture to stand for 10 minutes, add 50 mL of water, and titrate 2.50 g of the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period of time, should be restandardized.

Ammonium Thiocyanate, 0.1 mol/L
1000 mL of this solution contains 7.612 g of ammonium thiocyanate (NH$_4$SCN; 76.12).

Preparation—Dissolve 8 g of ammonium thiocyanate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the 0.1 mol/L silver nitrate VS, and add 50 mL of water, 2 mL of nitric acid and 2 mL of ammonium iron (III) sulfate TS. Titrate 2.50 g of the solution with the prepared ammonium thiocyanate solution to the first appearance of a persistent red-brown color with shaking. Calculate the molarity factor.

Note: Store protected from light.

Ammonium Thiocyanate, 0.02 mol/L
1000 mL of this solution contains 1.5224 g of ammonium thiocyanate (NH$_4$SCN; 76.12).

Preparation—Before use, dilute 0.1 mol/L ammonium thiocyanate VS with water to make exactly 5 times the initial volume.

Barium chloride, 0.1 mol/L
1000 mL of this solution contains 24.426 g of barium chloride dihydrate (BaCl$_2$.2H$_2$O; 244.26).

Preparation—Dissolve 24.5 g of barium chloride dihydrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 20 mL of the prepared solution, add 3 mL of hydrochloric acid, and warm the mixture. Add 40 mL of diluted sulfuric acid (1 in 130), previously warmed, heat the mixture on a water bath for 30 minutes, and allow it to stand overnight. Filter the mixture, wash the precipitate on the filter paper with water until the last washing shows no turbidity with silver nitrate TS, transfer the precipitate together with the filter paper to a tared crucible, and then heat strongly to ashes. After cooling, add 2 drops of sulfuric acid, and heat strongly again at about 700°C for 2 hours. After cooling, weigh accurately the residue as barium sulfate (BaSO$_4$), and calculate the molarity factor.

Each mL of 0.02 mol/L barium chloride VS
= 4.668 mg of BaSO$_4$

Barium Chloride, 0.01 mol/L
1000 mL of this solution contains 2.4426 g of barium chloride dihydrate (BaCl$_2$.2H$_2$O; 244.26).

Preparation—Before use, dilute 0.02 mol/L barium chloride VS with water to make exactly twice the initial volume.

Barium Perchlorate, 0.005 mol/L
1000 mL of this solution contains 1.6812 g of barium perchlorate [Ba(ClO$_4$)$_2$; 336.23].

Preparation—Dissolve 1.7 g of barium perchlorate in 200 mL of water, dilute with 2-propanol to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 20 mL of the prepared barium perchlorate solution, add 55 mL of methanol and 0.15 mL of arsenazo III TS. Titrate 2.50 g of the solution with 0.005 mol/L sulfuric acid VS until its purple color changes through red-purple to red. Calculate the molarity factor.

Bismuth Nitrate, 0.01 mol/L
1000 mL of this solution contains 4.851 g of bismuth nitrate pentahydrate [Bi(NO$_3$)$_3$.5H$_2$O; 485.07].

Preparation—Dissolve 4.86 g of bismuth nitrate pentahydrate in 60 mL of dilute nitric acid, add water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared bismuth nitrate solution, add 50 mL of water and 1 drop of xyleneorange TS, and titrate 2.50 g of the solution with 0.01 mol/L disodium dihydrogen ethylenediamine tetracetate VS until the red color changes to yellow. Calculate the molarity factor.

Bromine, 0.05 mol/L
1000 mL of this solution contains 7.990 g of bromine (Br; 79.90).

Preparation—Dissolve 2.8 g of potassium bromate and 15 g of potassium bromide in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared solution into an iodine flask. Add 120 mL of water, quickly add 5 mL of hydrochloric acid, stopper the flask immediately, and shake it gently. Then add 5 mL of potassium iodide TS, re-stopper immediately, shake the mixture gently, and allow to stand for 5 minutes. Titrate 2.50 g of the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.
Ceric Ammonium Sulfate, 0.1 mol/L
See cerium (IV) tetraammonium sulfate, 0.1 mol/L.

Ceric Ammonium Sulfate, 0.01 mol/L
See cerium (IV) tetraammonium sulfate, 0.01 mol/L.

Cerium (IV) Tetraammonium Sulfate, 0.1 mol/L
1000 mL of this solution contains 63.26 g of cerium (IV) tetraammonium sulfate dihydrate \([\text{Ce}(\text{NH}_3)_4(\text{SO}_4)_2 \cdot 2\text{H}_2\text{O}: 632.55]\).

**Preparation**—Dissolve 64 g of cerium (VI) tetraammonium sulfate dihydrate in 0.5 mol/L sulfuric acid VS to make 1000 mL, allow to stand for 24 hours, filter the solution through a glass filter (G3 or G4), if necessary, and standardize the solution as follows:

**Standardization**—Measure exactly 25 mL of the prepared cerium (IV) tetraammonium sulfate solution into an iodine flask. Add 20 mL of water and 20 mL of dilute sulfuric acid, then dissolve 1 g of potassium iodide in the mixture. Immediately titrate with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period of time, should be restandardized.

Cerium (IV) Tetraammonium Sulfate, 0.01 mol/L
1000 mL of this solution contains 6.326 g of cerium (IV) tetraammonium sulfate dihydrate \([\text{Ce}(\text{NH}_3)_4(\text{SO}_4)_2 \cdot 2\text{H}_2\text{O}: 632.55]\).

**Preparation**—Before use, dilute 0.1 mol/L cerium (IV) tetraammonium sulfate VS with 0.5 mol/L sulfuric acid VS to make exactly 10 times the initial volume.

Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.1 mol/L
1000 mL of this solution contains 37.224 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate \((\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}: 372.24)\).

**Preparation**—Dissolve 38 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Wash zinc (standard reagent) with dilute hydrochloric acid, water and then acetone, dry at 110°C for 5 minutes, and allow to cool in a desiccator (silica gel). Weigh accurately about 0.8 g of this zinc, add 12 mL of dilute hydrochloric acid and 5 drops of bromine TS, and dissolve it by gentle warming. Expel any excess of bromine by boiling, and add water to make exactly 200 mL. Pipet 25 mL of this solution, and neutralize with sodium hydroxide solution (1 in 50). Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate with 0.1 mol/L sodium hydroxide solution (1 in 50) to form a blank determination. Calculate the molarity factor.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

\[
= 3.269 \text{ mg of Zn}
\]

Note: Store in polyethylene bottles.
**Preparation**—Before use, dilute 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS with water to make exactly 10 times the initial volume.

**Ferric Ammonium Sulfate, 0.1 mol/L**
See Ammonium Iron (III) Sulfate, 0.1 mol/L.

**Ferrous Ammonium Sulfate, 0.1 mol/L**
See Ammonium Iron (II) Sulfate, 0.1 mol/L.

**Ferrous Ammonium Sulfate, 0.02 mol/L**
See Ammonium Iron (II) Sulfate, 0.02 mol/L.

**Hydrochloric Acid, 2 mol/L**
1000 mL of this solution contains 72.92 g of hydrochloric acid (HCl: 36.46).

**Preparation**—Dilute 180 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh about 1.5 g of sodium carbonate (standard reagent) accurately, and dissolve in 100 mL of water.

Each mL of 2 mol/L hydrochloric acid VS
\[= 106.0 \text{ mg of Na}_2\text{CO}_3\]

**Hydrochloric Acid, 1 mol/L**
1000 mL of this solution contains 36.461 g of hydrochloric acid (HCl: 36.46).

**Preparation**—Dilute 90 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.8 g of sodium carbonate (standard reagent), previously heated between 500°C and 650°C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve it in 50 mL of water, and titrate \(0.2500\) with the prepared hydrochloric acid to calculate the molarity factor (Indicator method: 3 drops of methyl red TS; or potentiometric titration). In the indicator method, when the end-point is approached, boil the content carefully, stopper the flask loosely, allow to cool, and continue the titration until the color of the solution changes to persistent orange to orange-red. In the potentiometric titration, titrate with vigorous stirring, without boiling.

Each mL of 1 mol/L hydrochloric acid VS
\[= 53.00 \text{ mg of Na}_2\text{CO}_3\]

**Hydrochloric Acid, 0.5 mol/L**
1000 mL of this solution contains 18.230 g of hydrochloric acid (HCl: 36.46).

**Preparation**—Dilute 45 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh accurately about 0.4 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

Each mL of 0.5 mol/L hydrochloric acid VS
\[= 26.50 \text{ mg of Na}_2\text{CO}_3\]

**Hydrochloric Acid, 0.2 mol/L**
1000 mL of this solution contains 7.292 g of hydrochloric acid (HCl: 36.46).

**Preparation**—Dilute 18 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh accurately about 0.15 g of sodium carbonate (standard reagent), and dissolve in 30 mL of water.

Each mL of 0.2 mol/L hydrochloric acid VS
\[= 10.60 \text{ mg of Na}_2\text{CO}_3\]

**Hydrochloric Acid, 0.1 mol/L**
1000 mL of this solution contains 3.6461 g of hydrochloric acid (HCl: 36.46).

**Preparation**—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly twice the initial volume.

Each mL of 0.1 mol/L hydrochloric acid VS
\[= 5.300 \text{ mg of Na}_2\text{CO}_3\]

**Hydrochloric Acid, 0.05 mol/L**
1000 mL of this solution contains 1.8230 g of hydrochloric acid (HCl: 36.46).

**Preparation**—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 4 times the initial volume.

**Hydrochloric Acid, 0.02 mol/L**
1000 mL of this solution contains 0.7292 g of hydrochloric acid (HCl: 36.46).

**Preparation**—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 10 times the initial volume.

**Hydrochloric Acid, 0.01 mol/L**
1000 mL of this solution contains 0.36461 g of hydrochloric acid (HCl: 36.46).

**Preparation**—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 20 times the initial volume.

**Hydrochloric Acid, 0.001 mol/L**
1000 mL of this solution contains 0.036461 g of hydrochloric acid (HCl: 36.46).

**Preparation**—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 200 times the initial volume.

**Iodine, 0.05 mol/L**
1000 mL of this solution contains 12.690 g of iodine (I: 126.90).

**Preparation**—Dissolve 13 g of iodine in 100 mL of a solution of potassium iodide (2 in 5), add 1 mL of dilute hydrochloric acid and water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 15 mL of the iodine solution, and titrate \(0.2500\) with 0.1 mol/L sodium thiosulfate VS (Indicator method: starch TS; or potentiometric titration: platinum electrode). In the indicator method, when the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS, and continue the titration...
until the blue color disappears. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period, should be restandardized before use.

**Iodine, 0.01 mol/L**

1000 mL of this solution contains 2.5381 g of iodine (I: 126.90).

**Preparation**—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 5 times the initial volume.

**Iodine, 0.005 mol/L**

1000 mL of this solution contains 1.2690 g of iodine (I: 126.90).

**Preparation**—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 10 times the initial volume.

**Iodine, 0.002 mol/L**

1000 mL of this solution contains 0.5076 g of iodine (I: 126.90).

**Preparation**—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 25 times the initial volume.

**Magnesium Chloride, 0.05 mol/L**

1000 mL of this solution contains 10.165 g of magnesium chloride hexahydrate (MgCl₂·6H₂O: 203.30).

**Preparation**—Dissolve 10.2 g of magnesium chloride hexahydrate in freshly boiled and cooled water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 25 mL of the prepared magnesium chloride solution. Add 50 mL of water, 3 mL of pH 10.7 ammonia-ammonium chloride buffer solution and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate with 0.05 mol/L disodium dihydrogen ethylenediamine tetracetate VS until the red-purple color of the solution changes to blue-purple. Calculate the molarity factor.

**Magnesium Chloride, 0.01 mol/L**

1000 mL of this solution contains 2.0330 g of magnesium chloride hexahydrate (MgCl₂·6H₂O: 203.30).

**Preparation**—Before use, dilute 0.05 mol/L magnesium chloride VS with water to make exactly 5 times the initial volume.

**Oxalic Acid, 0.05 mol/L**

1000 mL of this solution contains 6.303 g of oxalic acid (C₃H₂O₄·2H₂O: 126.07).

**Preparation**—Dissolve 6.3 g of oxalic acid dihydrate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 25 mL of the prepared oxalic acid solution in a 500-mL conical flask, and add 200 mL of diluted sulfuric acid (1 in 20), previously boiled for 10 to 15 minutes and then cooled to 27 ± 3°C. Transfer freshly standardized 0.02 mol/L potassium permanganate VS to a burette. Add quickly 22 mL of the 0.02 mol/L potassium permanganate VS to the oxalic acid solution from the burette under gentle stirring, and allow to stand until the red color of the mixture disappears. Heat the solution up to between 55°C and 60°C, and complete the titration with 0.03 mL of acetic anhydride with shaking at about 20°C. Calculate the molarity factor.

**Oxalic Acid, 0.005 mol/L**

1000 mL of this solution contains 0.6303 g of oxalic acid dihydrate (C₃H₂O₄·2H₂O: 126.07).

**Preparation**—Before use, dilute 0.05 mol/L oxalic acid VS with water to make exactly 10 times the initial volume.

**Perchloric Acid, 0.1 mol/L**

1000 mL of this solution contains 10.046 g of perchloric acid (HClO₄: 100.46).

**Preparation**—Add slowly 8.7 mL of perchloric acid to 1000 mL of acetic acid (100) while keeping the temperature at about 20°C. Allow the mixture to stand for about 1 hour. Perform quickly the test as directed under Water Determination with 3.0 mL of the mixture, and designate the water content as A (g/dL). To the rest mixture add slowly [(A – 0.03) × 52.2] mL of acetic anhydride with shaking at about 20°C. Allow the solution to stand for 24 hours, and standardize it as follows:

**Standardization**—Weigh accurately about 0.3 g of potassium hydrogen phthalate (standard reagent), previously dried at 105°C for 4 hours and allowed to cool in a desiccator (silica gel). Dissolve it in 50 mL of acetic acid (100), and titrate with 0.05 mol/L disodium dihydrogen ethylenediamine tetracetate VS until the red-purple color of the solution changes to blue-purple. Calculate the molarity factor.

**Perchloric Acid, 0.05 mol/L**

1000 mL of this solution contains 5.023 g of perchloric acid (HClO₄: 100.46).

**Preparation**—Before use, dilute 0.1 mol/L perchloric acid VS with acetic acid for nonaqueous titration to make exactly twice the initial volume. Perform quickly the test as directed under Water Determination with 8.0 mL of acetic acid for nonaqueous titration, and designate the water content as A (g/dL). If A is not less than 0.03, add [(A – 0.03) × 52.2] mL of acetic anhydride to 1000 mL of acetic acid for nonaqueous titration, and use it for the preparation.

**Perchloric Acid, 0.02 mol/L**

1000 mL of this solution contains 2.0092 g of perchloric acid (HClO₄: 100.46).

**Preparation**—Before use, dilute 0.1 mol/L perchloric acid VS with acetic acid for nonaqueous titration to make exactly 5 times the initial volume. Perform quickly the test as directed under Water Determination with 8.0 mL of acetic acid for nonaqueous titration, and designate the water content as A (g/dL). If A is not less than 0.03, add [(A – 0.03) × 52.2] mL of acetic anhydride to 1000 mL of acetic acid for nonaqueous titration, and use it for the preparation.

**Perchloric Acid-1,4-Dioxane, 0.1 mol/L**

1000 mL of this solution contains 10.046 g of perchloric acid (HClO₄: 100.46).
acids (HClO₄: 100.46).

**Preparation**—Dilute 8.5 mL of perchloric acid with 1,4-dioxane to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.5 g of potassium dichromate (standard reagent), previously dried at 105°C for 4 hours and allowed to cool in a desiccator (silica gel). Dissolve it in 80 mL of acetic acid for nonaqueous titration, and add 3 drops of crystal violet TS. Titrate ≤0.50 mL of the solution with the prepared perchloric acid-1,4-dioxane VS with 1,4-dioxane to make exactly twice the initial volume.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS with 1,4-dioxane to make exactly twice the initial volume.

**Perchloric Acid-1,4-Dioxane, 0.05 mol/L**
1000 mL of this solution contains 5.023 g of perchloric acid (HClO₄: 100.46).

**Preparation**—Before use, dilute 0.1 mol/L perchloric acid-1,4-dioxane VS with 1,4-dioxane to make exactly twice the initial volume.

**Perchloric Acid-1,4-Dioxane, 0.004 mol/L**
1000 mL of this solution contains 0.4018 g of perchloric acid (HClO₄: 100.46).

**Preparation**—Before use, dilute 0.1 mol/L perchloric acid-1,4-dioxane VS with 1,4-dioxane to make exactly 25 times the initial volume.

**Potassium Dichromate, 1/60 mol/L**
See Potassium Dichromate, 1/60 mol/L.

**Potassium Bromate, 1/60 mol/L**
1000 mL of this solution contains 2.7833 g of potassium bromate (KBrO₃: 187.00).

**Preparation**—Dissolve 2.8 g of potassium bromate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 25 mL of the prepared potassium bromate solution into an iodine flask. Add 2 g of potassium iodide and 5 mL of dilute sulfuric acid, stopper the flask, and allow the solution to stand for 5 minutes. Add 100 mL of water, and titrate ≤0.50 mL of the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

**Potassium Hexacyanoferrate (III), 0.05 mol/L**
1000 mL of this solution contains 16.462 g of potassium hexacyanoferrate (III) [K₃Fe(CN)₆: 329.24].

**Preparation**—Before use, dilute 0.1 mol/L potassium hexacyanoferrate (III) VS with water to make exactly twice the initial volume.

**Potassium Hydroxide, 1 mol/L**
1000 mL of this solution contains 56.11 g of potassium hydroxide (KOH: 56.11).

**Preparation**—Dissolve 65 g of potassium hydroxide in 950 mL of water. Add a freshly prepared, saturated solution of barium hydroxide octahydrate until no more precipitate is produced. Shake the mixture thoroughly, and allow it to stand for 24 hours in a tightly stoppered bottle. Decant the supernatant liquid or filter the solution through a glass filter (G3 or G4), and standardize the solution as follows:

**Standardization**—Weigh accurately about 2.5 g of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for about 48 hours. Dissolve it in 25 mL of freshly boiled and cooled water, and add 2 drops of bromothymol blue TS. Titrate ≤0.50 mL of the solution with the prepared potassium hydroxide solution until it acquires a green color. Calculate the molarity factor.

Note: Store in tightly stoppered bottles or in containers provided with a carbon dioxide-absorbing tube (soda-lime). This solution, if stored for a long period, should be restandardized.

**Potassium Hydroxide, 0.5 mol/L**
1000 mL of this solution contains 28.053 g of potassium hydroxide (KOH: 56.11).

**Preparation**—Weigh 32 g of potassium hydroxide, proceed as directed for preparation under 1 mol/L potassium hydroxide VS, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 1 mol/L potassium hydroxide VS, but weigh accurately about 1.3 g of amidosulfuric acid (standard reagent).
Each mL of 0.5 mol/L potassium hydroxide VS = 48.55 mg of HOSO₂NH₂

Note: Store as directed under 1 mol/L potassium hydroxide VS. This solution, if stored for a long period, should be restandardized.

Potassium Hydroxide-ethanol, 0.5 mol/L
1000 mL of this solution contains 28.053 g of potassium hydroxide (KOH: 56.11).

Preparation—Dissolve 35 g of potassium hydroxide in 20 mL of water, and add aldehyde-free ethanol to make 1000 mL. Allow the solution to stand for 24 hours in a tightly stoppered bottle. Then quickly decant the supernatant liquid, and standardize the solution as follows:

Standardization—Measure exactly 15 mL of 0.25 mol/L sulfuric acid VS, add 50 mL of water, and titrate with the prepared potassium-hydroxide-ethanol solution to calculate the molarity factor (indicator method: 2 drops of phenolphthalein TS or potentiometric titration). In the indicator method, titrate <2.50 mL until the solution acquires a pale red color.

Note: Store in tightly stoppered bottles, protected from light. Standardize before use.

Potassium Hydroxide-ethanol, 0.1 mol/L
1000 mL of this solution contains 5.611 g of potassium hydroxide (KOH: 56.11).

Preparation—Weigh 7 g of potassium hydroxide, proceed as directed for preparation under 0.5 mol/L potassium hydroxide-ethanol VS, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.5 mol/L potassium hydroxide-ethanol VS, but measure exactly 15 mL of 0.05 mol/L sulfuric acid VS.

Note: Store as directed under 0.5 mol/L potassium hydroxide-ethanol VS. Standardize before use.

Potassium Iodate, 0.05 mol/L
1000 mL of this solution contains 10.700 g of potassium iodate (KIO₃: 214.00).

Preparation—Weigh accurately about 10.700 g of potassium iodate (standard reagent), previously dried between 120°C and 140°C for 2 hours and allowed to cool in a desiccator (silica gel), and dissolve it in water to make exactly 1000 mL. Calculate the molarity factor.

Each mL of 0.02 mol/L potassium permanganate VS = 6.700 mg of Na₂C₂O₄

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

Potassium Permanganate, 0.002 mol/L
1000 mL of this solution contains 0.31607 g of potassium permanganate (KMnO₄: 158.03).

Preparation—Dissolve 3.2 g of potassium permanganate in water to make 1000 mL, and boil the solution for 15 minutes. Allow the solution to stand for at least 48 hours in a tightly stoppered flask, and filter it through a glass filter (G3 or G4). Standardize the solution as follows:

Standardization—Weigh accurately about 0.3 g of sodium oxalate (standard reagent), previously dried between 150°C and 200°C for 1 to 1.5 hours and allowed to cool in a desiccator (silica gel), transfer it to a 500 mL conical flask, dissolve in 30 mL of water, add 250 mL of diluted sulfuric acid (1 in 20), and warm the mixture between 30°C and 35°C. Transfer the prepared potassium permanganate solution to a buret, add quickly 40 mL of the solution under gentle stirring from the buret, and allow to stand until the red color of the mixture disappears. Warm the solution between 55°C and 60°C, and complete the titration <2.50 mL> with the potassium permanganate solution until a faint red color persists for 30 seconds. Add the last 0.5 to 1 mL dropwise before the end point, being particularly careful to allow the solution to be decolorized before the next drop is added. Calculate the molarity factor.

Each mL of 0.02 mol/L potassium permanganate VS = 6.700 mg of Na₂C₂O₄

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

Silver Nitrate, 0.1 mol/L
1000 mL of this solution contains 16.987 g of silver nitrate (AgNO₃: 169.87).

Preparation—Dissolve 17.0 g of silver nitrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 80 mg of sodium chloride (standard reagent), previously dried between 500°C and 650°C for 40 to 50 minutes and allowed to cool in
a desiccator (silica gel), dissolve it in 50 mL of water, and titrate \( \leq 2.50 \) under vigorous stirring with the prepared silver nitrate solution to calculate the molarity factor (Indicator method: 3 drops of fluorescein sodium TS; or potentiometric titration: silver electrode). In the indicator method, titrate until the color of the solution changes from yellow-green to orange through yellow.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

Note: Store protected from light.

### Silver Nitrate, 0.02 mol/L

1000 mL of this solution contains 3.3974 g of silver nitrate (AgNO₃: 169.87).

**Preparation**—Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 5 times the initial volume.

### Silver Nitrate, 0.01 mol/L

1000 mL of this solution contains 1.6987 g of silver nitrate (AgNO₃: 169.87).

**Preparation**—Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 10 times the initial volume.

### Silver Nitrate, 0.005 mol/L

1000 mL of this solution contains 0.8494 g of silver nitrate (AgNO₃: 169.87).

**Preparation**—Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 20 times the initial volume.

### Silver Nitrate, 0.001 mol/L

1000 mL of this solution contains 0.16987 g of silver nitrate (AgNO₃: 169.87).

**Preparation**—Dilute 0.1 mol/L silver nitrate VS with water to make exactly 100 times of the initial volume before use.

### Sodium Acetate, 0.1 mol/L

1000 mL of this solution contains 8.203 g of sodium acetate (CH₃COONa: 82.03).

**Preparation**—Dissolve 8.20 g of anhydrous sodium acetate in acetic acid (100) to make 1000 mL, and standardize the solution as follows:

**Standardization**—Pipet 25 mL of the prepared sodium acetate solution, add 50 mL of acetic acid (100) and 1 mL of p-naphtholbenzene TS, and titrate \( < 2.50 \) with 0.1 mol/L perchloric acid VS until a yellow-brown color changes through yellow to green. Perform a blank determination. Calculate the molarity factor.

### Sodium Acetate, 0.2 mol/L

1000 mL of this solution contains 19.42 mg of HOSO₂NH₂

**Preparation**—Weigh 9 g of sodium hydroxide, proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 0.3 g of amidosulfuric acid (standard reagent).

Each mL of 0.2 mol/L sodium hydroxide VS = 48.55 mg of HOSO₂NH₂

Note: Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

### Sodium Acetate, 0.5 mol/L

1000 mL of this solution contains 48.55 mg of HOSO₂NH₂

**Preparation**—Weigh 4.5 g of sodium hydroxide, proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 0.15 g of amidosulfuric acid (standard reagent).

Each mL of 0.5 mol/L sodium hydroxide VS = 97.09 mg of HOSO₂NH₂

Note: Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

### Sodium Acetate, 1 mol/L

1000 mL of this solution contains 97.09 mg of HOSO₂NH₂

**Preparation**—Weigh 42 g of sodium hydroxide in 950 mL of water. Add a freshly prepared, saturated solution of barium hydroxide octahydrate until no more precipitate is produced. Mix well the mixture, and allow to stand for 24 hours in a tightly stoppered bottle. Decant the supernatant liquid or filter the solution through a glass filter (G3 or G4), and standardize the solution as follows:

**Standardization**—Weigh accurately about 1.5 g of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for about 48 hours. Dissolve it in 25 mL of freshly boiled and cooled water, and titrate \( < 2.50 \) with the solution with the prepared sodium hydroxide solution to calculate the molarity factor (Indicator method: 2 drops of bromothymol blue TS; or potentiometric titration). In the indicator method, titrate until the solution acquires a green color.

Each mL of 1 mol/L sodium hydroxide VS = 9.709 mg of HOSO₂NH₂

Note: Store in tightly stoppered bottles or in containers provided with a carbon dioxide-absorbing tube (soda lime). This solution, if stored for a long period, should be restandardized.
Sodium Hydroxide, 0.05 mol/L
1000 mL of this solution contains 1.9999 g of sodium hydroxide (NaOH: 40.00).
Preparation—Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly twice the initial volume.

Sodium Hydroxide, 0.02 mol/L
1000 mL of this solution contains 0.7999 g of sodium hydroxide (NaOH: 40.00).
Preparation—Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

Sodium Hydroxide, 0.01 mol/L
1000 mL of this solution contains 0.39997 g of sodium hydroxide (NaOH: 40.00).
Preparation—Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly 10 times the initial volume.

Sodium Hydroxide-Ethanol, 0.025 mol/L
1000 mL of this solution contains 1.000 g of sodium hydroxide (NaOH: 40.00).
Preparation—Dissolve 2.1 g of sodium hydroxide in 100 mL of ethanol (99.5), stopper tightly, and allow to stand for a night. To 50 mL of the supernatant liquid add 650 mL of ethanol (99.5) and freshly boiled and cooled water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 25 mg of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for 48 hours. Dissolve in 30 mL of diluted ethanol (99.5) with fleshly boiled and cooled water (7 in 10), and titrate <2.50 with the prepared sodium hydroxide-ethanol to calculate the molarity factor (potentiometric titration).

Each mL of 0.025 mol/L sodium hydroxide-ethanol VS = 2.427 mg of HOSO₂NH₂
Note: Store in light-resistant, well-stoppered bottles. The standardization should be performed before using.

Sodium Lauryl Sulfate, 0.01 mol/L
1000 mL of this solution contains 2.8838 g of sodium lauryl sulfate (C₁₂H₂₅NaO₄S: 288.38).
Preparation—Dissolve 2.9 g of sodium lauryl sulfate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.3 g of papaerine hydrochloride for assay, previously dried, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a glass-stoppered conical flask, add 5 mL each of water and dilute sulfuric acid and 60 mL of dichloromethane, then add 5 to 6 drops of a solution of methyl yellow in dichloromethane (1 in 500) as indicator, and titrate <2.50 with the vigorous shaking, with the sodium lauryl sulfate solution prepeared above, using a buret with a minimum graduation of 0.02 mL. End point is reached when the color of the dichloromethane layer changes from yellow to orange-red after dropwise addition of the sodium lauryl sulfate solution, vigorous shaking and standing for a while.

Each mL of 0.01 mol/L sodium lauryl sulfate VS = 3.759 mg of C₁₂H₂₅NaO₄S·HCl

Sodium Methoxide, 0.1 mol/L
1000 mL of this solution contains 5.402 g of sodium methoxide (CH₃ONa: 54.02).
Preparation—Add little by little 2.5 g of freshly cut sodium pieces to 150 mL of methanol cooled in ice-water. After the sodium has dissolved, add benzene to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 3 g of benzoic acid, previously dried for 24 hours in a desiccator (silica gel), dissolve it in 80 mL of N,N-dimethylformamide, and add 3 drops of thymol blue-N,N-dimethylformamide TS. Titrate <2.50 the solution with the prepared sodium methoxide solution until a blue color appears. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium methoxide VS = 12.21 mg of CH₃COOH
Note: Store in a cold place, protected from moisture. Standardize before use.

Sodium Methoxide-Dioxane, 0.1 mol/L
See Sodium Methoxide-1,4-Dioxane, 0.1 mol/L.

Sodium Methoxide-1,4-Dioxane, 0.1 mol/L
1000 mL of this solution contains 5.402 g of sodium methoxide (CH₃ONa: 54.02).
Preparation—Add little by little 2.5 g of freshly cut sodium pieces to 150 mL of methanol cooled in ice-water. After the sodium has dissolved, add 1,4-dioxane to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.3 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 80 mL of N,N-dimethylformamide, and add 3 drops of thymol blue-N,N-dimethylformamide TS. Titrate <2.50 the solution with the prepared sodium methoxide-1,4-dioxane solution until a blue color appears. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium methoxide-1,4-dioxane VS = 12.21 mg of CH₃COOH
Note: Store in a cold place, protected from moisture. Standardize before use.

Sodium Nitrite, 0.1 mol/L
1000 mL of this solution contains 6.900 g of sodium nitrite (NaNO₂: 69.00).
Preparation—Dissolve 7.2 g of sodium nitrite in water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.44 g of sulfanilamide for titration of diazotization, previously dried at 105°C for 3 hours and allowed to cool in a desiccator (silica gel), dissolve in 10 mL of hydrochloric acid, 40 mL of water and 10 mL of a solution of potassium bromide (3 in 10), cool below 15°C, and titrate with the prepared sodium nitrite solution as directed in the potentiometric titration or amperometric titration under Endpoint Detection Methods in Titrimetry <2.50. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium nitrite VS = 17.22 mg of H₂N₂C₆H₄SO₂NH₂
Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

**Sodium Oxalate, 0.005 mol/L**

100 mL of this solution contains 0.6700 g of sodium oxalate (Na₂C₂O₄; 134.00).

Preparation—Weigh accurately about 0.6700 g of sodium oxalate (standard reagent), previously dried between 150°C and 200°C for 2 hours and allowed to cool in a desiccator (silica gel), dissolve it in water to make exactly 1000 mL, and calculate the molarity factor.

**Sodium Tetraphenylborate, 0.02 mol/L**

1000 mL of this solution contains 6.844 g of sodium tetraphenylborate [NaB(C₆H₅)₄; 342.22].

Preparation—Dissolve 7.0 g of sodium tetraphenylborate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh 0.5 g of potassium hydrogen phthalate (standard reagent), dissolve it in 100 mL of water, add 2 mL of acetic acid (31), and warm to 50°C in a water bath. Add slowly 50 mL of the prepared sodium tetraphenylborate solution under stirring from a buret, then cool the mixture quickly, and allow to stand for 1 hour at room temperature. Transfer the precipitate to a tared glass filter (G4), wash three 5 mL portions of potassium tetraphenylborate TS, dry at 105°C for 1 hour, and weigh accurately the glass filter. Calculate the molarity factor from the mass of potassium tetraphenylborate [KB(C₆H₅)₄; 358.32].

Each mL of 0.02 mol/L sodium tetraphenylborate VS = 7.166 mg of KB(C₆H₅)₄

Note: Prepare before use.

**Sodium Tetraphenylboron, 0.02 mol/L**

See Sodium Tetraphenylborate, 0.02 mol/L.

**Sodium Thiosulfate, 0.1 mol/L**

1000 mL of this solution contains 24.818 g of sodium thiosulfate pentahydrate (Na₂S₂O₃.5H₂O: 248.18).

Preparation—Dissolve 25 g of sodium thiosulfate and 0.2 g of anhydrous sodium carbonate in freshly boiled and cooled water to make 1000 mL, allow to stand for 24 hours, and standardize the solution as follows:

Standardization—Weigh accurately about 30 mg of potassium iodate (standard reagent), previously dried between 120°C and 140°C for 1.5 to 2 hours and allowed to cool in a desiccator (silica gel), and transfer to an iodine flask. Dissolve it in 25 mL of water, add 2 g of potassium iodide and 10 mL of dilute sulfuric acid, and stopper the flask. Allow the mixture to stand for 10 minutes, add 100 mL of water, and titrate with the prepared sulfuric acid solution (Indicator method; or potentiometric titration: platinum electrode). In the indicator method, when the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium thiosulfate VS = 3.567 mg of KI

Note: This solution, if stored for a long period, should be restandardized.
**Standardization**—Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 0.4 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

Each mL of 0.25 mol/L sulfuric acid VS = 26.50 mg of Na₂CO₃

**Sulfuric Acid, 0.1 mol/L**
1000 mL of this solution contains 9.808 g of sulfuric acid (H₂SO₄: 98.08).

**Preparation**—Add slowly, under stirring, 6 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 0.15 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

Each mL of 0.1 mol/L sulfuric acid VS = 10.60 mg of Na₂CO₃

**Sulfuric Acid, 0.05 mol/L**
1000 mL of this solution contains 4.904 g of sulfuric acid (H₂SO₄: 98.08).

**Preparation**—Add slowly, under stirring, 3 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 80 mg of sodium carbonate (standard reagent), and dissolve in 30 mL of water.

Each mL of 0.05 mol/L sulfuric acid VS = 5.300 mg of Na₂CO₃

**Sulfuric Acid, 0.025 mol/L**
1000 mL of this solution contains 2.4520 g of sulfuric acid (H₂SO₄: 98.08).

**Preparation**—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly twice the initial volume.

**Sulfuric Acid, 0.01 mol/L**
1000 mL of this solution contains 0.9808 g of sulfuric acid (H₂SO₄: 98.08).

**Preparation**—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 5 times the initial volume.

**Sulfuric Acid, 0.005 mol/L**
1000 mL of this solution contains 0.4904 g of sulfuric acid (H₂SO₄: 98.08).

**Preparation**—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 10 times the initial volume.

**Sulfuric Acid, 0.0005 mol/L**
1000 mL of this solution contains 0.04904 g of sulfuric acid (H₂SO₄: 98.08).

**Preparation**—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 100 times the initial volume.

**Tetraethylammonium Hydroxide, 0.1 mol/L**
1000 mL of this solution contains 25.947 g of tetrabutyl ammonium hydroxide [C₄H₉]₄NOH: 259.47.

**Preparation**—Before use, dilute a volume of 10% tetrabutylammonium hydroxide-methanol TS, equivalent to 26.0 g of tetrabutylammonium hydroxide, with 2-propanol to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.3 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 50 mL of acetone, and titrate ≤0.5 ml the solution with the prepared tetrabutylammonium hydroxide solution (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS = 12.21 mg of C₆H₅COOH

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

**Tetraethylammonium Hydroxide, 0.2 mol/L**
1000 mL of this solution contains 18.231 g of tetrethylammonium hydroxide [C₄H₉]₄NOH: 91.15.

**Preparation**—Before use, dilute a volume of tetrethylammonium hydroxide-methanol TS, equivalent to 18.4 g of tetrethylammonium hydroxide, with water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.4 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 60 mL of N,N-dimethylformamide, and titrate ≤0.5 ml the solution with the prepared 0.2 mol/L tetrethyl ammonium hydroxide solution (Indicator method: 3 drops of thymol blue-N,N-dimethylformamide TS; or potentiometric titration). In the indicator method, titrate until a blue color is produced. Perform a blank determination in the same manner. Calculate the molarity factor.

Each mL of 0.2 mol/L tetrethylammonium hydroxide VS = 24.42 mg of C₆H₅COOH

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

**Tetraethylammonium Hydroxide, 0.1 mol/L**
1000 mL of this solution contains 9.115 g of tetrethylammonium hydroxide [C₄H₉]₄NOH: 91.15.

**Preparation**—Before use, dilute a volume of tetrethylammonium hydroxide-methanol TS, equivalent to 9.2 g of tetrethylammonium hydroxide, with water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.2 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 30 mL of N,N-dimethylformamide and titrate ≤0.5 ml the solution with the prepared 0.1 mol/L tetrethyl ammonium hydroxide solution (Indicator method: 3 drops of thymol blue-N,N-dimethylformamide TS; or potentiometric titration). In the indicator method, titrate until a blue color is produced. Perform a blank determination in the same manner. Calculate the molarity factor.

Each mL of 0.1 mol/L tetrethylammonium hydroxide VS = 12.21 mg of C₆H₅COOH

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

**Tetraethylammonium Hydroxide, 0.02 mol/L**
1000 mL of this solution contains 1.8231 g of tetrethylammonium hydroxide [C₄H₉]₄NOH: 91.15.

**Preparation**—Before use, dilute 0.1 mol/L tetrethylammonium hydroxide VS with freshly boiled and cooled water to make exactly 5 times the initial volume.
Tetramethylammonium Hydroxide-Methanol, 0.1 mol/L
1000 mL of this solution contains 15.425 g of tetramethylammonium hydroxide [(CH₃)₄NOH: 91.15].

Preparation—Before use, dilute a volume of tetramethylammonium hydroxide-methanol TS, equivalent to 9.2 g of tetramethylammonium hydroxide, with methanol to make 1000 mL, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.1 mol/L tetramethylammonium hydroxide VS.

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

Titanium (III) Chloride, 0.1 mol/L
1000 mL of this solution contains 15.425 g of titanium (III) chloride (TiCl₃: 154.23).

Preparation—Add 75 mL of hydrochloric acid to 75 mL of titanium (III) chloride (20), and dilute with freshly boiled and cooled water to make 1000 mL. Transfer the solution into a buret provided with a reservoir protected from light, replace the air with hydrogen, and allow to stand for 48 hours. Before use, standardize the solution as follows:

Standardization—Weigh 3 g of ammonium iron (II) sulfate hexahydrate in a wide-mouthed, 500 mL conical flask. Passing carbon dioxide through the flask, dissolve it in 50 mL of freshly boiled and cooled water, and add 25 mL of diluted sulfuric acid (27 in 100). Rapidly add exactly 40 mL of 0.02 mol/L potassium permanganate VS to the mixture, while passing carbon dioxide through the flask. Titrate <2.50> with the prepared titanium (III) chloride solution until the calculated end point is approached, then add 5 g of ammonium thiocyanate immediately, and continue the titration with the prepared titanium (III) chloride solution until the color of the solution disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store after the air has been displaced with hydrogen.

Titanium Trichloride, 0.1 mol/L
See Titanium (III) Chloride, 0.1 mol/L.

Zinc, 0.1 mol/L
1000 mL of this solution contains 6.538 g of zinc (Zn: 65.38).

Preparation—To 6.538 g of zinc (standard reagent), previously washed with dilute hydrochloric acid, with water and then acetone, and cooled in a desiccator (silica gel) after drying at 110°C for 5 minutes, add 80 mL of dilute hydrochloric acid and 2.5 mL of bromine TS, dissolve by gentle warming, evaporate excess bromine by boiling, and add water to make exactly 1000 mL.

Zinc Acetate, 0.05 mol/L
1000 mL of this solution contains 10.975 g of zinc acetate dihydrate [Zn(CH₃COO)₂·2H₂O: 219.50].

Preparation—Dissolve 11.1 g of zinc acetate dihydrate in 40 mL of water and 4 mL of dilute acetic acid, add water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 20 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and add 50 mL of water, 3 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride reagent. Titrate <2.50> the solution with the prepared zinc acetate solution until the blue color changes to blue-purple. Calculate the molarity factor.

Zinc Acetate, 0.02 mol/L
1000 mL of this solution contains 4.390 g of zinc acetate dihydrate [Zn(CH₃COO)₂·2H₂O: 219.50].

Preparation—Dissolve 4.43 g of zinc acetate dihydrate in 20 mL of water and 2 mL of dilute acetic acid, add water to make 1000 mL, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.05 mol/L zinc acetate VS, but measure exactly 20 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS.

Zinc Sulfate, 0.1 mol/L
1000 mL of this solution contains 28.755 g of zinc sulfate heptahydrate (ZnSO₄·7H₂O: 287.55).

Preparation—Dissolve 28.8 g of zinc sulfate heptahydrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Pipet 25 mL of the prepared zinc sulfate solution, add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate <2.50> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue-purple. Calculate the molarity factor.

Zinc sulfate, 0.02 mol/L
1000 mL of this solution contains 5.7510 g of zinc sulfate heptahydrate (ZnSO₄·7H₂O: 287.55).

Preparation—Before use, dilute 0.1 mol/L zinc sulfate VS with water to make exactly 5 times the initial volume.

9.22 Standard Solutions

Standard Solutions are used as the standard for the comparison in a text of the Pharmacopoeia.

Borate pH Standard Solution See pH Determination <2.54>.

Calcium Hydroxide pH Standard Solution See pH Determination <2.54>.

Carbonate pH Standard Solution See pH Determination <2.54>.

Formalin stock suspension To 25 mL of hexamethylenetetramine TS add 25 mL of hydrazinium sulfate TS, mix, and use after allowing to stand at 25 ± 3°C for 24 hours. Store in a glass container free from surface defects. Use within 2 months. Shake thoroughly before use.

Oxalate pH Standard Solution See pH Determination <2.54>.

pH Standard Solution, Borate See pH Determination <2.54>.

pH Standard Solution, Calcium Hydroxide See pH Determination <2.54>.

pH Standard Solution, Carbonate See pH Determination <2.54>.

pH Standard Solution, Oxalate See pH Determination <2.54>.
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<pH Standard Solution, Phosphate</p>
See pH Determination 2.54.

<pH Standard Solution, Phthalate</p>
See pH Determination 2.54.

Phosphate pH Standard Solution
See pH Determination 2.54.

Phthalate pH Standard Solution
See pH Determination 2.54.

Standard Aluminum Stock Solution
Weigh 1.0 g of aluminum, add 60 mL of dilute hydrochloric acid (1 in 2), dissolve by heating, cool, add water to make exactly 1000 mL. Each mL of this solution contains 0.2698 mg of aluminum (Al).

Standard Ammonium Solution
Dissolve 2.97 g of ammonium chloride, exactly weighed, in water for ammonium limit test to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of ammonium (NH₄⁺).

Standard Arsenic Solution
See Arsenic Limit Test 1.11.

Standard Arsenic Stock Solution
See Arsenic Limit Test 1.11.

Standard Boron Solution
Weigh exactly 0.286 g of boric acid, previously dried in a desiccator (silica gel) to constant mass, and dissolve in water to make exactly 1000 mL. Each mL of this solution contains 0.5 μg of boron (B).

Standard Cadmium Solution
Measure exactly 4.840 g of standard cadmium stock solution, and add diluted nitric acid (1 in 3) to make exactly 1000 mL. Each mL of this solution contains 0.001 mg of cadmium (Cd). Prepare before use.

Standard Cadmium Stock Solution
Dissolve 1.000 g of cadmium ground metal, exactly weighed, in 100 mL of dilute nitric acid by gentle heating, cool, and add dilute nitric acid to make exactly 1000 mL.

Standard Calcium Solution
Weigh exactly 0.250 g of calcium carbonate, add 5 mL of dilute hydrochloric acid and 25 mL of water, and dissolve by heating. After cooling, add water to make exactly 1000 mL. Each mL of this solution contains 0.1 mg of calcium (Ca).

Standard Calcium Solution for Atomic Absorption Spectrophotometry
Weigh accurately 0.250 g of calcium carbonate, and add 1 mol/L hydrochloric acid TS to make exactly 1000 mL. Each mL of this solution contains 1.00 mg of calcium (Ca).

Standard Copper Solution
Weigh exactly 1.000 g of copper (standard reagent), add 100 mL of dilute nitric acid, and dissolve by heating. After cooling, add water to make exactly 1000 mL.

Standard Copper Stock Solution
Weigh exactly 1.000 g of copper (standard reagent), add 100 mL of dilute nitric acid, and dissolve by heating. After cooling, add water to make exactly 1000 mL.

Standard Cyanide Solution
Measure exactly a volume of standard cyanide solution, equivalent to 10 mg of cyanide (CN), add 100 mL of potassium hydroxide TS and water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of cyanide (CN). Prepare before use.

Standard Cyanide Stock Solution
Dissolve exactly 4.840 g of potassium cyanide in water to make exactly 1000 mL. Measure exactly 100 mL of this solution, add 0.5 mL of 4-dimethylaminobenzylidene rhodanine TS, and titrate 2.50 with 0.01 mol/L silver nitrate VS until the solution shows a red color.

Each mL of 0.1 mol/L silver nitrate VS
= 5.204 mg of CN

Standard Fluorine Solution
See Oxygen Flask Combustion Method 1.00.

Standard Gold Solution for Atomic Absorption Spectrophotometry
To exactly 25 mL of standard gold solution, exactly measured, add water to make exactly 1000 mL. Each mL of this solution contains 0.025 mg of gold (Au).

Standard Gold Stock Solution
Dissolve exactly 10 mL of standard gold solution, and add water to make exactly 200 mL. Prepare before use. Each mL of this solution contains 0.250 mg of iron (Fe).

Standard Iron Solution
Dissolve exactly 10 mL of standard iron solution, and add water to make exactly 200 mL. Prepare before use. Each mL of this solution contains 0.01 mg of iron (Fe).

Standard Lead Solution
Measure exactly 10 mL of standard lead solution, and add water to make exactly 100 mL. Each mL of this solution contains 0.01 mg of lead (Pb). Prepare before use.
Standard Lead Stock Solution  Weigh exactly 159.8 mg of lead (II) nitrate, dissolve in 10 mL of dilute nitric acid, and add water to make exactly 1000 mL. Prepare and store this solution using glass containers, free from soluble lead salts.

Standard Liquids for Calibrating Viscosimeters [JIS, Standard Liquids for Calibrating Viscosimeters (Z 8809)]

Standard Magnesium Solution for Atomic Absorption Spectrophotometry  To exactly 1 mL of Standard Magnesium Stock Solution add water to make exactly 100 mL. Prepare before use. Each mL of this solution contains 0.0100 mg of magnesium (Mg).

Standard Magnesium Stock Solution  Dissolve exactly 8.365 g of magnesium chloride hexahydrate in 2 mol/L hydrochloric acid TS to make exactly 1000 mL.

Standard Mercury Solution  Weigh exactly 13.5 mg of mercury (II) chloride, previously dried for 6 hours in a desiccator (silica gel), dissolve in 10 mL of dilute nitric acid, and add water to make exactly 1000 mL. Pipet 10 mL of this solution, and add 10 mL of dilute nitric acid and water to make exactly 1000 mL. Each mL of this solution contains 0.1 μg of mercury (Hg). Prepare before use.

Standard Methanol Solution  See Methanol Test <1.12>.

Standard Nickel Solution  Dissolve 6.73 g of ammonium nickel (II) sulfate hexahydrate, exactly weighed, in water to make exactly 1000 mL. Pipet 5 mL of this solution, add water to make exactly 1000 mL. Each mL of this solution contains 0.005 mg of nickel (Ni).

Standard Nitric Acid Solution  Measure exactly 72.2 mg of potassium nitrate, dissolve in water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of nitrogen (N).

Standard Phosphoric Acid Solution  Weigh exactly 0.358 g of potassium dihydrogen phosphate, previously dried to constant mass in a desiccator (silica gel), and add 10 mL of dilute sulfuric acid (3 in 10) and water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.025 mg of phosphoric acid (as PO₄).

Standard Potassium Stock Solution  Weigh exactly 9.534 g of potassium chloride, previously dried at 130°C for 2 hours, and dissolve in water to make exactly 1000 mL. Each mL of this solution contains 5.00 mg of potassium (K).

Standard Selenium Solution  To exactly 1 mL of Standard Selenium Stock Solution add water to make exactly 1000 mL. Prepare before use. It contains 1.0 μg of selenium (Se) per mL.

Standard Selenium Stock Solution  Dissolve exactly 1.405 g of selenium dioxide in 0.1 mol/L nitric acid to make exactly 1000 mL.

Standard Silver Solution for Atomic Absorption Spectrophotometry  Measure exactly 10 mL of Standard Silver Stock Solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of silver (Ag). Prepare before use.

Standard Silver Stock Solution  Dissolve 1.575 g of silver nitrate, exactly weighed, in water to make exactly 1000 mL. Each mL of this solution contains 1.00 mg of silver (Ag).

Standard Sodium Dodecylbenzene Sulfonate Solution  Weigh exactly 1.000 g of sodium dodecylbenzene sulfonate, and dissolve in water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of sodium dodecylbenzene sulfonate [CH₃(CH₂)₁₄C₆H₄SO₃Na].

Standard Sodium Stock Solution  Weigh exactly 2.542 g of sodium chloride (standard reagent), previously dried at 130°C for 2 hours, and dissolve in water to make exactly 1000 mL. Each mL of this solution contains 1.00 mg of sodium (Na).

Standard Tin Solution  Weigh exactly 0.250 g of tin, and dissolve in 10 mL of sulfuric acid by heating. After cooling, transfer this solution with 400 mL of diluted hydrochloric acid (1 in 5) to a 500-mL volumetric flask, and add diluted hydrochloric acid (1 in 5) to make 500 mL. Pipet 10 mL of this solution, and add diluted hydrochloric acid (1 in 5) to make exactly 1000 mL. Each mL of this solution contains 0.005 mg of tin (Sn). Prepare before use.

Standard Vinyl Chloride Solution  Transfer about 190 mL of ethanol for gas chromatography into a 200-mL volumetric flask, and stopper with a silicone rubber stopper. Cooling this volumetric flask in a methanol-dry ice bath, inject 0.20 g of vinyl chloride, previously liquidized, through the silicone rubber stopper, and then inject ethanol for gas chromatography, previously cooled in a methanol-dry ice bath, through the silicone rubber stopper to make 200 mL. Pipet 1 mL of this solution, add ethanol for gas chromatography, cooled previously in a methanol-dry ice bath to make exactly 100 mL. Preserve in a hermetic container, at a temperature not exceeding –20°C. This solution contains 10 μg of vinyl chloride per mL.

Standard Water-Methanol Solution  See Water Determination <2.48>.

Standard Zinc Solution  Measure exactly 25 mL of Standard Zinc Stock Solution, and add water to make exactly 1000 mL. Prepare before use. Each mL of this solution contains 0.025 mg of zinc (Zn).

Standard Zinc Solution for Atomic Absorption Spectrophotometry  See Test for Rubber Closure for Aqueous Infusions <7.03>.

Standard Zinc Stock Solution  Dissolve exactly 1.000 g of zinc (standard reagent), in 100 mL of water and 5 mL of hydrochloric acid with the aid of gentle heating, cool, and add water to make exactly 1000 mL.

9.23 Matching Fluids for Color

Matching Fluids for Color are used as the reference for the comparison of color in a text of the Pharmacopoeia. They are prepared from the following colorimetric stock solutions. Colorimetric stock solutions are prepared by the following procedures and stored in glass-stoppered bottles. When the color of the solution is compared with Matching Fluids for Color, unless otherwise specified, transfer both
**Table 9.23-1 Matching fluid for color**

<table>
<thead>
<tr>
<th>Matching fluid for color</th>
<th>Parts of cobalt (II) Chloride CS (mL)</th>
<th>Parts of iron (III) Chloride CS (mL)</th>
<th>Parts of copper (II) Sulfate CS (mL)</th>
<th>Parts of water (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.1</td>
<td>0.4</td>
<td>0.1</td>
<td>4.4</td>
</tr>
<tr>
<td>B</td>
<td>0.3</td>
<td>0.9</td>
<td>0.3</td>
<td>3.5</td>
</tr>
<tr>
<td>C</td>
<td>0.1</td>
<td>0.6</td>
<td>0.1</td>
<td>4.2</td>
</tr>
<tr>
<td>D</td>
<td>0.3</td>
<td>0.6</td>
<td>0.4</td>
<td>3.7</td>
</tr>
<tr>
<td>E</td>
<td>0.4</td>
<td>1.2</td>
<td>0.3</td>
<td>3.1</td>
</tr>
<tr>
<td>F</td>
<td>0.3</td>
<td>1.2</td>
<td>—</td>
<td>3.5</td>
</tr>
<tr>
<td>G</td>
<td>0.5</td>
<td>1.2</td>
<td>0.2</td>
<td>3.1</td>
</tr>
<tr>
<td>H</td>
<td>0.2</td>
<td>1.5</td>
<td>—</td>
<td>3.3</td>
</tr>
<tr>
<td>I</td>
<td>0.4</td>
<td>2.2</td>
<td>0.1</td>
<td>2.3</td>
</tr>
<tr>
<td>J</td>
<td>0.4</td>
<td>3.5</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>K</td>
<td>0.5</td>
<td>4.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>L</td>
<td>0.8</td>
<td>3.8</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>M</td>
<td>0.1</td>
<td>2.0</td>
<td>0.1</td>
<td>2.8</td>
</tr>
<tr>
<td>N</td>
<td>—</td>
<td>4.9</td>
<td>0.1</td>
<td>—</td>
</tr>
<tr>
<td>O</td>
<td>0.1</td>
<td>4.8</td>
<td>0.1</td>
<td>4.3</td>
</tr>
<tr>
<td>P</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Q</td>
<td>0.2</td>
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<td>4.4</td>
</tr>
<tr>
<td>R</td>
<td>0.3</td>
<td>0.4</td>
<td>0.2</td>
<td>4.1</td>
</tr>
<tr>
<td>S</td>
<td>0.2</td>
<td>0.1</td>
<td>—</td>
<td>4.7</td>
</tr>
<tr>
<td>T</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
<td>3.6</td>
</tr>
</tbody>
</table>

solutions and fluids to Nessler tubes and view transversely against a white background.

**Cobalt (II) Chloride CS** Weigh 65 g of cobalt (II) chloride hexahydrate, and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 250 mL. Measure exactly 25 mL of the solution, add 75 mL of water and 0.05 g of mulexide–sodium chloride indicator, and add dropwise diluted ammonia solution (28) (1 in 10) until the color of the solution changes from green to purple. Titrate $2.50\%$ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to yellow. Titrate $2.50\%$ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from green to purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate

$$= 2.379 \text{ mg of CoCl}_2 \cdot 6\text{H}_2\text{O}$$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 62.4 mg of cobalt (II) chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}: 237.93$) in each mL, and use this solution as the colorimetric stock solution.

**Cobaltous Chloride CS** See Cobalt (II) Chloride CS.

**Copper (II) Sulfate CS** Weigh 65 g of copper (II) sulfate pentahydrate, and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 250 mL. Measure exactly 25 mL of the solution, add 75 mL of water, 10 mL of a solution of ammonium chloride (3 in 50), 2 mL of diluted ammonia solution (28) (1 in 10) and 0.05 g of mulexide–sodium chloride indicator. Titrate $2.50\%$ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from green to purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate

$$= 2.497 \text{ mg of CuSO}_4 \cdot 5\text{H}_2\text{O}$$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 62.4 mg of copper (II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}: 249.69$) in each mL, and use this solution as the colorimetric stock solution.

**Copper Sulfate CS** See Copper (II) Sulfate CS.

**Iron (III) Chloride CS** Weigh 55 g of iron (III) chloride hexahydrate, and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Measure exactly 10 mL of this solution, transfer to an iodine flask, add 15 mL of water and 3 g of potassium iodide, stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 mL of water to the mixture, and titrate $2.50\%$ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS

$$= 27.03 \text{ mg of FeCl}_3 \cdot 6\text{H}_2\text{O}$$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 45.0 mg of iron (II) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}: 270.30$) in each mL, and use this solution as the colorimetric stock solution.

**Matching Fluids for Color** Measure exactly the volume of colorimetric stock solutions and water shown in the following table with a buret or a pipet graduated to less than 0.1 mL, and mix.

### Reagents, Test Solutions, etc.

**9.41 Reagents, Test Solutions**

Reagents are the substances used in the tests of the Pharmacopoeia. The reagents that are described as “Standard reagent for volumetric analysis”, “Special class”, “First class”, “For water determination”, etc. in square brackets meet the corresponding requirements of the Japan Industrial Standards (JIS). The tests for them are performed according to the test methods of JIS. In the case where the reagent name in the Pharmacopoeia differs from that of JIS, the JIS name is given in the brackets. The reagents for which a monograph’s title is given in the brackets meet the requirements of the corresponding monograph. In the case of the reagents that are described merely as test items, the corresponding test method of the Pharmacopoeia is applied.

Test Solutions are the solutions prepared for use in the tests of the Pharmacopoeia.

**Acemetacin** $\text{C}_{21}\text{H}_{18}\text{ClNO}_6$ [Same as the namesake monograph]

**Acemetacin for assay** $\text{C}_{21}\text{H}_{18}\text{ClNO}_6$ [Same as the monograph Acemetacin. When dried, it contains not less than 99.5% of acemetacin ($\text{C}_{21}\text{H}_{18}\text{ClNO}_6$) meeting the following additional specifications.]

**Purity** Related substances—Dissolve 40 mg of acemetacin for assay in 10 mL of methanol, and use this solution as
the sample solution. Pipet 1 mL of this solution, and add methanol to make exactly 10 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.02> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than acemetacin obtained from the sample solution is not larger than 1/2 times the peak area of acemetacin obtained from the standard solution, and the total area of the peaks other than the peak of acemetacin is not larger than the peak area of acemetacin from the standard solution.

Operating conditions
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Acemetacin Tablets.

Time span of measurement: About 4 times as long as the retention time of Acemetacin.

System Suitability Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of acemetacin obtained from 10 μL of this solution is equivalent to 3 to 7% of that of acemetacin from 10 μL of the standard solution.

System performance: Dissolve 75 mg of acemetacin and 75 mg of indometacin in 50 mL of methanol. To 4 mL of this solution add 1 mL of a solution of hexyl parahydroxybenzoate in methanol (1 in 250), and add methanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, acemetacin, indometacin and hexyl parahydroxybenzoate are eluted in this order with the resolutions between the peaks of acemetacin and indometacin and between the peaks of indometacin and hexyl parahydroxybenzoate being not less than 3, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of acemetacin is not more than 1.5%.

Acenaphthene C_{12}H_{10} White to pale yellowish white crystals or crystalline powder, having a characteristic aroma. Freely soluble in diethyl ether and in chloroform, soluble in acetone, sparingly soluble in methanol, and practically insoluble in water.

Identification—Determine the infrared absorption spectrum of acenaphthene according to the paste method under Infrared Spectrophotometry <2.25>, with 5 mg of acenaphthene: it exhibits absorption at the wave numbers of about 1605 cm\(^{-1}\), 840 cm\(^{-1}\), 785 cm\(^{-1}\) and 750 cm\(^{-1}\).

Melting point <2.60>: 93 – 96°C

Purity—Dissolve 0.1 g of acenaphthene in 5 mL of chloroform, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of acenaphthene by the area percentage method: it shows a purity of not less than 98.0%.

Operating conditions
Detector: Hydrogen flame-ionization detector.

Acenaphthene is equivalent to 3 to 7% insoluble in water. Freely soluble in diethyl ether and in chloroform, soluble in crystals or crystalline powder, having a characteristic aroma.

1605 cm\(^{-1}\); it exhibits absorption at the wave numbers of about 168168 cm\(^{-1}\). The time span of measurement: About 4 times as long as the retention time of acenaphthene beginning after the solvent peak.

Residue on ignition <2.44>—Not more than 0.1% (1 g).

Acetal C_2H_4O_2 A clear and colorless volatile liquid. Miscible with water and with ethanol (95).

Refractive index <2.45> n_D\(^20\): about 1.382

Specific gravity <2.56> d_D\(^20\): about 0.824

Boiling point <2.57>: about 103°C

Acetaldehyde CH_3CHO [K 8030, First class]

Acetaldehyde for assay Distil 100 mL of acetaldehyde under reduced pressure, discard the first 20 mL of the distillate, and use the subsequent. Prepare before use.

Acetaldehyde for gas chromatography C_2H_4O A clear and colorless, flammable liquid. Miscible with water and with ethanol (95).

Refractive index <2.45> n_D\(^20\): about 1.332

Specific gravity <2.56> d_D\(^20\): about 0.788

Boiling point <2.57>: about 21°C

2-Acetamidoglutarimide C_{10}H_{18}N_2O_3: 170.17

Identification—Determine the infrared absorption spectrum of 2-acetamidoglutarimide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3350 cm\(^{-1}\), 1707 cm\(^{-1}\), 1639 cm\(^{-1}\) and 1545 cm\(^{-1}\).

Purity Related substances—Dissolve 10 mg of 2-acetamidoglutarimide in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Proceed with exactly 20 μL each of the sample solution and standard solution as directed in the Purity (3) under Acetoglutarimide Aluminiun: the total of the peak areas other than 2-acetamidoglutarimide from the sample solution is not larger than the peak area from the standard solution.

Content: not less than 98.0%. Assay—Weigh accurately about 20 mg of 2-acetamidoglutarimide, and perform the test as directed under Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS = 0.8509 mg of C_6H_8N_2O_3

Acetaminophen C_{6}H_{8}NO [Same as the namesake monograph]

Acetanilide C_8H_9NO White, crystals or crystalline powder.

Melting point <2.60>: 114 – 117°C
**p-Acetanisidine** \(C_9H_{11}NO_2\) White to purplish white, crystals or crystalline powder, having a characteristic odor. It is freely soluble in ethanol (95) and in acetonitrile, and very slightly soluble in water.

**Melting point** 2.050\(^{\circ}\) 126 – 132\(^{\circ}\)

**Content:** not less than 98.0%. Assay—Dissolve 0.1 g of \(p\)-acetanisidine in 5 mL of ethanol (95). Perform the test with 2 mL of this solution as directed under Gas Chromatography \(< 2.02\) according to the following conditions, and determine the area of each peak by the automatic integration method.

\[
\text{Content (\%)} = \left(\frac{\text{Peak area of } p\text{-acetanisidine}}{\text{total of all peak areas}}\right) \times 100
\]

**Operating conditions**
- **Detector:** Hydrogen flame-ionization detector.
- **Column:** A glass tube 3 mm in inside diameter and 2 m in length, packed with acid-treated and silanized siliceous earth for gas chromatography coated with alkylene glycol phthalate ester for gas chromatography in 1% (177–250 µm in particle diameter).
- **Column temperature:** A constant temperature of about 210\(^{\circ}\).
- **Carrier gas:** Nitrogen.
- **Flow rate:** Adjust to a constant flow rate of between 30 and 50 mL per minute and so that the retention time of \(p\)-acetanisidine is between 11 and 14 minutes.
- **Time span of measurement:** About 3 times as long as the retention time of \(p\)-acetanisidine beginning after the solvent peak.

**Acetate buffer solution, pH 3.5** Dissolve 50 g of ammonium acetate in 100 mL of 6 mol/L hydrochloric acid TS, adjust to pH 3.5 with ammonia TS or 6 mol/L hydrochloric acid TS, if necessary, and add water to make 200 mL.

**Acetate buffer solution, pH 4.5** Dissolve 63 g of anhydrous sodium acetate in a suitable amount of water, add 90 mL of acetic acid (100) and water to make 1000 mL.

**0.01 mol/L Acetate buffer solution, pH 5.0** Dissolve 385 g of ammonium acetate in 900 mL of water, add acetic acid (31) to adjust the pH to 5.0, and then add water to make 1000 mL.

**Acetate buffer solution, pH 5.4** To 5.78 mL of acetic acid (100) add water to make 1000 mL (solution A). Dissolve 8.2 g of anhydrous sodium acetate in water to make 1000 mL (solution B). Mix 176 mL of the solution A and 824 mL of the solution B, and adjust, if necessary, the pH to 5.4 with the solution A or the solution B.

**Acetate buffer solution, pH 5.5** Dissolve 2.72 g of sodium acetate trihydrate in water to make 1000 mL, and adjust the pH to 5.5 with diluted acetic acid (100) (3 in 2500).

**Acetic acid** See acetic acid (31).

**Acetic acid-ammonium acetate buffer solution, pH 3.0** Add acetic acid (31) to ammonium acetate TS, and adjust the pH to 3.0.

**Acetic acid-ammonium acetate buffer solution, pH 4.5** Dissolve 77 g of ammonium acetate in about 200 mL of water, adjust the pH to 4.5 by adding acetic acid (100), and add water to make 1000 mL.

**Acetic acid-ammonium acetate buffer solution, pH 4.8** Dissolve 77 g of ammonium acetate in about 200 mL of water, and add 57 mL of acetic acid (100) and water to make 1000 mL.

**Acetic acid buffer solution containing 0.1% bovine serum albumin** Dissolve 0.1 g of bovine serum albumin in sodium acetate trihydrate solution (1 in 100) to make exactly 100 mL, and adjust the pH to 4.0 with 1 mol/L hydrochloric acid TS.

**Acetic acid, dilute** Dilute 6 g of acetic acid (100) with water to make 100 mL (1 mol/L).

**Acetic acid-potassium acetate buffer solution, pH 4.3** Dissolve 14 g of potassium acetate in 20.5 mL of acetic acid (100), and add water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution, pH 4.0** Dissolve 5.44 g of sodium acetate trihydrate in 900 mL of water, adjust the pH 4.0 with acetic acid (100), and add water to make 1000 mL.

**0.05 mol/L Acetic acid-sodium acetate buffer solution, pH 4.0** To 3.0 g of acetic acid (100) add water to make 1000 mL. Adjust to pH 4.0 with a solution prepared by dissolving 3.4 g of sodium acetate trihydrate in water to make 500 mL.

**0.1 mol/L Acetic acid-sodium acetate buffer solution, pH 4.0** Dissolve 13.61 g of sodium acetate trihydrate in 900 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution, pH 4.5** To 80 mL of sodium acetate TS add 120 mL of dilute acetic acid and water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution, pH 4.5, for iron limit test** Dissolve 75.4 mL of acetic acid (100) and 11 g of sodium acetate trihydrate in 1000 mL of water.

**Acetic acid-sodium acetate buffer solution, pH 4.7** Dissolve 27.2 g of sodium acetate trihydrate in 900 mL of water, adjust the pH to 4.7 by adding acetic acid (100) dropwise, and add water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution, pH 5.0** To 140 mL of sodium acetate TS add 60 mL of dilute acetic acid.
and water to make 1000 mL.

1 mol/L Acetic acid-sodium acetate buffer solution, pH 5.0 To sodium acetate TS add dilute acetic acid, and adjust the pH to 5.0.

Acetic acid-sodium acetate buffer solution, pH 5.5 Dissolve 20 g of sodium acetate trihydrate in 80 mL of water, adjust the pH to 5.5 by adding acetic acid (100) dropwise, and add water to make 100 mL.

Acetic acid-sodium acetate TS Mix 17 mL of 1 mol/L sodium hydroxide VS with 40 mL of dilute acetic acid, and add water to make 100 mL.

Acetic acid-sodium acetate TS, pH 7.0 Dissolve 4.53 g of sodium acetate trihydrate in water to make 100 mL, and adjust the pH to 7.0 with diluted acetic acid (100) (1 in 50).

0.02 mol/L Acetic acid-sodium acetate TS Dissolve 2.74 g of sodium acetate trihydrate in a suitable amount of water, and add 2 mL of acetic acid (100) and water to make 1000 mL.

0.25 mol/L Acetic acid TS To 3 g of acetic acid (100) add water to make 200 mL.

6 mol/L Acetic acid TS Dilute 36 g of acetic acid (100) with water to make 100 mL.

Acetic acid (100) CH₃COOH [K 8355, Acetic Acid, Special class]

Acetic acid (100)-sulfuric acid TS To 5 mL of acetic acid (100) add cautiously 5 mL of sulfuric acid while cooling in an ice bath, and mix.

Acetic acid (31) Dilute 31.0 g of acetic acid (100) with water to make 100 mL (5 mol/L).

Acetic anhydride (CH₃CO)₂O [K 8886, Special class]

Acetic anhydride-pyridine TS Place 25 g of acetic anhydride in a 100 mL volumetric flask, add pyridine to make 100 mL, and mix well. Preserve in light-resistant containers, protected from air. This solution may be used even if it becomes colored during storage.

Acetone CH₃COCH₃ [K 8034, Special class]

Acetone for nonaqueous titration Add potassium permanganate to acetone in small portions, and shake. When the mixture keeps its purple color after standing for 2 to 3 days, distil, and dehydrate with freshly ignited anhydrous potassium carbonate. Distill by using a fractionating column under protection from moisture, and collect the fraction distilling at 56°C.

Acetone for purity of crude drug CH₃COCH₃ [K 8034, Acetone, Special class] Use acetone meeting the following additional specification. Evaporate 300.0 mL of acetone to be tested in vacuum at a temperature not higher than 40°C, add the acetone to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of γ-BHC in hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane for purity of crude drug to make exactly 100 mL. Further pipet 2 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1 μL each of the sample solution and standard solution (1) as directed under Gas Chromatography C2.02< according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the solvent peak from the sample solution is not larger than the peak area of γ-BHC starting from the beginning of the standard solution (1).

Operating conditions Proceed the operating conditions in the Purity (2) under Crude Drugs Test <5.01D except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of γ-BHC obtained from 1 μL of the standard solution (2) can be measured by the automatic integration method, and the peak height of γ-BHC from 1 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About three times as long as the retention time of γ-BHC beginning after the solvent peak.

Acetonitrile CH₃CN [K 8032, Special class]

Acetonitrile for liquid chromatography CH₃CN Colorless and clear liquid. Mixable with water.

Purity Ultraviolet light absorbing substances—Determine the absorbances at the following wavelengths as directed under Ultraviolet-visible Spectrophotometry C2.24>, using water as the control; not more than 0.07 at 200 nm, not more than 0.046 at 210 nm, not more than 0.027 at 220 nm, not more than 0.014 at 230 nm and not more than 0.009 at 240 nm.

Acetric acid C₄H₄I₃NO₃ White powder.

Purity Related substances—Dissolve 60 mg of acetric acid in a solution of meglumine (3 in 1000) to make 100 mL. To 10 mL of this solution add water to make 100 mL, and use this solution as the sample solution. Proceed the test with 5 μL of the sample solution as directed in the Assay under Meglumine Sodium Amidotrizoate Injection: any peaks other than the principal peak are not observed.

Acetylace ton CH₃COCH₂COCH₃ [K 8027, Special class]

Acetylace ton TS Dissolve 150 g of ammonium acetate in a sufficient quantity of water, and add 3 mL of acetic acid (100), 2 mL of acetylace ton and water to make 1000 mL. Prepare before use.

Acetylene See dissolved acetylene.

Achyranthes root for thin-layer chromatography A heat-dried, pulverized root of Achyranthes fauriei Leveillé et Vaniot (Amaranthaceae) meeting the following additional specifications.

Identification (1) To 2 g of pulverized achyranthes root for thin-layer chromatography add 10 mL of water, shake for 10 minutes, add 5 mL of 1-butanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of chikuset-
susaponin IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $2.07$. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (5:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 105°C for 10 minutes: the standard solution shows a deep purplish red spot at around $R_f$ value of 0.35, and the sample solution shows spots equivalent to those described below:

<table>
<thead>
<tr>
<th>$R_f$ value</th>
<th>Color and shape of the spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Around 0</td>
<td>A weak spot, black</td>
</tr>
<tr>
<td>Around 0.1</td>
<td>A weak, tailing spot, strong purplish red</td>
</tr>
<tr>
<td>Around 0.2</td>
<td>A strong spot, deep purplish red</td>
</tr>
<tr>
<td>Around 0.25</td>
<td>A leading spot, deep purplish red</td>
</tr>
<tr>
<td>Around 0.45</td>
<td>A weak spot, dull yellow</td>
</tr>
<tr>
<td>Around 0.5</td>
<td>A weak spot, grayish purplish red</td>
</tr>
<tr>
<td>Around 0.7</td>
<td>A weak spot, grayish red</td>
</tr>
<tr>
<td>Around 0.9</td>
<td>A weak spot, dull red</td>
</tr>
</tbody>
</table>

(2) Perform the test as directed in the operating conditions under (1), except using a mixture of 1-propanol, ethyl acetate and water (4:4:3) as the developing solvent: the standard solution shows a deep purplish red spot at around $R_f$ value of 0.45, and the sample solution shows spots equivalent to those described below:

<table>
<thead>
<tr>
<th>$R_f$ value</th>
<th>Color and shape of the spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Around 0.25</td>
<td>A weak spot, strongly purplish red</td>
</tr>
<tr>
<td>Around 0.25 – 0.3</td>
<td>A leading spot or two strong spots, strongly purplish red</td>
</tr>
<tr>
<td>Around 0.35</td>
<td>A deep purplish red spot</td>
</tr>
<tr>
<td>Around 0.4</td>
<td>A weak spot, dull red</td>
</tr>
<tr>
<td>Around 0.42</td>
<td>A dark red spot</td>
</tr>
<tr>
<td>Around 0.45</td>
<td>A weak spot, grayish red</td>
</tr>
<tr>
<td>Around 0.65</td>
<td>A weak spot, dull greenish yellow</td>
</tr>
<tr>
<td>Around 0.7</td>
<td>A weak spot, grayish red</td>
</tr>
<tr>
<td>Around 0.85</td>
<td>A weak spot, grayish red</td>
</tr>
<tr>
<td>Around 0.95</td>
<td>A weak spot, dull yellow-red</td>
</tr>
</tbody>
</table>

**Acidic ferric chloride TS** See iron (III) chloride TS, acidic.

**Acidic potassium chloride TS** See potassium chloride TS, acidic.

**Acidic potassium permanganate TS** See potassium permanganate TS, acidic.

**Acidic stannous chloride TS** See tin (II) chloride TS, acidic.

**Acid-treated gelatin** See gelatin, acid-treated.

**Aconitine for purity C$_{34}$H$_{47}$NO$_{11}$** White, crystals or crystalline powder. Slightly soluble in acetonitrile and in ethanol (99.5), slightly soluble in diethyl ether, and practically insoluble in water. Melting point: about 185°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of aconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry $2.25$: it exhibits absorption at the wave numbers of about 3500 cm$^{-1}$, 1718 cm$^{-1}$, 1278 cm$^{-1}$, 1111 cm$^{-1}$, 1097 cm$^{-1}$ and 717 cm$^{-1}$.

**Absorbance** $2.24$: $E_{10}$% (230 nm): 211 – 243 [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), ethanol (99.5), 200 mL].

**Purity** Related substances—

(1) Dissolve 5.0 mg of aconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $2.07$. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of aconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography $2.07$: according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of aconitine and the solvent obtained with the sample solution is not larger than the peak area of aconitine with the standard solution.

**Operating conditions**

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity under Processed Aconite Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust the flow rate so that the retention time of aconitine is about 26 minutes.

Time span of measurement: About 3 times as long as the retention time of aconitine.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of aconitine obtained from 10 μL of this solution is equivalent to 3.5 to 6.5 μL each of the sample solution and standard solution.

System performance: Dissolve 1 mg each of aconitine for purity, hypaconitine for purity and mesaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10 μL of this solution under the above operating conditions, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aconitine is not more than 1.5%.
Water \(<2.48\): not more than 1.0% [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), coulometric titration].

Aconitum diester alkaloids standard TS for purity It is a solution containing 10 mg of aconitine for purity, 10 mg of jesaconitine for purity, 30 mg of hypaconitine for purity and 20 mg of mesaconitine for purity in 1000 mL of a mixture of water (95), and sparingly soluble in water.

Melting point \(<2.60\): 151 – 154°C

Content: not less than 98.0%. Assay—Weigh accurately about 1 g of adipic acid, and 100 mL of water, dissolve by warming, cool, and titrate \(<2.50\) with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS = 73.07 mg of C\(_6\)H\(_{10}\)O\(_4\).

Agar [K 8263, Special class. Same as the monograph Agar or Agar Powder. Loss on drying is not more than 15%.

Agar medium, ordinary See ordinary agar medium.

Agar slant Dispense portions of about 10 mL of ordinary agar medium into test tubes, and sterilize by autoclaving. Before the medium congeals, allow to stand in a slanting position, and solidify. When the coagulating water is lost, reprepare by dissolving with the aid of heat.

Ajmaline for assay C\(_{20}\)H\(_{26}\)N\(_2\)O\(_2\). [Same as the monograph Ajmaline. When dried, it contains not less than 99.0% of ajmaline (C\(_{20}\)H\(_{26}\)N\(_2\)O\(_2\)).

Alacepril C\(_{20}\)H\(_{26}\)N\(_2\)O\(_5\)S [Same as the namesake monograph]

Alacepril for assay [Same as the monograph Alacepril. When dried, it contains not less than 99.0% of alacepril (C\(_{20}\)H\(_{26}\)N\(_2\)O\(_5\)S)].

\(\beta\)-Alanine C\(_6\)H\(_5\)NO\(_2\) Colorless crystals or a white crystalline powder. Freely soluble in water, very slightly soluble in methanol, and practically insoluble in ethanol (99.5) and in diethyl ether.

Purity Related substances—Dissolve 5.0 mg in 10 mL of diluted methanol (4 in 5), and use this as the sample solution. Pipet 1 mL of this solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.63\). Spot 2 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1:butanol, water and acetic acid (100) (5:2:2) to a distance of about 8 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

L-Alanine C\(_6\)H\(_5\)NO\(_2\) [K 9101, Special class]

Albiflorin C\(_{23}\)H\(_{28}\)O\(_{11}\). White powder having no odor. Freely soluble in water, in methanol and in ethanol (99.5).

Identification—Determine the absorption spectrum of a solution of albiflorin in diluted methanol (1 in 2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\): it exhibits a maximum between 230 nm and 234 nm.

Purity (1) Related substances 1—Dissolve 1 mg of albiflorin in 1 mL of methanol, and perform the test with 10 \(\mu\)L of this solution as directed in the Identification (2) under Peony Root: any spot other than the principal spot which appears at around Rf 0.2 does not appear.
(2) Related substances 2—Dissolve 1 mg of albiflorin in 10 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed in the Assay under Peony Root, and measure the peak areas about 2 times as long as the retention time of peoniflorin: the total area of the peaks other than albiflorin from the sample solution is not larger than 1/10 times the total area of the peaks other than the solvent peak.

Albumin TS Carefully separate the white from the yolk of a fresh hen’s egg. Shake the white with 100 mL of water until the mixture is thoroughly mixed, and filter. Prepare before use.

Alcian blue 8 GX C18H28Cl2CuN16S4 Dark blue-purple powder.

Alcian blue staining solution Dissolve 0.5 g of alcian blue 8 GX in 100 mL of diluted acetic acid (100) (3 in 100). White to pale yellow powder. Very soluble in methanol, practically insoluble in water.

Aldehyde dehydrogenase Each mg contains not less than 2 enzyme activity units. White powder.

Assay—Dissolve about 20 mg of aldehyde dehydrogenase, accurately weighed, in 1 mL of water, add ice-cold solution of bovine serum albumin (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. In a spectrophotometric cell, place 2.50 mL of pyrophosphate buffer solution, pH 9.0, 0.20 mL of a solution prepared by dissolving 20.0 mg of β-nicotinamide adenine dinucleotide (NAD) to make exactly 1 mL, 0.10 mL of a pyrazole solution (17 in 2500) and 0.10 mL of the sample solution, stir, stopper tightly, determine every 30 seconds the absorbance at 340 nm as directed under Ultraviolet-visible Spectrophotometry <2.26>, and calculate a change (ΔA) in absorbance in minute starting from the spot where the relation of time and absorbance is shown with a straight line. One enzyme activity unit means an amount of enzyme which oxidizes 1 μmol of acetaldehyde per minute when the test is conducted under the conditions of the Procedure.

Enzyme activity unit (unit/mg) of aldehyde dehydrogenase

\[
\text{Enzyme activity unit (unit/mg) of aldehyde dehydrogenase} = \frac{2.91 \times \Delta A \times 200}{6.3 \times M \times 0.10 \times 1000}
\]

M: Amount (g) of sample

Aldehyde dehydrogenase TS Dissolve an amount equivalent to 70 aldehyde dehydrogenase units in 10 mL of water. Prepare before use.

Aldehyde-free ethanol See ethanol, aldehyde-free.

Alendronate sodium hydrate C13H12NNaO7.P2.3H2O [Same as the namesake monograph]

Alisol A for thin-layer chromatography C30H50O5 A white to pale yellow powder. Very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

Optical rotation <2.49> [α]D20: +86° – +106° (5 mg previously dried on silica gel for 24 hours, methanol, 1 mL, 50 mm).

Purity Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 5 μL of this solution as directed in the Identification (6) under Saireito Extract: no spot appears other than the principal spot of around Rf 0.3.

Alizarin complexone C14H24N2O4 1,2-Dihydroxy-3-quinolone-2,4-naphthalene-N,N-diacetate A yellow-brown powder. Soluble in ammonia TS, and practically insoluble in water, in ethanol (95) and in diethl ether.

Sensitivity—Dissolve 0.1 g of alizarin complexone by adding 2 drops of ammonia solution (28), 2 drops of ammonium acetate TS and 20 mL of water. To 10 mL of this solution add acetic acid-potassium acetate buffer solution, pH 4.3, to make 100 mL. Place 1 drop of this solution on a white spot plate, add 1 drop of a solution of sodium fluoride (1 in 100,000) and 1 drop of cerium (III) nitrate hexahydrate TS, stir, and observe under scattered light after 1 minute: a blue-purple color is produced, and the color of the control solution is red-purple. Use a solution prepared in the same manner, to which 1 drop of water is added in place of a solution of sodium fluoride, as the control solution.

Alizarin complexone TS Dissolve 0.390 g of alizarin complexone in 20 mL of a freshly prepared solution of sodium hydroxide (1 in 50), then add 800 mL of water and 0.2 g of sodium acetate trihydrate, and dissolve. Adjust the pH to 4 to 5 with 1 mol/L hydrochloric acid VS, and add water to make 1000 mL.

Alizarin red S C13H12NaO7 [K 8057, Special class]

Alizarin red S TS Dissolve 0.1 g of alizarin red S in water to make 100 mL, and filter if necessary.

Alizarin S See alizarin red S.

Alizarin S TS See alizarin red S TS.

Alizarin yellow GG C13H12N4NaO7 [K 8056, Special class]

Alizarin yellow GG-thymolphthalein TS Mix 10 mL of alizarin GG TS with 20 mL of thymolphthalein TS.

Alizarin yellow GG TS Dissolve 0.1 g of alizarin yellow GG in 100 mL of ethanol (95), and filter if necessary.

Alkali copper TS Dissolve 70.6 g of disodium hydrogen phosphate dodecahydrate, 40.0 g of potassium sodium tartrate tetrahydrate and 180.0 g of anhydrous sodium sulfate in 600 mL of water, and add 20 mL of a solution of sodium hydroxide (1 in 5). To this mixture add, with stirring, 100 mL of a solution of copper (II) sulfate (2 in 25), 33.3 mL of 0.05 mol/L potassium iodate VS and water to make 1000 mL.

Alkali blue tetrazolium TS See blue tetrazolium TS, alkaline.

Alkaline copper solution See alkaline copper TS for protein content determination.

Alkali copper TS Dissolve 2 g of anhydrous sodium carbonate in 100 mL of 0.1 mol/L sodium hydroxide TS. To 50 mL of this solution add 1 mL of a mixture of a solution of copper (II) sulfate pentahydrate (1 in 100) and a solution of potassium tartrate (1 in 50) (1:1), and mix.

Alkaline copper TS for protein content determination Dissolve 0.8 g of sodium hydroxide in water to make 100 mL. Dissolve 4 g of anhydrous sodium carbonate in this solution to make solution A. Combine 1 mL of copper (II) sulfate pentahydrate solution (1 in 50) and 1 mL of sodium tar-
trate dihydrate solution (1 in 25) to make solution B. Mix 50 mL of solution A and 1 mL of solution B. Prepare at the time of use.

Alkaline copper (II) sulfate solution See copper (II) sulfate solution, alkaline.

Alkaline copper (II) TS Dissolve 20 g of anhydrous sodium carbonate in dilute sodium hydroxide TS to make 1000 mL, and use this solution as solution A. Dissolve 0.5 g of copper (II) sulfate pentahydrate in potassium sodium tartrate tetrahydrate solution (1 in 100) to make 100 mL, and use this solution as solution B. To 50 mL of solution A add 1 mL of solution B. Prepare before use.

Alkaline glycerin TS To 200 g of glycerin add water to make 235 g, and add 142.5 mL of sodium hydroxide TS and 47.5 mL of water.

Alkaline hydroxylamine TS See hydroxylamine TS, alkaline.

Alkaline m-dinitrobenzene TS See 1,3-dinitrobenzene TS, alkaline.

Alkaline phosphatase Obtained from bovine small intestine, a white to grayish white or yellow-brown, freeze-dried powder having no odor. Alkaline phosphatase contains not less than 1 unit per mg and no salts. One unit of alkaline phosphatase indicates an amount of the enzyme which produces 1 µmol of 4-nitrophenol in 1 minute at 37°C and pH 9.8, from 4-nitrophenylphosphate as the substrate.

Alkaline phosphatase TS Dissolve 0.1 g of alkaline phosphatase in 10 mL of boric acid-magnesium chloride buffer solution, pH 9.0. Prepare before use.

Alkaline picric acid TS See 2,4,6-trinitrophenol TS, alkaline.

Alkaline potassium ferricyanide TS See potassium hexacyanoferrate (III) TS, alkaline.

Alkylene glycol phthalate ester for gas chromatography Prepared for gas chromatography.

Allopurinol C7H7N3 White to light yellow crystals or crystalline powder, having a characteristic odor. Melting point: 232 – 238°C.

Amidosulfuric acid (standard reagent) HOSO2NH2 [K 8005, Standard substance for volumetric analysis]

Amidotrizoic acid for assay C11H9I3N2O4 Same as the monograph Amidotrizoic Acid. It contains not less than 99.9% of amidotrizoic acid (C11H9I3N2O4), calculated on the dried basis.

p-Aminoacetophenone See 4-aminoacetophenone.

p-Aminoacetophenone TS See 4-aminoacetophenone TS.

4-Aminoacetophenone H2NC6H4COCH3 Light yellow, crystals or crystalline powder, having a characteristic odor. Melting point <2.60º: 105 – 108°C

4-Aminoacetophenone TS Dissolve 0.100 g of 4-aminoacetophenone in methanol to make exactly 100 mL.

4-Aminoantipyrine C13H17NO2 [K 8048, Special class]


4-Aminoantipyrine hydrochloride TS Dissolve 1 g of 4-aminoantipyrine hydrochloride in 25 mL of water: the solution is almost clear.

Alternative thioglycolate medium See Sterility Test <4.06> under the General Tests, Processes and Apparatus.

Aluminon C22H23N3O4 [K 8011, Special class]

Aluminon TS Dissolve 0.1 g of aluminon in water to make 100 mL, and allow this solution to stand for 24 hours.

Aluminum (Al) [K 8069, Special class]

Aluminum chloride See aluminum (III) chloride hexahydrate.

Aluminum chloride TS See Aluminum (III) chloride TS.

Aluminum (III) chloride TS Dissolve 64.7 g of aluminum (III) chloride hexahydrate in 71 mL of water, add 0.5 g of activated charcoal, then shake for 10 minutes, and filter. Adjust the pH of the filtrate to 1.5 with a solution of sodium hydroxide (1 in 100) with stirring, and filter if necessary.

Aluminum (III) chloride hexahydrate AlCl₃.6H₂O [K 8114, Special class]

Aluminum oxide Al₂O₃ White crystals, crystalline powder, or powder. Boiling point: about 3000°C. Melting point: about 2000°C.

Aluminum potassium sulfate dodecahydrate AlK(SO₄)₂.12H₂O [K 8255, Special class]

6-Amidino-2-naphthyl methanesulfonate C₁₁H₁₀N₂O₆.CH₃SO₂H A white to pale yellow crystalline powder. Melting point: about 233°C (with decomposition).

Purity—A solution obtained by dissolving 0.5 g of 6-amidino-2-naphthyl methanesulfonate in 10 mL of methanol is clear.

Amidosulfuric acid (standard reagent) HOSO₂NH₂ [K 8005, Standard substance for volumetric analysis]

Amidotrizoic acid for assay C₁₁H₉I₃N₂O₄ Same as the monograph Amidotrizoic Acid. It contains not less than 99.9% of amidotrizoic acid (C₁₁H₉I₃N₂O₄), calculated on the dried basis.

p-Aminoacetophenone See 4-aminoacetophenone.

p-Aminoacetophenone TS See 4-aminoacetophenone TS.

4-Aminoacetophenone H₂NC₆H₄COCH₃ Light yellow, crystals or crystalline powder, having a characteristic odor. Melting point <2.60º: 105 – 108°C

4-Aminoacetophenone TS Dissolve 0.100 g of 4-aminoacetophenone in methanol to make exactly 100 mL.

4-Aminoantipyrine C₁₃H₁₇NO₂ [K 8048, Special class]


Purity Clarity of solution—Dissolve 1 g of 4-aminoantipyrine hydrochloride in 25 mL of water: the solution is almost clear.

Content: 100.6 – 108.5%. Assay—Weigh accurately about 0.5 g of 4-aminoantipyrine hydrochloride, dissolve in 50 mL of water, and, if necessary, neutralize with 0.1 mol/L sodium hydroxide VS (indicator: red litmus paper). Add 4 drops of dichlorofluorescein TS, and titrate <2.50º> with 0.1 mol/L silver nitrate VS.

Each mL of 0.1 mol/L silver nitrate VS = 23.97 mg of C₁₃H₁₇N₃O.HCl

4-Aminoantipyrine hydrochloride TS Dissolve 1 g of 4-aminoantipyrine hydrochloride in water to make 50 mL.

4-Aminoantipyrine TS Dissolve 0.1 g of 4-aminoantipyrine in 30 mL of water, add 10 mL of a solution of sodium carbonate decahydrate (1 in 5), 2 mL of sodium hydroxide TS and water to make 100 mL. Prepare before use.

2-Aminobenzimidazole C₇H₇N₁ White to light yellow
Aminobenzoate derivatization TS To 0.28 g of ethyl aminobenzoate add 600 μL of methanol, warm at about 50°C to dissolve, and add 170 μL of acetic acid and 145 μL of borane-pyridine complex.

p-Aminobenzoic acid See 4-aminobenzoic acid.

4-Aminobenzoic acid C₆H₄N₂COOH White to very pale yellow crystalline powder.

Purity Clarity of solution—Dissolve 0.1 g of 4-aminobenzoic acid in 10 mL of ethanol (95): the solution is clear.

2-Amino-1-butanol CH₃CH₂CH(NH₂)CH₂OH Clear, colorless to light yellow liquid. Miscible with water and dissolves in methanol.

Refractive index <2.45> nD²₀: 1.450 – 1.455

Specific gravity <2.56> dD²₀: 0.944 – 0.950

Purity Related substances—Dissolve 50 mg of 2-amino-1-butanol in 10 mL of methanol, measured exactly, and perform the test with 2 μL of this solution as directed in the Purity (4) under Ethambutol Hydrochloride: any spot other than the principal spot at the Rf value of about 0.3 does not appear.

4-Aminobutylglycine C₄H₁₁NO₃.HCl White crystals or crystalline powder. Melting point: about 200°C (with decomposition).

2-Amino-5-chlorobenzenophenone for thin-layer chromatography C₁₅H₁₂ClNO Yellow, crystalline powder.

Melting point <2.60>: 97 – 101°C

Purity Related substances—Dissolve 10 mg of 2-amino-5-chlorobenzenophenone for thin-layer chromatography in methanol to make exactly 200 mL, and perform the test with this solution as directed in the Purity (4) under Ethambutol Hydrochloride: any spot other than the principal spot at the Rf value about 0.7 does not appear.

4-Aminodiethylamine sulfate monohydrate C₆H₁₂N₂SO₄.H₂O White to slightly colored powder. It dissolves in water.

Melting point <2.60>: 173 – 176°C

Residue on ignition <2.44>: not more than 0.1% (1 g).

4-Amino-4-N,N-diethylaniline sulfate TS Dissolve 0.2 g of 4-amino-N,N-diethylaniline sulfate monohydrate in water to make 100 mL. Prepare before use, protected from light.

2-Aminoethylhydrochloride C₅H₁₀N₂SH.HCl White crystal or granule.

Melting point <2.60>: 65 – 71°C

2-Aminoethanol NH₂CH₂CH₂OH [K 8109, Special class]

N-Aminohexamethylenamine (CH₃)₆N≡N Cleared, colorless to pale yellow liquid.

Refractive index <2.45> nD²₀: 1.482 – 1.487

Specific gravity <2.56> dD²₀: 0.936 – 0.942

2-Amino-2-hydroxymethyl-1,3-propanediol C₆H₁₁NO₃ [K 9704, Special class]

2-Amino-2-hydroxymethyl-1,3-propanediol hydrochloride C₆H₁₁NO₃.HCl White crystals or crystalline powder

4-(Aminomethyl)benzoic acid C₈H₁₀NO₂ A white powder.

Purity—Dissolve 10 mg of 4-(aminomethyl)benzoic acid in 100 mL of water, and use this as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.0F> according to the operating conditions as directed in the Purity (5) under Tranexamic Acid, and determine each peak area by the automatic integration method: each of the peak other than 4-(aminomethyl)benzoic acid obtained from the sample solution is not larger than the peak area of 4-(aminomethyl)benzoic acid from the standard solution.

1-Amino-2-methylnaphthalene C₁₁H₁₁N Pale yellow to pale brown masses or liquid.

2-Aminomethylpyridine C₆H₁₄N A colorless or light yellowish clear liquid, having an amine like characteristic odor.

Identification—Determine the infrared absorption spectrum as directed in the liquid film method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3280 cm⁻¹, 1600 cm⁻¹, 1440 cm⁻¹, 1120 cm⁻¹ and 840 cm⁻¹.

Purity Related substances—Perform the test with 0.8 μL of 2-aminomethylpyridine as directed under Gas Chromatography <2.0E>. Determine each peak area by the automatic integration method, and calculate these amounts by the area percentage method: the total amount of the peaks other than 2-aminomethylpyridine is not more than 1.5%.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (150 – 180 μm) coated with 10% of polyethylene glycol 20M for gas chromatography and 2% of potassium hydrogen oxide.

Column temperature: 100°C at beginning, and increase to 200°C by 10°C per minute after injection.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of 2-aminomethylpyridine is about 5 minutes.

Time span of measurement: About 2 times as long as the retention time of 2-aminomethylpyridine.

3-(2-Aminomethyl)indole C₅H₁₈N₂ Yellowish-brown crystals.

Melting point <2.60>: about 118°C.

1-Amino-2-naphthol-4-sulfonic acid C₁₀H₉NO₄S [K 8050, Special class]

1-Amino-2-naphthol-4-sulfonic acid TS Mix thoroughly 5 g of anhydrous sodium sulfate, 94.3 g of sodium bisulfite and 0.7 g of 1-amino-2-naphthol-4-sulfonic acid. Before use, dissolve 1.5 g of this mixture in water to make 10 mL.

m-Aminophenol See 3-aminophenol.

3-Aminophenol C₆H₄NH₂OH White, crystals or crystalline powder.

Melting point <2.60>: about 121 – 125°C

Content: not less than 97.0%. Assay—Weigh accurately about 0.2 g, dissolve in 50 mL of acetic acid for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid...
VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
                   = 10.91 mg of H₂NCl₂H₂O

*p*-Aminophenol hydrochloride See 4-aminophenol hydrochloride.

4-Aminophenol hydrochloride  HOC₆H₄NH₂.HCl
White to pale colored crystals. Freely soluble in water and in ethanol (95). Melting point: about 306°C (with decomposition).

Content: not less than 99.0%. Assay—Weigh accurately about 0.17 g of 4-aminophenol hydrochloride, dissolve in 50 mL of acetic acid for nonaqueous titration and 5 mL of mercury (II) acetate TS for nonaqueous titration, and titrate ≤2.50 with 0.1 mol/L perchloric acid-1,4-dioxane VS (indicator: 1 mL of p-naphtholbenzeine TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS
                   = 14.56 mg of C₆H₄NOC₁

Storage—Preserve in tight, light-resistant containers.

Aminopropylsilanized silica gel for pretreatment Prepared for pretreatment.

Aminopyrine  C₁₃H₁₇N₃O White to pale yellow crystals or crystalline powder. Odorless.

Melting point <2.60°: 107 – 109°C

6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate
C₁₄H₁₁N₃O₄ Prepared for amino acid analysis or biochemistry.

1,2-Aminosuberic acid  C₄H₈NO₄ White, crystals or crystalline powder. Odorless.

Optical rotation <2.49° [α]D°: +19.1 – +20.1° (after drying, 0.1 g, 5 mol/L hydrochloric acid TS, 100 mm).

Loss on drying <2.47°: not more than 0.3% (1 g, 105°C, 2 hours).

Assay—Weigh accurately about 0.3 g of 1,2-aminosuberic acid, previously dried, add exactly 6 mL of formic acid to dissolve, then add exactly 50 mL of acetic acid (100), and titrate ≤2.50 with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
                   = 18.92 mg of C₈H₁₅NO₄

Amiodarone hydrochloride for assay  C₂₅H₂₉I₂NO₃.HCl
[Same as the monograph Amiodarone Hydrochloride. When dried, it contains not less than 99.5% of amiodarone hydrochloride (C₂₅H₂₉I₂NO₃.HCl).]

Ammonia-ammonium chloride buffer solution, pH 8.0
To ammonium acetate TS add ammonia TS dropwise to adjust the pH to 8.0.

Ammonia-ammonium chloride buffer solution, pH 8.5
Dissolve 50 g of ammonium acetate in 800 mL of water and 200 mL of ethanol (95), and add ammonia solution (28) to adjust the pH to 8.5.

Ammonia-ammonium chloride buffer solution, pH 8.0
Dissolve 1.07 g of ammonium chloride in water to make 100 mL, and adjust the pH to 8.0 by adding diluted ammonia TS (1 in 30).

Ammonia-ammonium chloride buffer solution, pH 10.0
Dissolve 70 g of ammonium chloride in water, add 100 mL of ammonia solution (28), dilute with water to make 1000 mL, and add ammonia solution (28) dropwise to adjust the pH to 10.0.

Ammonia-ammonium chloride buffer solution, pH 10.7
Dissolve 67.5 g of ammonium chloride in water, add 570 mL of ammonia solution (28), and dilute with water to make 1000 mL.

Ammonia-ammonium chloride buffer solution, pH 11.0
Dissolve 53.5 g of ammonium chloride in water, add 480 mL of ammonia solution (28), and dilute with water to make 1000 mL.

Ammonia copper TS To 0.5 g of cupric carbonate monohydrate add 10 mL of water, triturate, and add 10 mL of ammonia solution (28).

Ammonia-ethanol TS To 20 mL of ammonia solution (28) add 100 mL of ethanol (99.5).

Ammonia gas NH₃ Prepare by heating ammonia solution (28).

Ammonia-saturated 1-butanol TS To 100 mL of 1-butanol add 60 mL of diluted ammonia solution (28) (1 in 100), shake vigorously for 10 minutes, and allow to stand. Use the upper layer.

Ammonia solution (28) NH₃OH [K 8085, Ammonia Water, Special class, Density: 0.90 g/mL, Content: 28 – 30%]

Ammonia TS To 400 mL of ammonia solution (28) add water to make 1000 mL (10%).

1 mol/L Ammonia TS To 65 mL of ammonia solution (28) add water to make 1000 mL.

13.5 mol/L Ammonia TS To exactly 9 mL of water add ammonia solution (28) to make exactly 50 mL.

Ammonia water See ammonia TS.

1 mol/L Ammonia water See 1 mol/L ammonia TS.

13.5 mol/L Ammonia water See 13.5 mol/L ammonia TS.

Ammonia water, strong See ammonia solution (28).

Ammonium acetate  CH₃COONH₄ [K 8359, Special class]

Ammonium acetate TS Dissolve 10 g of ammonium acetate in water to make 100 mL.

0.5 mol/L Ammonium acetate TS Dissolve 38.5 g of ammonium acetate in water to make 1000 mL.

Ammonium amidosulfate NH₄OSO₂NH₂ [K 8588, Special class]

Ammonium amidosulfate TS Dissolve 1 g of ammonium amidosulfate in water to make 40 mL.

Ammonium amminetrichloroplatinate for liquid chromatography Cl₂H₅N₂Pt To 20 g of cisplatin add 600 mL of 6 mol/L hydrochloric acid TS, and heat under a reflux con-
Ammonium chloride - NH₄Cl [K 8116, Special class]

Ammonium chloride-ammonia TS To ammonia solution (28) add an equal volume of water, and saturate this solution with ammonium chloride.

Ammonium chloride buffer solution, pH 10 Dissolve 5.4 g of ammonium chloride in water, and add 21 mL of ammonia solution (28) and water to make 100 mL.

Ammonium chloride TS Dissolve 10.5 g of ammonium chloride in water to make 100 mL (2 mol/L).

Ammonium citrate See diammmonium hydrogen citrate.

Ammonium dihydrogenphosphate - NH₄H₂PO₄ [K 9006, Special class]

0.02 mol/L Ammonium dihydrogenphosphate TS Dissolve 2.30 g of ammonium dihydrogenphosphate in water to make 1000 mL.

Ammonium formate HCOONH₄ Colorless crystals. Very soluble in water. Melting point <2.60°: 116 – 119°C

0.05 mol/L Ammonium formate buffer solution, pH 4.0 Dissolve 3.15 g of ammonium formate in 750 mL of water, adjust to pH 4.0 with formic acid, and add water to make 1000 mL.

Ammonium hydrogen carbonate - NH₄HCO₃ White or semi-transparency crystals, crystalline powder or masses, having an ammonia odor.

Ammonium iron (II) sulfate hexahydrate FeSO₄(NH₄)₂SO₄.6H₂O [K 8979, Special class]

Ammonium iron (III) citrate [Same as the monograph Ferric Ammonium Citrate in the Japanese Standards of Food Additives]

Ammonium iron (III) sulfate dodecahydrate FeNH₄(SO₄)₂.12H₂O [K 8982, Special class]

Ammonium iron (III) sulfate TS Dissolve 8 g of ammonium iron (III) sulfate dodecahydrate in water to make 100 mL.

Ammonium iron (III) sulfate, acidic Dissolve 20 g of ammonium iron (III) sulfate dodecahydrate in a suitable amount of water, add 9.4 mL of sulfuric acid, and add water to make 100 mL.

Ammonium iron (III) sulfate, dilute To 2 mL of ammonium iron (III) sulfate TS add 1 mL of 1 mol/L hydrochloric acid TS and water to make 100 mL.

Ammonium molybdate See hexaammonium heptamolybdate tetrahydrate.

Ammonium molybdate-sulfuric acid TS See hexaammonium heptamolybdate-sulfuric acid TS

Ammonium molybdate TS See hexaammonium heptamolybdate TS.

Ammonium nickel (II) sulfate See ammonium nickel (II) sulfate hexahydrate.

Ammonium nickel (II) sulfate hexahydrate

denser for 4 – 6 hours to boil while stirring. After cooling, evaporate the solvent, and dry the orange residue at room temperature under reduced pressure. To the residue so obtained add 300 mL of methanol, and heat at about 50°C to dissolve. Filter, separate insoluble yellow solids, and wash the solids with 10 mL of methanol. Combine the filtrate and the washing, heat at about 50°C, and add slowly 100 mL of ethyl acetate while stirring. Cool the mixture to room temperature avoiding exposure to light, and allow to stand at –10°C for 1 hour. Filter the mixture to take off the formed crystals, wash the crystals with 100 mL of acetone, combine the washing, heat at about 50°C, and add slowly 100 mL of ethyl acetate while stirring. Cool the mixture to room temperature under reduced pressure. It is a yellow-brown crystalline powder.

Identification - Determine the infrared absorption spectrum of the substance to be examined, previously dried at 80°C for 3 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3480 cm⁻¹, 3220 cm⁻¹, 1622 cm⁻¹, 1408 cm⁻¹ and 1321 cm⁻¹.

Purity Related substances - Cisplatin Conduct this procedure using light-resistant vessels. Dissolve 10 mg in N,N-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of cisplatin in N,N-dimethylformamide to make exactly 50 mL. Pipet 5 mL of this solution, add N,N-dimethylformamide to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak area of cisplatin by the automatic integration method: the peak area from the sample solution is not larger than that from the standard solution.

Operating conditions
Proced as directed in the operating conditions in the Assay under Cisplatin.

System suitability
System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cisplatin are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cisplatin is not more than 5.0%.

Ammonium aurintricarboxylate See aluminon.

Ammonium carbonate [K 8613, Special class]

Ammonium carbonate TS Dissolve 20 g of ammonium carbonate in 20 mL of ammonia TS and water to make 100 mL.
(NH₄)₂Ni(SO₄)₂·6H₂O Green crystals or crystalline powder.

**Identification**—(1) Dissolve 1 g of ammonium nickel (II) sulfate hexahydrate in 20 mL of water, and use this as the sample solution. To 5 mL of the sample solution add 1 mL of barium chloride TS: a white precipitate is produced.

(2) To 5 mL of the sample solution obtained in (1) add 5 mL of 8 mol/L sodium hydroxide TS: a green precipitate is formed, and the liquid evolves ammonia on heating.

(3) To 5 mL of the sample solution obtained in (1) add 1 mL of ammonium TS and dimethyldiethanolamine TS: a red precipitate is formed.

**Content:** not less than 99.0%. Assay—Weigh accurately about 1 g of ammonium nickel (II) sulfate hexahydrate, add 100 mL of water and 5 mL of ammonium chloride TS, then add exactly 20 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetaacetate VS, warm to 50 – 60°C, add 10 mL of diluted ammonia solution (28) (1 in 2), and titrate with 0.1 mol/L disodium dihydrogen ethylenediamine tetaacetate VS until the color of the solution is changed from green to blue-purple (indicator: 0.05 g of murexide-sodium chloride indicator).

Each mL of disodium dihydrogen ethylenediamine tetaacetate VS = 39.50 mg of (NH₄)₂Ni(SO₄)₂·6H₂O

**Ammonium nitrate** NH₄NO₃ [K 8545, Special class]

**Ammonium oxalate** See ammonium oxalate monohydrate.

**Ammonium oxalate monohydrate** (NH₄)₂C₂O₄·H₂O [K 8521, Special class]

**Ammonium oxalate TS** Dissolve 3.5 g of ammonium oxalate monohydrate in water to make 100 mL (0.25 mol/L).

**Ammonium peroxodisulfate** (NH₄)₂S₂O₈ [K 8522, Special class]

10% **Ammonium peroxodisulfate TS** Dissolve 1 g of ammonium peroxodisulfate in water to make 10 mL.

**Ammonium persulfate** See ammonium peroxodisulfate.

**Ammonium polysulfide TS** (NH₄)₂Sₓ [K 8943, Ammonium Sulfide Solution (yellow), First class]

**Ammonium sodium hydrogenphosphate tetrahydrate** NaNH₄HPO₄·4H₂O [K 9013, Special class]

**Ammonium sulfamate** See ammonium amidosulfate.

**Ammonium sulfamate TS** See ammonium amidosulfate TS.

**Ammonium sulfate** (NH₄)₂SO₄ [K 8960, Special class]

**Ammonium sulfate buffer solution** Dissolve 264 g of ammonium sulfate in 1000 mL of water, add 1000 mL of 0.5 mol/L sulfuric acid TS, shake, and filter. The pH of this solution is about 1.

**Ammonium sulfide TS** (NH₄)₂S [K 8943, Ammonium Sulfide Solution, (colorless), First class] Store in small, well-filled containers, protected from light.

**Ammonium tartrate** See L-ammonium tartrate.

**L-Ammonium tartrate** C₄H₇N₂O₆ [K 8534, (+) Ammonium tartrate, Special class]
solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of amygdalin obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (3) under Keishibukuryogon Extract.

Amygdalin for component determination See amygdalin for assay.

Amygdalin for thin-layer chromatography \( \text{C}_{20}\text{H}_{27}\text{NO}_{11} \), A white, odorless powder. Soluble in water, sparingly soluble in methanol, and practically insoluble in diethyl ether, anesthetic.

Identification—Determine the absorption spectrum of a solution of amygdalin for thin-layer chromatography in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, between 261 nm and 265 nm, and between 267 nm and 271 nm.

Purity Related substances—Dissolve 5 mg of amygdalin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL of each of the sample solution and standard solution as directed in the Identification under Peach Kernel: any spot other than the principal spot at the Rf value of about 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

\( n \)-Amyl alcohol \( \text{CH}_3(\text{CH}_2)_4\text{OH} \), Clear, colorless liquid, having a characteristic odor. Sparingly soluble in water, and miscible with ethanol (95% unresolved) and with diethyl ether. Refractive index <2.45> nD 20: 1.409 – 1.411

Specific gravity <2.56> dD 20: 0.810 – 0.820

Distilling range <2.57>: 135 – 140°C, not less than 95 vol%.

\( t \)-Amyl alcohol \( \text{CH}_3(\text{CH}_2)_4\text{CH}2\text{CH}_3 \), Clear, colorless liquid, having a characteristic odor. Miscible with tert-butanol and with 2-butanol, and freely soluble in water. Specific gravity <2.56> dD 20: 0.808 – 0.815

Purity Acid and ester—To 20 mL of \( t \)-amyl alcohol add 20 mL of ethanol (95%) and 5.0 mL of 0.1 mol/L sodium hydroxide VS, and heat gently under a reflux condenser in a water bath for 10 minutes. Cool, add 2 drops of phenolphthalein TS, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS. Perform a blank determination: not more than 1.25 mL of 0.1 mol/L sodium hydroxide VS is consumed.

Nonvolatile residue—Evaporate 50 mL of \( t \)-amyl alcohol, and dry at 105°C for 1 hour: the residue is not more than 1.6 mg.

Distilling range <2.57>: 100 – 103°C, not less than 95 vol%.

\( t \)-Amyl alcohol See \( t \)-amyl alcohol.

Amyl alcohol, iso See 3-methyl-1-butanol.

Anesthetic ether See ether, anesthetic.

Anhydrous caffeine See caffeine, anhydrous.

Anhydrous cupric sulfate See copper (II) sulfate (anhydrous).

Anhydrous dibasic sodium phosphate See disodium hydrogen phosphate.

Anhydrous dibasic sodium phosphate for pH determination See disodium hydrogen phosphate for pH determination.

Anhydrous hydrazine for amino acid analysis Prepared for amino acid analysis.

Anhydrous lactose \( \text{C}_{12}\text{H}_{22}\text{O}_{11} \) [Same as the monograph Anhydrous Lactose]

Anhydrous potassium carbonate See potassium carbonate.

Anhydrous sodium acetate See sodium acetate, anhydrous.

Anhydrous sodium carbonate See sodium carbonate, anhydrous.

Anhydrous sodium sulfate See sodium sulfate, anhydrous.

Anhydrous sodium sulfite See sodium sulfite, anhydrous.

Aniline \( \text{C}_{6}\text{H}_5\text{NH}_2 \) [K 8042, Special class]

Animal tissue peptone See peptone, animal tissue.

p-Anisaldehyde See 4-methoxybenzaldehyde.

p-Anisaldehyde-acetic acid TS See 4-methoxybenzaldehyde-acetic acid TS.

p-Anisaldehyde-sulfuric acid TS See 4-methoxybenzaldehyde-sulfuric acid TS.

Anisole \( \text{C}_7\text{H}_8\text{O} \), A colorless liquid. Boiling point: about 155°C. Specific gravity <2.56> dD 20: 0.995 – 1.001.

14-Anisoylacetone hydrochloride for assay \( \text{C}_3\text{H}_5\text{NO}_{11}.\text{HCl},\text{H}_2\text{O} \), White crystalline powder or powder. Freely soluble in methanol, sparingly soluble in water and in ethanol (99.5%). Melting point: about 210°C (with decomposition).

Absorbance <2.24> E1% (258 nm): 276 – 294 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

Purity (1) Related substances—To 1.0 mg of 14-anisoylacetone hydrochloride for assay add exactly 1 mL of ethanol (99.5%). Perform the test with 5 μL of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principle spot at around Rf value of 0.5 appears.

(2) Related substances—Dissolve 5.0 mg of 14-anisoylacetone hydrochloride for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of 14-anisoylacetone obtained from the sample solution is not larger than the peak area of 14-anisoylacetone from the standard solution.

Operating conditions
Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.
Detector: An ultraviolet absorption photometer (wavelength: 245 nm).
Time span of measurement: About 4 times as long as the retention time of 14-anisoylaconine.

System suitability
Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of 14-anisoylaconine obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of 14-anisoylaconine from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylpyaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylpyaconine and 14-anisoylaconine are not more than 1.5%, respectively.

14-Anisoylaconine hydrochloride for component determination
See 14-anisoylaconine hydrochloride for assay.

Anode solution A for water determination
Dissolve 100 g of diethanolamine in 900 mL of a mixture of methanol for water determination and chloroform for water determination (1:1), pass dried sulfur dioxide gas through this solution while cooling until the mass increase of the solution reaches 64 g. Then add 20 g of iodine, and add water until the color of the solution changes from brown to yellow. To 600 mL of this solution add 400 mL of chloroform for water determination.

Anthrone
C14H10O
Light yellow crystals or crystalline powder.
Melting point
≤2.60°: 154 – 160°C.
Preserve in a light-resistant tight container.

Anthrone TS
Dissolve 35 mg of anthrone in 100 mL of sulfuric acid.

Anti-A type antibody for blood typing
Conforms to the requirements of antibody for blood typing.

Anti-B type antibody for blood typing
Conforms to the requirements of antibody for blood typing.

Antibody fragment (Fab')
Purify E. coli protein antibody by affinity chromatography using Staphylococcus aureus protein A as a ligand, and fractionate IgG. Digest this fraction using pepsin, remove the pepsin and Fc fragment by gel filtration chromatography, and obtain F(ab')2 fragment after removing undigested IgG by affinity chromatography with protein A as ligand. Reduce this with 2-mercaptoethanol.

Anti-bradykinin antibody
A colorless to light brown, clear solution prepared by dissolving rabbit origin anti-bradykinin antibody in 0.04 mol/L phosphate buffer solution, pH 7.0 containing 1 mg/mL of bovine serum albumin.

Performance test—To a suitable amount of anti-bradykinin antibody to be tested add 0.04 mol/L phosphate buffer solution, pH 7.0 containing 1 mg/mL bovine serum albumin to make a 1 vol% solution. Perform the test with 0.1 mL of this solution as directed in the Purity (2) under Kallidinogenase, and determine the absorbances at 490 – 492 nm, A1 and A2, of the standard solution (1) and the standard solution (7): the value, A2 – A1, is not less than 1.

Anti-bradykinin antibody TS
To 0.15 mL of anti-bradykinin antibody, 15 mg of bovine serum albumin, 2.97 mg of sodium dihydrogen phosphate dihydrate, 13.5 mg of disodium hydrogen phosphate dodecahydrate and 13.5 mg of sodium chloride add water to make 15 mL, and lyophilize. Dissolve this in 15 mL of water. Prepare before use.

Anti-E. coli protein antibody stock solution
Taking E. coli protein stock solution as the immunogen, mix with Freund’s complete adjuvant, and immunize rabbits by subcutaneous injection at 3 week intervals to obtain antiserum. Treat the antiserum obtained by ammonium sulfate precipitation.

Protein concentration: Dilute anti-E. coli protein antibody stock solution with 0.05 mol/L tris hydrochloride buffer solution (pH 7.5), measure the absorbance at 280 nm using 0.05 mol/L tris hydrochloride buffer solution (pH 7.5) as a control as direct under Ultraviolet-visible Spectrophotometry <2.24>, and determine the protein concentration (absorbance 1.0 = 0.676 mg/mL).

Antimony (III) chloride
SbCl3 [K 8400, Special class]
Antimony (III) chloride TS
Wash chloroform with an equal volume of water twice or three times, add freshly ignited and cooled potassium carbonate, and allow to stand overnight in a well-closed container protected from light. Separate the chloroform layer, and distil it, preferably with protection from light. With this chloroform, wash the surface of antimony (III) chloride until the rinsing solution becomes clear, add the chloroform to this antimony (III) chloride to make a saturated solution, and place in light-resistant, glass-stoppered bottles. Prepare before use.

Antimony trichlorid
See antimony (III) chloride.

Antimony trichlorid TS
See antimony (III) chloride TS.

Antipyrine
C11H12N2O [Same as the namesake monograph]
Anti-rabbit antibody-coated wells
Wells of a polystyrene microplate coated with goat origin anti-rabbit IgG antibody.

Anti-thrombin III
A white powder.
Water <2.48>: not more than 5%.
Content: not less than 80% and not more than 130% of the labeled amount.

Anti-thrombin III TS
Dissolve 10 unit of anti-thrombin III in 10 mL of water.

Anti-ulinastatin rabbit serum
To a suitable amount of Ulinastatin having the specific activity of more than 3000 Units per mg protein add isotonic sodium chloride solution so that each mL of the solution contains about 1 mg of protein. To 1 mL of this solution add 1 mL of Freund’s complete adjuvant, and emulsify completely. Intracutaneously, inject the emulsion so obtained into a rabbit weighing about
2 kg. Repeat the injection at least 4 times at one-week intervals, and draw the blood of the animal from the carotid artery after the antibody titer reaches 16 times or more. Separate the serum after the blood has coagulated. Preserve at below 20°C.

**Anti-urokinase serum** Take Urokinase containing not less than 140,000 Unit per mg of protein, dissolve in isotonic sodium chloride solution to make a solution containing 1 mg of protein per mL, and emulsify with an equal volume of Freund’s complete adjuvant. Inject intracutaneously three 2-mL portions of the emulsion to a healthy rabbit weighed between 2.5 kg and 3.0 kg in a week interval. Collect the blood from the rabbit at 7 to 10 days after the last injection, and prepare the anti-serum.

**Performance test**—Dissolve 1.0 g of agar in 100 mL of boric acid-sodium hydroxide buffer solution, pH 8.4, by warming, and pour the solution into a Petri dish to make a depth of about 2 mm. After cooling, bore three of a pair-well 2.5 mm in diameter with a space of 6 mm each other. In one of the wells of each pair-well, place 10 μL of anti-urokinase serum, and in each another well, place 10 μL of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution, 10 μL of human serum and 10 μL of human urine, respectively, and allow to stand overnight: a precipitin line appears between anti-urokinase serum and urokinase, and not appears between anti-urokinase serum and human serum or human urine.

α-Apoxytetracycline C_{22}H_{22}N_{2}O_{8} Yellow-brown to green powder.

Melting point <2.60°: 200 – 205°C

β-Apoxytetracycline C_{22}H_{22}N_{2}O_{8} Yellow-brown to brown powder.

**Purity** Related substances—Dissolve 8 mg of β-apoxygenyctetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as the sample solution. Proceed the test with 20 μL of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than β-apoxygenyctetracycline is not more than 10%.

**Aprimidine hydrochloride for assay** C_{22}H_{30}N_{2}HCl

[Same as the monograph Aprimidine Hydrochloride. When dried, it contains not less than 99.5% of aprimidine hydrochloride (C_{22}H_{30}N_{2}HCl).]

Aprotinin A clear and colorless liquid containing aprotinin extracted from the lung or parotid gland of a healthy cat.

The pH is between 5.0 and 7.0.

**Content**: not less than 15,000 KIE Units and not more than 25,000 KIE Units of aprotinin per mL. Assay—(i) Trypsin solution: Weigh an amount of crystalline trypsin equivalent to about 250 FIP Units of trypsin according to the labeled FIP Units, and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 10 mL. Prepare before use, and preserve in ice. (ii) Sample solution: Dilute a suitable quantity of aprotinin with sodium tetraborate-calcium chloride buffer solution, pH 8.0 so that each mL of the solution contains 800 KIE Units of aprotinin, and use this solution as the sample solution. (iii) Apparatus: Use a glass bottle as a reaction reservoir, 20 mm in inside diameter and 50 mm in height, equipped with a rubber stopper for attachment to a glass/silver-silver chloride electrode, a nitrogen-induction tube and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature of the bath at 25 ± 0.1°C by means of a precise thermoregulator. (iv) Procedure: To 5.0 mL of N-α-benzoyl-L-arginine ethyl ester TS add 45.0 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0, and use this solution as the substrate solution. Pipet 1 mL of the trypsin solution, add sodium tetraborate-calcium chloride buffer solution, pH 8.0 to make exactly 10 mL, and use this solution as the test solution I. Transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00 by adding dropwise 0.1 mol/L sodium hydroxide VS while stirring and passing a current of nitrogen, add exactly 1 mL of the test solution I previously allowed to stand at 25 ± 0.1°C for 10 minutes, then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50-μL micropipet (minimum graduation of 1 μL), while stirring, to keep the reaction solution at pH 8.00, and read the amount of 0.1 mol/L sodium hydroxide VS consumed and the reaction time when the pH reached 8.00. Continue this procedure up to 6 minutes. Separately, pipet 2 mL of the trypsin solution and 1 mL of the sample solution, add sodium tetraborate-calcium chloride buffer solution, pH 8.0 to make exactly 10 mL, and use this solution as the test solution II. Transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00, while stirring and passing a current of nitrogen, add exactly 1 mL of the test solution II, previously allowed to stand at 25 ± 0.1°C for 10 minutes, and proceed in the same manner. Separately, transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00, while stirring and passing a current of nitrogen, add 1 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0, previously allowed to stand at 25 ± 0.1°C for 10 minutes, and perform a blank determination in the same manner. (v) Calculation: Plot the amount of consumption (f) of 0.1 mol/L sodium hydroxide VS against the reaction time (minutes), select linear reaction times, t₁ and t₂, designate the corresponding consumption amount of 0.1 mol/L sodium hydroxide VS as v₁ and v₂, respectively, and designate μmol of sodium hydroxide consumed per minute as D.

\[
D (\mu\text{mol NaOH/min}) = \frac{v_2 - v_1}{t_2 - t_1} \times \frac{1}{10} \times f
\]

f: Factor of 0.1 mol/L sodium hydroxide VS

KIE Units per mL of aprotinin to be tested

\[
= \frac{2(D_A - D_S) - (D_B - D_S)}{L} \times n \times 32.5
\]

L: Amount (mL) of the sample solution added to the test solution II
n: Dilution coefficient of aprotinin to be tested
Dₐ: μmol of sodium hydroxide consumed in 1 minute when the test solution I is used
Dₐ: μmol of sodium hydroxide consumed in 1 minute when the test solution II is used
Dₐ: μmol of sodium hydroxide consumed in 1 minute when the solution for blank determination is used
32.5: Equivalent coefficient for calculation of KIE Units from FIP Units
One KIE Unit means an amount of aprotinin making a reduction of 50% off the potency of 2 Units of kallidinogenase at pH 8.0 and room temperature for 2 hours.

Storage—Preserve in a light-resistant, hermetic container and in a cold place.

Aprotinin TS Measure an appropriate amount of aprotinin, and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 50 KIE Units per mL.

Aquæ regiæ Add 1 volume of nitric acid to 3 volumes of hydrochloric acid. Prepare before use.

L-Arabinose C_{6}H_{17}O_{7} A white crystalline powder. Melting point <2.600: 155 - 160°C.

Optical rotation <2.490 [α]_{D}^{20}: +103.0 - +105.5° Weigh accurately about 5 g of L-arabinose, previously dried at 105°C for 2 hours, dissolve in 30 mL of water, add 0.4 mL of ammonia TS, and add water to make exactly 50 mL. Allow to stand for 1 hour, and determine using a 100-mm cell.

Arbutin for assay Use arbutin for thin-layer chromatography meeting the following additional specifications.

Absorbance <2.240 E_{1%}^{10} (280 nm): 70 - 76 [4 mg, previously dried in a desiccator (in vacuum, silica gel), 12 hours, water, 100 mL].

Purity Related substances—Dissolve 40 mg of arbutin for assay in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μL of the sample solution and standard solution (1) as directed in the operating conditions and measure each peak area of the both solutions by the automatic integration method: the total area of the peaks other than that of arbutin from the sample solution is not larger than the peak area of arbutin from the standard solution (1).

Operating conditions Proceed the operating conditions in the Assay under Bearberry Leaf except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add water to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of arbutin obtained from 10 μL of the standard solution (2) can be measured by the automatic integration method and the peak height of arbutin obtained from 10 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of arbutin beginning after the solvent peak.

Arbutin for component determination See arbutin for assay.

Arbutin for thin-layer chromatography C_{13}H_{19}O_{7}·nH_{2}O Colorless to white crystals or crystalline powder, and odorless. Freely soluble in water, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in ethyl acetate and in chloroform.

Melting point <2.600: 199 - 201°C

Purity Related substances—Dissolve 1.0 mg of arbutin for thin-layer chromatography in exactly 1 mL of a mixture of ethanol (95) and water (7:3). Perform the test with 20 μL of this solution as directed in the Identification (2) under Bearberry Leaf: any spot other than the main spot at the Rf value of about 0.4 does not appear.

Acorus japonicus hydrobromide for thin-layer chromatography C_{12}H_{16}O_{7}·HBr White crystals. Freely soluble in water, soluble in methanol, and practically insoluble in diethyl ether.

Melting point <2.600: 169 - 171°C

Purity Related substances—Dissolve 5 mg of aconitum hydrobromide for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 10 μL of this solution as directed in the Identification under Area: any spot other than the principal spot at the Rf value of about 0.6 does not appear.

L-Arginine C_{6}H_{14}N_{2}O_{2} White, crystals or crystalline powder. It has a characteristic odor.

Optical rotation <2.490 [α]_{D}^{20}: +26.9 - +27.9° (After drying, 4 g, 6 mol/L hydrochloric acid TS, 50 mL, 200 mm). Loss on drying <2.410: not more than 0.50% (1 g, 105°C, 3 hours).

Content: not less than 98.0% and not more than 102.0%. Assay—Weigh accurately about 0.15 g of L-arginine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.500 with 0.1 mol/L perchloric acid VS until the color of the solution changes to green through yellow (indicator: 10 drops of p-naphtholbenzein TS). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 8.710 mg of C_{6}H_{14}N_{2}O_{2}

L-Arginine hydrochloride C_{6}H_{14}N_{2}O_{2}·HCl [Same as the namesake monograph]

Aristolochic acid I for crude drugs purity test

C_{7}H_{12}O_{7} Yellow crystalline powder. Melting point: about 280°C (with decomposition).

Absorbance <2.240 E_{1%}^{10} (318 nm): 384 - 424 (1 mg, methanol, 100 mL).

Purity Related substances—Dissolve 1.0 mg of aristolochic acid I for crude drugs purity test in 100 mL of diluted methanol (3 in 4), and use this solution as the sample solution. Pipet 1 mL of this solution, add diluted methanol (3 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.010 according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than aristolochic acid I obtained from the sample solution is not larger than the peak area of aristolochic acid I from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Asiasarum Root.

Time span of measurement: About 3 times as long as the retention time of aristolochic acid I beginning after the solvent peak.

System suitability

Proceed as directed in the system suitability in the Purity.
L-Ascorbic acid \( \text{C}_{6}\text{H}_8\text{O}_6 \) [K 9502, L(+)-Ascorbic Acid, Special class]

Ascorbic acid for iron limit test
See L-ascorbic acid.

0.012 g/dL L-Ascorbic acid-hydrochloric acid TS
Dissolve 15 mg of L-ascorbic acid in 25 mL of methanol, add carefully 100 mL of hydrochloric acid, and mix. Prepare before use.

0.02 g/dL L-Ascorbic acid-hydrochloric acid TS
Dissolve 25 mg of L-ascorbic acid in 25 mL of methanol, add carefully 100 mL of hydrochloric acid, and mix. Prepare before use.

0.05 g/dL L-Ascorbic acid-hydrochloric acid TS
Dissolve 0.05 g of L-ascorbic acid in 30 mL of methanol, add carefully hydrochloric acid to make 100 mL. Prepare before use.

DL-Aspartic acid \( \text{C}_4\text{H}_7\text{NO}_4 \)
A white crystalline powder that is sparingly soluble in water. Melting point: 270 to 271°C.

L-Aspartic acid \( \text{C}_4\text{H}_7\text{NO}_4 \) [K 9045, Special class]

Aspartic acid See L-aspartic acid.

Aspirin \( \text{C}_9\text{H}_8\text{O}_4 \) [Same as the namesake monograph]

Aspirin See L-aspirin.

Atropine sulfate \( \text{C}_{17}\text{H}_{23}\text{NO}_3 \cdot \text{H}_2\text{SO}_4 \).
See atropine sulfate hydrate.

Atropine sulfate for assay See atropine sulfate hydrate for assay.

Atropine sulfate for thin-layer chromatography
See atropine sulfate hydrate.

Atropine sulfate hydrate \( \text{C}_{17}\text{H}_{23}\text{NO}_3 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O} \) [Same as the namesake monograph]

Atropine sulfate hydrate for assay \( \text{C}_{17}\text{H}_{23}\text{NO}_3 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O} \) [Same as the monograph Atropine Sulfate Hydrate.
When dried, it contains not less than 99.0% of atropine sulfate \([\text{C}_{17}\text{H}_{23}\text{NO}_3 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O} ] \).]
A tropine sulfate hydrate for thin-layer chromatography
(C\textsubscript{17}H\textsubscript{22}NO\textsubscript{3})\textsubscript{2}.H\textsubscript{2}SO\textsubscript{4}.H\textsubscript{2}O Use atropine sulfate hydrate for assay meeting the following additional specifications. Weigh accurately about 50 mg of atropine sulfate hydrate for assay, dissolve in ethanol (95) to make 10 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography \( \mu \text{L} \). Spot 50 \( \mu \text{L} \) of the solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of chloroform and diethylamine (9:1) to a distance of about 10 cm, air-dry the plate, and spray evenly chloroplatinic acid-potassium iodide TS on the plate: any spot other than the spot at the \( Rf \) value of about 0.4 does not appear.

**A-type erythrocyte suspension** Prepare a suspension containing 1 vol\% of erythrocyte separated from human A-type blood in isotonic sodium chloride solution.

Azelaistine hydrochloride for assay C\textsubscript{21}H\textsubscript{18}O\textsubscript{11}.H\textsubscript{2}O Light yellow odorless powder. Slightly soluble in water, slightly soluble in ethanol (95), and practically insoluble in methanol, and practically insoluble in water and in diethyl ether. Melting point: about 206°C (with decomposition).

**Purity** Related substance—Dissolve 1.0 mg of azelaistine hydrate for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 10 \( \mu \text{L} \) of this solution as directed in the Identification (2) under Scutellaria Root: any spot other than the principal spot at the \( Rf \) value of about 0.4 does not appear.

**Bakumondo** [Same as the namesake monograph]

Balsam Canada balsam for microscopy. Before use, dilute to a suitable concentration with xylene.

**Bamethan sulfate** (C\textsubscript{17}H\textsubscript{23}NO\textsubscript{3})\textsubscript{2}.H\textsubscript{2}SO\textsubscript{4} [Same as the namesake monograph]

**Barbaloin for assay** C\textsubscript{21}H\textsubscript{22}O\textsubscript{9} Use barbaloin for thin-layer chromatography in exactly 1 mL of methanol.

**Barbaloin for thin-layer chromatography** C\textsubscript{21}H\textsubscript{22}O\textsubscript{9}.H\textsubscript{2}O Light yellow odorless powder. Freely soluble in methanol, practically insoluble in water and in diethyl ether.

**Barbital** C\textsubscript{8}H\textsubscript{12}N\textsubscript{2}O\textsubscript{3} [Same as the namesake monograph]

**Barbital buffer solution** Dissolve 15 g of barbital sodium in 700 mL of water, adjust the pH to 7.6 with dilute hydrochloric acid, and filter.

**Barbital sodium** C\textsubscript{8}H\textsubscript{11}N\textsubscript{2}NaO\textsubscript{3}. White, odorless crystals of crystalline powder, having a bitter taste. Freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

**pH** \( \text{H} \rightarrow \text{L} \). The pH of a solution of barbital sodium (1 in 200) is between 9.9 and 10.3.

**Loss on drying** \( \text{L} \). Assay—Weigh accurately about 0.5 g of barbital sodium, previously dried, transfer to a separator, dissolve in 20 mL of water, add 5 mL of ethanol (95) and 10 mL of dilute hydrochloric acid, and extract with 50 mL of chloroform. Then extract with three 25-mL portions of chloroform, combine the total extract, wash with two 5-mL portions of water, and extract the washings with two 10-mL portions of chloroform. Combine the chloroform extracts, and filter into a conical flask. Wash the filter paper with three 5-mL portions of chloroform, combine the filtrate and the washings, add 10 mL of ethanol (95), and titrate with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow to purple through light purple (indicator: 2 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 20.62 mg of C\textsubscript{8}H\textsubscript{11}N\textsubscript{2}NaO\textsubscript{3}.

**Barium chloride** See barium chloride dihydrate.

**Barium chloride dihydrate** BaCl\textsubscript{2}.2H\textsubscript{2}O [K 8155, Special class]

**Barium chloride TS** Dissolve 12 g of barium chloride dihydrate in water to make 100 mL (0.5 mol/L).

**Barium hydroxide** See barium hydroxide octahydrate.
Barium hydroxide octahydrate \( \text{Ba(OH)}_2 \cdot 8\text{H}_2\text{O} \) [K 8577, Special class] Store in tightly stoppered containers.

Barium hydroxide TS Saturate barium hydroxide octahydrate in freshly boiled and cooled water (0.25 mol/L). Prepare before use.

Barium nitrate \( \text{Ba(NO_3)}_2 \) [K 8565, Special class]

Barium nitrate TS Dissolve 6.5 g of barium nitrate in water to make 100 mL (0.25 mol/L).

Barium oxide \( \text{BaO} \) A white to yellowish or grayish white, powder.

Identification (1) Dissolve 0.5 g in 15 mL of water and 5 mL of hydrochloric acid, and add 10 mL of dilute sulfuric acid: white precipitates appear.

(2) Perform the test as directed under Flame Coloration Test (1) \(<1.04>\); a green color appears.

Barium perchlorate \( \text{Ba(ClO}_3)_2 \) [K 9551, Special class]

Becamycin sulfate \( \text{C}_{18}\text{H}_{37}\text{N}_{3}\text{O}_{6}\cdot\text{xH}_2\text{SO}_4 \) [Same as the namesake monograph]

Beclometasone dipropionate \( \text{C}_{28}\text{H}_{31}\text{N}_{3}\text{O}_6\cdot\text{HCl} \) [Same as the namesake monograph]

Benidipine hydrochloride \( \text{C}_{28}\text{H}_{31}\text{N}_{3}\text{O}_6\cdot\text{HCl} \) [Same as the namesake monograph]

Benidipine hydrochloride for assay \( \text{C}_{28}\text{H}_{31}\text{N}_{3}\text{O}_6\cdot\text{HCl} \) [Same as the monograph Benidipine Hydrochloride. When dried, it contains not less than 99.5% of benidipine hydrochloride (\( \text{C}_{28}\text{H}_{31}\text{N}_{3}\text{O}_6\cdot\text{HCl} \)).

Benzaldehyde \( \text{C}_6\text{H}_5\text{CHO} \) [K 8857, First class]

Benzalkonium chloride [Same as the namesake monograph]

Benzaldehyde \( \text{C}_6\text{H}_5\text{CHO} \) [K 8857, First class]

Benzalkonium chloride [Same as the namesake monograph]

Benzaldehyde \( \text{C}_6\text{H}_5\text{CHO} \) [K 8857, First class]

Benzaldehyde \( \text{C}_6\text{H}_5\text{CHO} \) [K 8857, First class]

Benzathion chloride for assay \( \text{C}_6\text{H}_6\text{ClN}_2\text{O}_2 \) [Same as the monograph Benzathion Chloride. When dried, it contains not less than 99.0% of benzathion chloride (\( \text{C}_6\text{H}_6\text{ClN}_2\text{O}_2 \)).

Benzoic acid \( \text{C}_6\text{H}_5\text{COOH} \) [K 8073, Special class]

Benzoin \( \text{C}_6\text{H}_5\text{CH(OH)}\text{COC}_6\text{H}_5 \) White to pale yellow, crystals or powder.

Melting point \(<2.60>\): 132 – 137°C

Benzenophene \( \text{C}_6\text{H}_5\text{COC}_6\text{H}_5 \) Colorless crystals, having a characteristic odor.

Melting point \(<2.60>\): 48 – 50°C

Benz[\(a\)]anthracene \( \text{C}_{20}\text{H}_{12} \) Light yellow to green-yellow crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 176 – 181°C.

Identification—Perform the test with benz[\(a\)]anthracene as directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak \((m/z\ 252)\) and a fragment ion peak \((m/z\ 125)\).

Purity Related substances—Dissolve 3.0 mg of benz[\(a\)]anthracene in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 1 \(\mu\)L of this solution as directed under Gas Chromatography \(<2.02>\) according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than benz[\(a\)]anthracene is not more than 3.0%.

Operating conditions

Detector: A mass spectrophotometer (EI).

Mass scan range: 15.00 – 300.00.

Time of measurement: 12 – 30 minutes.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 0.25 – 0.5 \(\mu\)m.

Column temperature: Inject at a constant temperature of about 45°C, raise the temperature to 240°C at a rate of 40°C per minute, maintain at 240°C for 5 minutes, raise to 300°C at a rate of 4°C per minute, raise to 320°C at a rate of 10°C per minute, and maintain at 320°C for 3 minutes.
Injection port temperature: A constant temperature of about 250°C.

Interface temperature: A constant temperature of about 300°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of benzoprene is about 22 minutes.

Split ratio: Splitless.

System suitability
Test for required detectability: Pipet 1 mL of the sample solution, add methanol to make exactly 10 mL. Confirm that the peak area of benzo[pyrene obtained from 1 µL of this solution is equivalent to 5 to 15% of that of benzoprene from 1 µL of the sample solution.

p-Benzooquinone \( \text{C}_4\text{H}_6\text{O}_2 \) Yellow to yellow-brown, crystals or crystalline powder, having a pungent odor. Soluble in ethanol (95) and in diethyl ether, slightly soluble in water. It is gradually changed to a blackish brown color by light.

Melting point \(< 220^\circ\text{C} \): 111 - 116°C

Content: not less than 98.0%. Assay—Weigh accurately about 0.1 g of p-benzoquinone, place in an iodine bottle, add exactly 25 mL of water and 25 mL of diluted sulfuric acid (1 in 5), dissolve 3 g of potassium iodide by shaking, and titrate \( \text{C}_4\text{H}_6\text{O}_2 \) with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS = 5.405 mg of \( \text{C}_4\text{H}_6\text{O}_2 \).

p-Benzooquinone TS Dissolve 1 g of p-benzoquinone in 5 mL of acetic acid (100), and add ethanol (95) to make 100 mL.

**N-α-Benzoyl-L-arginine ethyl ester hydrochloride** \( \text{C}_{21}\text{H}_{22}\text{N}_{6}\text{O}_4\cdot\text{HCl} \) White crystals or crystalline powder. Freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

Melting point \(< 220^\circ\text{C} \): 129 - 133°C

Optical rotation \( < 249^\circ \) \([\alpha]_D^- \): -15.5 - -17.0° (2.5 g, water, 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.1 g of \( \text{N-α-benzoyl-L-arginine ethyl ester hydrochloride} \) in 20 mL of water: the solution is clear and colorless.

(2) Related substances—Weigh 0.10 g of \( \text{N-α-benzoyl-L-arginine ethyl ester hydrochloride} \), dissolve in 6 mL of water, add 4 mL of hydrochloric acid, heat in a boiling water bath for 5 minutes to decompose, and use this solution as the sample solution. Perform the test with the sample solution as directed under Paper Chromatography. Spot 5 µL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:1:1) to a distance of about 10 cm, and air-dry the plate. Exposure the plate to a vapor of iodine: only one spot appears.

**N-α-Benzoyl-L-arginine-4-nitroanilide hydrochloride** \( \text{C}_{31}\text{H}_{43}\text{NO}_9\cdot\text{HCl} \) Light yellow crystalline powder.

**Liquid chromatography**—Dissolve 0.1 g of \( \text{N-α-benzoyl-L-arginine-4-nitroanilide hydrochloride} \) in 10 mL of \( \text{N,N-dimethylformamide} \), and use this solution as the sample solution.

Perform the test with this solution as directed under Liquid Chromatography \( < 2.0^\circ \). Spot 10 µL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:1:1) to a distance of about 10 cm, and air-dry the plate. Exposure the plate to a vapor of iodine: only one spot appears.

**N-α-Benzoyl-L-arginine-4-nitroanilide TS** Dissolve 0.1 g of \( \text{N-α-benzoyl-L-arginine-4-nitroanilide hydrochloride} \) in water to make 100 mL.

**Benzoyl chloride** \( \text{C}_8\text{H}_7\text{COCl} \) A clear and colorless fuming liquid. Specific gravity: about 1.2 g/mL.

**Identification**—Determine the infrared absorption spectrum as directed in the liquid film method as directed under Infrared Spectrophotometry \( < 2.25^\circ \): it exhibits absorption at the wave numbers of about 1775 cm\(^{-1}\), 1596 cm\(^{-1}\), 1450 cm\(^{-1}\), 1307 cm\(^{-1}\), 1206 cm\(^{-1}\), 873 cm\(^{-1}\), 776 cm\(^{-1}\) and 671 cm\(^{-1}\).

**Benzoylhyponcacin hydrochloride for assay** \( \text{C}_{19}\text{H}_{22}\text{N}_{6}\text{O}_4\cdot\text{HCl} \) White crystals or crystalline powder. Freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5). Melting point: about 230°C (with decomposition).

Absorbance \( < 2.24^\circ \) \( E_1^1\text{cm} \): 225 - 240 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

Purity (1) Related substances—To 1.0 mg of benzoylhyponcacin hydrochloride for assay add exactly 1 mL of ethanol (99.5). Perform the test with 5 µL of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principal spot at around RF value of 0.5 appears.

(2) Related substance—Dissolve 5.0 mg of benzoylhyponcacin hydrochloride for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL of the sample solution and standard solution as directed under Liquid Chromatography \( < 2.0^\circ \) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of benzoylhyponcacin obtained from the sample solution is not larger than the peak area of benzoylhyponcacin from the standard solution.

Operating conditions
Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.
Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Time span of measurement: About 5 times as long as the retention time of benzoylhyacoline.

System suitability
Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of benzoylhyacoline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of benzoylmesaconine from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylhyacoline and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhyacoline and 14-anisoylaconine are not more than 1.5%, respectively.

**Benzoylmesaconine hydrochloride for component determination** See benzoylmesaconine hydrochloride for assay.


Absorbance ≤2.24 E₁%₁₀₀ (316 nm): 166 – 184 (10 mg, water, 300 mL).

**Benzoylmesaconine hydrochloride for assay** Benzoylmesaconine hydrochloride for thin-layer chromatography meeting the following additional specifications.

**Purity** Related substances—Dissolve 5.0 mg of benzoylmesaconine hydrochloride for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ≤2.0 for the following conditions. Determine each peak area of both solutions by the automatic integration method; the total area of the peaks other than the peak of benzoylmesaconine obtained from the sample solution is not larger than the peak area of benzoylmesaconine from the standard solution.

Operating conditions
Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Time span of measurement: About 6 times as long as the retention time of benzoylmesaconine.

System suitability
Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of benzoylmesaconine obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of benzoylmesaconine from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylhyacoline and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhyacoline and 14-anisoylaconine are not more than 1.5%, respectively.

**Benzoylmesaconine hydrochloride for component determination** See benzoylmesaconine hydrochloride for assay.

**Benzoylmesaconine hydrochloride for thin-layer chromatography** C₃₁H₄₃NO₁₀.HCl.₈H₂O White crystals or crystalline powder. Soluble in water and in ethanol (99.5) and sparingly soluble in methanol. Melting point: about 250°C (with decomposition).

Absorbance ≤2.24 E₁%₁₀₀ (230 nm): 217 – 231 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

**Purity** Related substances—Dissolve 1.0 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in exactly 1 mL of ethanol (99.5). Perform the test with 5 μL of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principal spot at around Rf value of 0.4 appears.

**Benzoyl peroxide, 25% water containing** (C₆H₅COOCH₂C₆H₅)₂O. White moist crystals or powder. Soluble in diethylene and in chloroform, and very slightly soluble in water and in ethanol (95). Melting point: 103 – 106°C (dried substance) (with decomposition).

Loss on drying ≤2.41: not more than 30% (0.1 g, in vacuum, silica gel, constant mass).

**Benzyl alcohol** C₆H₅CH₂OH Clear and colorless liquid, having a characteristic odor.

**Specific gravity** ≤2.560 d₂₀°: 1.045 – 1.050.

Preserve in a light-resistant tight container.

**Benzyl benzoate** C₆H₅COOCH₂C₆H₅ A colorless oily liquid. Congealing point: about 18°C. Boiling point: about 323°C.

**Specific gravity** ≤2.560 d₂₀°: 1.118 – 1.123.

Preserve in a light-resistant tight container.

**Benzyl parahydroxybenzoate** HOCH₂H₂COOCH₂C₆H₅ White, odorless, fine crystals or crystalline powder. Freely soluble in ethanol (95), in acetone and in diethylene ether, and very slightly soluble in water.

**Melting point** ≤2.60: 109 – 112°C

**Residue on ignition** ≤2.40: not more than 0.1%.

**Content**: not less than 99.0%.—Assay—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS = 228.2 mg of C₆H₅O₃

**Benzylpenicillin benzathine** See benzylpenicillin benzathine hydrate.

**Benzylpenicillin benzathine hydrate** (C₁₆H₁₆N₂O₁₂S₂)₅.C₁₆H₂₅N₂.H₂O [Same as the namesake
monograph]

Benzylopenicillin potassium C_{16}H_{17}KNO_{5}S [Same as the monograph Benzylpenicillin Potassium]

Benzy l- p-hydroxybenzoate See benzy l parahydroxyben zoate.

p-Benzylphenol C_{12}H_{10}ClCH_{2}OH White to pale yellowish white crystals or crystalline powder.

Melting point <2.60°: 80 – 85°C

Beraprost sodium C_{24}H_{29}NaO_{5} [Same as the namesake monograph]

Beraprost sodium for assay C_{24}H_{29}NaO_{3} [Same as the monograph Beraprost Sodium. When dried it contains not less than 99.0% of beraprost sodium (C_{24}H_{29}NaO_{3}).]

Berberine chloride See berberine chloride hydrate.

Berberine chloride hydrate C_{20}H_{18}ClNO_{4} [Same as the namesake monograph]

Berberine chloride hydrate for thin-layer chromatography See berberine chloride hydrate for thin-layer chromatography.

Berberine chloride hydrate for thin-layer chromatography [Same as the monograph Berberine Chloride Hydrate. Use berberine chloride hydrate for thin-layer chromatography.]

Bergenin for thin-layer chromatography C_{14}H_{16}O_{9} White crystals or crystalline powder. Slightly soluble in ethanol (99.5), very slightly soluble in water, and practically insoluble in diethyl ether.

Identification—Dissolve 10 mg of bergenin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL of each of the sample solution and standard solution as directed in the Identification (2) under Phellodendron Bark: any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Bergenin for thin-layer chromatography C_{14}H_{16}O_{9}.xH_{2}O White crystals or crystalline powder. Slightly soluble in ethanol (99.5), very slightly soluble in water, and practically insoluble in diethyl ether.

Identification—Determine the absorption spectrum of a solution of bergenin for thin-layer chromatography in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 217 nm and 221 nm, and between 273 nm and 277 nm, and a minimum between 241 nm and 245 nm.

Purity Related substances—Dissolve 1.0 mg of bergenin for thin-layer chromatography in 20 mL of methanol, and add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL of this solution and standard solution as directed in the Identification (2) under Phellodendron Bark: any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Betahistine mesilate C_{12}H_{15}N_{2}.2CH_{3}O_{2}S [Same as the namesake monograph]

Betahistine mesilate for assay [Same as the monograph Betahistine Mesilate. When dried, it contains not less than 99.0% of betahistine mesilate (C_{12}H_{15}N_{2}.2CH_{3}O_{2}S).]

Bezafibrate for assay C_{20}H_{27}CINO_{4} [Same as the monograph Bezafibrate. When dried it contains not less than 99.0% of bezafibrate (C_{20}H_{27}CINO_{4}).]

BGLB Dissolve 10 g of peptone and 10 g of lactose monohydrate in 500 mL of water, add 200 mL of fresh ox bile or a solution prepared by dissolving 20 g of dried ox bile powder in 200 mL of water and adjusted the pH to between 7.0 and 7.5, then add water to make 975 mL, and again adjust to pH 7.4. Then add 13.3 mL of a solution of brilliant green (1 in 1000) and water to make 1000 mL in total volume, and filter through absorbent cotton. Dispense 10 mL portions of the filtrate into tubes for fermentation, and sterilize by autoclaving at 121°C for not more than 20 minutes, then cool quickly, or sterilize fractionally on each of three successive days for 30 minutes at 100°C.

α-BHC (α-Hexachlorocyclohexane) C_{12}H_{13}Cl_{6} Melting point <2.60°: 157 – 159°C

Purity Related substances—Dissolve 10 mg of α-BHC in 5 mL of acetone for purity of crude drug, and add hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1 μL each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak area other than α-BHC from the sample solution is not larger than the peak area of α-BHC from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Purity (2) under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make 20 mL, and use this solution as standard solution (2). Adjust the detection sensitivity so that the peak area of α-BHC obtained from 1 mL of the standard solution (2) can be measured by the automatic integration method, and the peak height of α-BHC from 1 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of α-BHC beginning after the peak of solvent.

β-BHC (β-Hexachlorocyclohexane) C_{12}H_{13}Cl_{6} Melting point <2.60°: 308 – 310°C

Purity Related substances—Proceed as directed in the Purity under α-BHC using the following standard solution (1).

Standard solution (1): Pipet 2 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

γ-BHC (γ-Hexachlorocyclohexane) C_{12}H_{13}Cl_{6} Melting point <2.60°: 112 – 114°C

Purity Related substances—Proceed as directed in the Purity under α-BHC.

δ-BHC (δ-Hexachlorocyclohexane) C_{12}H_{13}Cl_{6} Melting point <2.60°: 137 – 140°C

Purity Related substances—Proceed as directed in the Purity under α-BHC using the following standard solution (1).

Standard solution (1): Pipet 5 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.
Bifonazole \( C_{22}H_{18}N_2 \) [Same as the namesake monograph]

Bile salts See Microbial Limit Test for Crude Drugs <5.02>.

**2-(4-Biphenylyl)propionic acid** \( C_{13}H_{14}O_2 \) Light yellowish white powder.

**Melting point** <2.60°: 145 – 148°C

**Purity**—Dissolve 1 mg of 2-(4-biphenylyl) propionic acid in a mixture of water and acetonitrile (11:9) to make 50 mL. Perform the test with 20 μL of this solution as directed under Liquid Chromatography <2.01> according to the operating conditions of the Related substances in the Purity (3) under Flurbiprofen. Determine each peak area of the solution in about twice as long as the retention time of the main peak by the automatic integration method, and calculate the amount of 2-(4-biphenylyl)propionic acid by the area percentage method: it is not less than 98.0%.

**Content:** not less than 98.0%. Assay—Weigh accurately about 0.5 g of 2-(4-biphenylyl)propionic acid, previously dried in vacuum over silica gel for 4 hours, and titrate <2.50° with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS is 22.63 mg of \( C_{13}H_{14}O_2 \).

**2,2'-Bipyridyl** \( C_{14}H_{8}N_2 \) [K 8486, Special class]

**Bis(cis-3,3,5-trimethylcyclohexyl) phthalate**

\( C_{16}H_{20}I_3N_3O_8 \) White crystalline powder. **Melting point** <2.60°: 91 – 94°C

**Bisdemethoxycurcumin** \( C_{10}H_{12}O_4 \) Yellow to orange crystalline powder. Sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

**Melting point:** 213 – 217°C.

**Identification**—Determine the absorption spectrum of a solution of bisdemethoxycurcumin in methanol (1 in 400000) as directed under Ultraviolet-visible Spectrophotometry <2.24°> it exhibits a maximum between 413 nm and 417 nm.

**Purity** Related substances—(1) Dissolve 4 mg of bisdemethoxycurcumin in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07°>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at RT value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of bisdemethoxycurcumin in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> under the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of bisdemethoxycurcumin obtained from the sample solution is not larger than the peak area of bisdemethoxycurcumin from the standard solution.

**Operating conditions**

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Turmeric.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of bisdemethoxycurcumin obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of bisdemethoxycurcumin from 10 μL of the standard solution.

**System performance and system repeatability:** Proceed as directed in the operating conditions in the Assay under Turmeric.

**4,4'-Bis(diethylamino)benzophenone**

\( C_{12}H_{12}NCO_2 \) Light yellow crystals.

**Content:** not less than 98%. Assay—Weigh accurately 0.25 g of 4,4'-bis(diethylamino)benzophenone, dissolve in 50 mL of acetic acid (100), and titrate <2.50° with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank titration in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS is 16.22 mg of \( C_{12}H_{12}NCO_2 \).

**N,N'-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide** \( C_{19}H_{16}O_4 \) White crystalline powder.

**Identification** (1) Heat 0.1 g of \( N,N'-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide \) over free flame: a purple colored gas evolves.

(2) Determine the infrared absorption spectrum of \( N,N'-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide \) over free flame: a purple colored gas evolves.

**Purity**—Dissolve 0.10 g of \( N,N'-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide \) in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total area of the peaks other than the peak of bisdemethoxycurcumin obtained from the sample solution is not larger than the peak area of bisdemethoxycurcumin from the standard solution.
peak area of N,N'-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetamino-2,4,6-triiodoisophthalamide obtained from the standard solution.

Operating conditions
Proceed the operating conditions in the Purity (6) under Iopamidol.

System suitability
Proceed the system suitability in the Purity (6) under Iopamidol.

Bismuth nitrate See bismuth nitrate pentahydrate.

Bismuth nitrate pentahydrate Bi(NO₃)₃.5H₂O [K 8566, Special class]

Bismuth nitrate-potassium iodide TS Dissolve 0.35 g of bismuth nitrate pentahydrate in 4 mL of acetic acid (100) and 16 mL of water (solution A). Dissolve 8 g of potassium iodide in 20 mL of water (solution B). To 20 mL of a mixture of solution A and solution B (1:1) add 80 mL of dilute sulfuric acid and 0.2 mL of hydrogen peroxide (30). Prepare before use.

Bismuth nitrate TS Dissolve 5.0 g of bismuth nitrate pentahydrate in acetic acid (100) to make 100 mL.

Bismuth potassium iodide TS Dissolve 10 g of l-tartaric acid in 40 mL of water, add 0.85 g of bismuth subnitrate, shake for 1 hour, add 20 mL of a solution of potassium iodide (2 in 5), shake thoroughly, allow to stand for 24 hours, and filter (solution A). Separately, dissolve 10 g of l-tartaric acid in 50 mL of water, add 5 mL of solution A, and preserve in a light-resistant, glass-stoppered bottle.

Bismuth sodium trioxide NaBiO₃ A yellow-brown powder.

Identification—(1) To 10 mg of bismuth sodium trioxide add 5 mL of a solution of manganese (II) nitrate hexahydrate (4 in 125) and 1 mL of diluted nitric acid (1 in 3), and shake vigorously for 10 seconds: a red-purple color is developed.

(2) Dissolve 10 mg of bismuth sodium trioxide in 2 mL of diluted hydrochloric acid (1 in 2): this solution responds to the Qualitative Tests <1.09> (1) for sodium salt.

Bismuth subnitrate [Same as the namesake monograph]

Bismuth subnitrate TS Dissolve 10 g of l-tartaric acid in 40 mL of water, add 0.85 g of bismuth subnitrate, shake for 1 hour, then add 20 mL of a solution of potassium iodide (2 in 5), and shake well. After standing for 24 hours, filter, and preserve the filtrate in a light-resistant bottle.

Bismuth subnitrate-potassium iodide TS for spraying, dilute Dissolve 10 g of l-tartaric acid in 50 mL of water, and add 5 mL of bismuth subnitrate TS.

Bismuth sulfite indicator Prepared for microbial test.

Bisoprolol fumarate for assay (C₁₇H₁₉NO₄)₂.C₄H₇O₄
[Same as the monograph Bisoprolol Fumarate. However, when dried, it contains not less than 99.0% of bisoprolol fumarate (C₁₇H₁₉NO₄)₂.C₄H₇O₄]. Also, when performing the Purity (2) under Bisoprolol Fumarate, the total area of the peaks other than bisoprolol is not larger than 1/5 times the peak area of bisoprolol from the standard solution.

Purify as follows if needed.

Purification method—Dissolve, with heating, 2 g of Bisoprolol Fumarate in 200 mL of ethyl acetate, add 0.5 g of activated carbon, shake well, and filter using a glass filter (G4). Place the filtrate in ice water for 2 hours while occasional shaking. Collect the crystals that precipitate out using a glass filter (G3). Dry the crystals obtained in vacuum at 80°C for 5 hours using phosphorus (V) oxide as a desiccant.

Bis-(1-phenyl-3-methyl-5-pyrazolone) C₁₇H₁₇B₂O₃
White to pale yellow crystals or crystalline powder. It dissolves in mineral acids and in alkali hydroxides, and it does not dissolve in water, in ammonia TS, or in organic solvents. Melting point: not below 300°C.

Nitrogen content <1.08>: 15.5 – 16.5%
Residue on ignition <2.44>: not more than 0.1%.

Bis(1,1-trifluoroacetoxy)iodobenzene C₁₇H₁₇F₂IO₄
Prepared for amino acid analysis or biochemistry.

Bis-trimethyl silyl acetamide CH₃CON[Si(CH₃)₃]₂
Colorless liquid.
Refractive index <2.45> nD20: 1.414 – 1.418
Specific gravity <2.56> d30: 0.825 – 0.835
Boiling point <2.57>: 71 – 73°C

Bitter orange peel [Same as the namesake monograph]

Block buffer solution Dissolve 4 g of blocking agent in 100 mL of water, and add 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS, pH 7.4.

Blocking agent Powder whose main ingredient is bovine-derived lactoprotein. For immunological research purposes.

Blood agar medium Sterilize 950 mL of heart infusion agar medium under increased pressure. Allow the media to cool to about 50°C, add 50 mL of horse or sheep defibrinated blood, dispense in sterilized Petro dishes, and make them as plate media.

1% blood suspension Wash a defibrinated animal blood in isotonic solution, and make it into suspension to contain 1 vol%. Prepare before use.

Blue tetrazolium C₄₀H₃₂Cl₂N₈O₂ 3,3’-(3,5-diphenyl) tetrazolium chloride] Light yellow crystals. Freely soluble in methanol, in ethanol (95) and in chloroform, slightly soluble in water, and practically insoluble in acetone and in ether. Melting point: about 245°C (with decomposition).

Absorbance <2.24> E₁%cm (252 nm): not less than 826 (methanol).

Blue tetrazolium TS, alkaline To 1 volume of a solution of blue tetrazolium in methanol (1 in 200) add 3 volumes of a solution of sodium hydroxide in methanol (3 in 25). Prepare before use.

Borane-pyridine complex C₅H₈BN
Content: not less than 80%. Assay—Accurately weigh about 30 mg of borane-pyridine complex, dissolve in 40 mL of 0.05 mol/L iodide solution, add 10 mL of diluted sulfuric acid (1 in 6), and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS = 1.549 mg of C₅H₈BN

Borate-hydrochloric acid buffer solution, pH 9.0 Dis-
solve 19.0 g of sodium borate in 900 mL of water, adjust the pH to exactly 9.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

Borax See sodium tetraborate decahydrate.

Boric acid $\text{H}_3\text{BO}_3$ [K 8863, Special class]

Boric acid-magnesium chloride buffer solution, pH 9.0
Dissolve 3.1 g of boric acid in 210 mL of dilute sodium hydroxide, and add 10 mL of magnesium chloride hexahydrate (1 in 50) and water to make 1000 mL. Adjust the pH to 9.0, if necessary.

Boric acid-methanol buffer solution Weigh exactly 2.1 g of boric acid, dissolve in 28 mL of sodium hydroxide TS, and add 36 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0
To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 21.30 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.2
To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 26.70 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6
To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 36.85 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0
To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 43.90 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.2
To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 50 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.4
Dissolve 12.376 g of boric acid and 24.736 g of boric acid in 0.1 mol/L sodium hydroxide TS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.6
Dissolve 19.0 g of sodium borate in 900 mL of water, adjust the pH to exactly 9.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

Borate

Bradykinin $\text{C}_8\text{H}_{17}\text{N}_2\text{O}_{11}$ A white powder. Freely soluble in water and in acetic acid (31), and practically insoluble in diethyl ether. Optical rotation $<2.49\text{ }^\circ$ $[\alpha]_D^20: -80$ to $-90^\circ$ (15 mg, water, 5 mL, 100 mm).
**Purity** Related substances—Dissolve 2.0 mg of bradykinin in 0.2 mL of water, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography 〈2.02〉. Prepare 5 μL of the sample solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (31:15:12:10:3) to a distance of about 10 cm, and dry the plate at 60°C. Spray evenly a solution of ninhydrin in 1-butanol (1 in 1000) on the plate, and heat at 60°C for 30 to 60 minutes: any spot other than the principal spot arisen from bradykinin does not appear.

**Brilliant green** C₂₇H₃₄N₂O₄S Fine, glistening, yellow crystals. It dissolves in water and in ethanol (95). The wavelength of absorption maximum: 623 nm.

**Bromine** Br [K 8529, Special class]

**Bromine-acetic acid TS** Dissolve 10 g of sodium acetate trihydrate in acetic acid (100) to make 100 mL, add 5 mL of bromine, and shake. Preserve in light-resistant containers, preferably in a cold place.

**Bromine-carbon tetrachloride TS** To 0.1 g of bromine add carbon tetrachloride to make 100 mL, and dilute a 2 mL portion of this solution with carbon tetrachloride to make 100 mL. Prepare before use.

**Bromine-cyclohexane TS** Dissolve 0.1 g of bromine in cyclohexane to make 100 mL. To 2 mL of this solution add cyclohexane to make 10 mL. Prepare before use.

**Bromine-sodium hydroxide TS** To 100 mL of a solution of sodium hydroxide (3 in 100) add 0.2 mL of bromine. Prepare before use.

**Bromine TS** Prepare by saturating water with bromine as follows: Transfer 2 to 3 mL of bromine to a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum, add 100 mL of cold water, insert the stopper, and shake. Preserve in light-resistant containers, preferably in a cold place.

**Bromocresol green** C₂₁H₁₄Br₄O₅S [K 8840, Special class]

**Bromocresol green-sodium hydroxide TS** Triturate 0.2 g of bromocresol green with 2.8 mL of 0.1 mol/L sodium hydroxide VS in a mortar, add water to make 200 mL, and filter if necessary.

**Bromocresol green TS** Dissolve 50 mg of bromocresol green in 100 mL of ethanol (95), and filter if necessary.

**Bromocresol purple** C₁₂H₁₆Br₂O₅S [K 8841, Special class]

**Bromocresol purple-dipotassium hydrogenphosphatocitric acid TS** Mix 30 mL of bromocresol purple-sodium hydroxide TS and 30 mL of dibasic potassium phosphate-citric acid buffer solution, pH 5.3, and wash with three 60-mL portions of chloroform.

**Bromocresol purple-sodium hydroxide TS** Triturate 0.4 g of bromocresol purple with 6.3 mL of dilute sodium hydroxide TS in a mortar, add water to make 250 mL, and filter if necessary.

**Bromocresol purple TS** Dissolve 0.05 g of bromocresol purple in 100 mL of ethanol (95), and filter if necessary.

**Bromophenol blue** C₁₉H₁₀Br₄O₅S [K 8844, Special class]

**Bromophenol blue-potassium biphthalate TS** Dissolve 0.1 g of bromophenol blue in potassium biphthalate buffer solution, pH 4.6, to make 100 mL.

**Bromophenol blue TS** Dissolve 0.1 g of bromophenol blue in 100 mL of dilute ethanol, and filter if necessary.

**0.05% Bromophenol blue TS** Dissolve 0.01 g of bromophenol blue in water to make 20 mL.

**Bromophenol blue TS, dilute** Dissolve 0.05 g of bromophenol blue in 100 mL of ethanol (99.5). Prepare before use.

**Bromophenol blue TS, pH 7.0** Mix 10 mL of bromophenol blue TS and 10 mL of ethanol (95), and adjust the pH to 7.0 with dilute sodium hydroxide TS.

**N-Bromosuccinimide** C₇H₇BrNO₂ [K 9553, Special class]

**N-Bromosuccinimide TS** Dissolve 1 g of N-bromosuccinimide in 1000 mL of water.

**Bromothymol blue** C₂₂H₂₃Br₂O₂S [K 8842, Special class]

**Bromothymol blue-sodium hydroxide TS** To 0.2 g of powdered bromothymol blue add 5 mL of dilute sodium hydroxide TS and a small quantity of water, dissolve by shaking in a water bath at 50°C, then add water to make 100 mL.

**Bromothymol blue-sodium hydroxide-ethanol TS** Dissolve 50 mg of bromothymol blue in 4 mL of diluted 0.2 mol/L sodium hydroxide TS (1 in 10) and 20 mL of ethanol (99.5), and add water to make 100 mL.

**Bromothymol blue TS** Dissolve 0.1 g of bromothymol blue in 100 mL of dilute ethanol, and filter if necessary.

**Bromovalerylurea** C₈H₁₁BrN₂O₂ [Same as the name-sake monograph]

**Brucine** See brucine n-hydrate.

**Brucine dihydrate** See brucine n-hydrate.
Brucine n-hydrate \( \text{C}_2\text{H}_{10}\text{N}_4\text{O}_2\cdot n\text{H}_2\text{O} \) [K 8832, Special class]

B-type erythrocyte suspension Prepare a suspension containing 1 vol% of erythrocyte separated from human B-type blood in isotonic sodium chloride solution.

Bucillamine \( \text{C}_7\text{H}_{12}\text{NO}_3\text{S}_2 \) [Same as the namesake monograph]

Bucillamine for assay \( \text{C}_7\text{H}_{12}\text{NO}_3\text{S}_2 \) [Same as the monograph Bucillamine. However, when dried, it contains not less than 99.0% of bucillamine \( (\text{C}_7\text{H}_{12}\text{NO}_3\text{S}_2) \). Furthermore, it conforms to the following test.]

Purity Related substances—Dissolve 60 mg of bucillamine for assay in 20 mL of a mixture of water and methanol (1:1) and use this solution as the sample solution. Pipet 1 mL of this solution, add the mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. When the test is performed according to the Purity (3) under Bucillamine, the total area of the peaks other than the bucillamine peak from the sample solution is not larger than the peak area of bucillamine from the standard solution.

Bufalin for assay \( \text{C}_2\text{H}_3\text{O}_6\cdot x\text{H}_2\text{O} \) White, odorless, crystalline powder.

Absorbance \(<2.24\times 10^{-1}\%\) (300 nm): 143 – 153 (10 mg, methanol, 250 mL). Use the sample dried in a desiccator (silica gel) for 24 hours for the test.

Purity Related substances—Dissolve 40 mg of bufalin for assay in 5 mL of chloroform and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\times 10^{-1}\%\). Spot 5 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and chloroform (4:3:3) to a distance of about 14 cm, and air-dry. Spray evenly sulfuric acid, and heat at 100°C for 2 to 3 minutes: any spot other than the principal spot obtained from the sample solution is not larger and not more intense than the peak area from the standard solution.

Content: not less than 99.0%. Assay—Weigh accurately about 10 mg of bufalin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20 \( \mu L \) of the sample solution as directed under Liquid Chromatography \(<2.07\times 10^{-1}\%\) according to the following conditions. Measure the peak area by the automatic integration method, and calculate the amount of bufalin by the area percentage method.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 300 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of bufalin is about 6 minutes.

Selection of column: Dissolve 0.01 g each of bufalin for assay, cinobufagin for assay and resibufogenin for assay in methanol to make 200 mL. Proceed with 20 \( \mu L \) of this solution according to the above conditions. Use a column giving elution of bufalin, cinobufagin and resibufogenin in this order and completely resolving these peaks.

Detection sensitivity: Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of bufalin obtained from 20 \( \mu L \) of the standard solution (2) can be measured by the automatic integration method, and the peak height of bufalin from 20 \( \mu L \) of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of bufalin beginning after the solvent peak.

Bufalin for component determination See bufalin for assay.

Buffer solution for celmoleukin Combine 12.5 mL of 0.5 mol/L tris buffer solution, pH 6.8, 10 mL of sodium lauryl sulfate solution (1→10), 10 mL of glycerin, and 17.5 mL of water, shake, and then add and dissolve 5 mg of bromophenol blue.

Storage—Store in a cool place, shielded from light.

Buformin hydrochloride for assay \( \text{C}_8\text{H}_{12}\text{N}_2\text{HCl} \) [Same as the monograph Buformin Hydrochloride. When dried, it contains not less than 99.5% of buformin hydrochloride (\( \text{C}_8\text{H}_{12}\text{N}_2\text{HCl} \)).]

n-Butanol See 1-butanol.

sec-Butanol See 2-butanol.

1-Butanol \( \text{CH}_3(\text{CH}_2)_2\text{CH}_2\text{OH} \) [K 8810, Special class]

2-Butanol \( \text{CH}_3\text{CH}_2\text{CH}(\text{OH})\text{CH}_3 \) [K 8812, Special class]

2-Butanone \( \text{CH}_3\text{COC}_2\text{H}_3 \) [K 8900, Special class]

Butenafine hydrochloride for assay \( \text{C}_2\text{H}_3\text{N}_2\text{HCl} \) [Same as the monograph Butenafine Hydrochloride]

\( \text{N}-\text{t}-\text{Butoxytocarboxyl}-1\text{-glutamic acid-}\alpha\text{-phenyl ester} \) \( \text{C}_{16}\text{H}_{29}\text{NO}_4 \) White powder.

Melting point \(<2.60\times 95-104^\circ\text{C}\)

Purity Related substances—Dissolve 10 mg of \( \text{N}-\text{t}-\text{butoxytocarboxyl}-1\text{-glutamic acid-}\alpha\text{-phenyl ester} \) in 5 mL of dilute ethanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.05\times\) according to the following conditions. Measure the peak area by the automatic integration method, and calculate the amount of butenafine hydrochloride on the standard solution on three plates of silica gel with fluorescent indicator for thin-layer chromatography. Develop the first plate with a mixture of chloroform, ethyl acetate and acetic acid (100) (25:25:1), the second plate with a mixture of benzene, 1,4-dioxane and acetic acid (100) (95:25:4), and the third plate with a mixture of chloroform, methanol and acetic acid (100) (45:4:1) to a distance of about 12 cm, and air-dry these plates. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard so-
lution in all plates.

**n-Butyl acetate** CH₃COOCH₂CH₂CH₃CH₃ [K 8377, Special class]

**t-Butyl alcohol** (CH₃)₃COH A crystalline solid, having a characteristic odor. A colorless liquid at above an ordinary temperature.

Specific gravity <2.56> d₂₀°: about 0.78; Boiling point: about 83°C; Melting point: about 25°C.

Identification—Determine the infrared absorption spectrum as directed in the liquid film method as directed under Infrared Spectrophotometry <2.25> it exhibits absorption at the wave numbers of about 3370 cm⁻¹, 2970 cm⁻¹, 1471 cm⁻¹, 1202 cm⁻¹, 1022 cm⁻¹, 913 cm⁻¹ and 749 cm⁻¹.

**n-Butylamine** CH₃CH₂CH₂CH₂NH₂ A colorless liquid, having an amine-like, characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether. The solution in water shows alkalinity and rapidly absorbs carbon dioxide from the air.

Specific gravity <2.56> d₂₀°; 0.740 - 0.747
Distilling range <2.57>: 76.5 - 79°C, not less than 96 vol.%

**Butyl benzoate** C₆H₅COOCH₂CH₂CH₃CH₃ A clear and colorless liquid.

Refractive index <2.45> nD₂₀: 1.495 - 1.500
Specific gravity <2.56> d₁₅°: 1.066 - 1.013

**n-Butyboronic acid** CH₃H₂BO₂ White flakes.
Melting point <2.60>: 90 - 92°C

**n-Butyl chloride** See 1-chlorobutane.

**n-Butyl formate** HCOO(CH₂)₃CH₃ Clear and colorless liquid, having a characteristic odor.
Specific gravity <2.56> d₂₀°: 0.884 - 0.904

**tert-Butyl methyl ether** (CH₃)₂COCH₃ Clear colorless liquid, having a specific odor.
Refractive index <2.45> nD₂₀: 1.3689
Specific gravity <2.56> d₁₅°: 0.7404

**Butyl parahydroxybenzoate** HOCH₃COOCH₂CH₂CH₂CH₃ [Same as the namesake monograph]

**Butyrolactone** C₄H₇O₂ Clear, colorless to practically colorless liquid.
Specific gravity <2.56> d₁₅°: 1.128 - 1.135
Boiling point <2.57>: 198 - 208°C

**Cadmium acetate** See cadmium acetate dihydrate.

**Cadmium acetate dihydrate** Cd(CH₃COO)₂·2H₂O White crystals or crystalline powder.

Identification—(1) Dissolve 0.2 g of cadmium acetate dihydrate in 20 mL of water, and use this as the sample solution. To 10 mL of the sample solution add 2 mL of iron (III) chloride TS: a red-brown color is produced.

(2) To 10 mL of the sample solution obtained in (1) add 1 mL of sodium sulfide TS: a yellow precipitate is produced.

**Cadmium ground metal** Cd [H 2113, First class]

**Cadmium-ninhydrin TS** Dissolve 0.05 g of cadmium acetate dihydrate in 5 mL of water and 1 mL of acetic acid (100), add 2-butanol to make 50 mL, and dissolve 0.1 g of ninhydrin in this solution. Prepare before use.

**Cadralazine for assay** C₁₂H₁₇N₂O₃ [Same as the monograph Cadralazine. When dried, it contains not less than 99.0% of cadralazine (C₁₂H₁₇N₂O₃).]

**Caffeine** See caffeine hydrate.

**Caffeine hydrate** C₈H₁₀N₄O₂·H₂O [Same as the namesake monograph]

**Caffeine, anhydrous** C₈H₁₀N₄O₂ [Same as the monograph Anhydrous Caffeine]

**Calcium acetate monohydrate** (CH₃COO)₂Ca·H₂O [K 8364, Special class]

**Calcium carbonate** CaCO₃ [K 8617, Special class]

**Calcium carbonate for assay** CaCO₃ [Same as the monograph Precipitated Calcium Carbonate. When dried, it contains not less than 99.0% of calcium carbonate (CaCO₃).]

**Calcium chloride** See calcium chloride dihydrate.

**Calcium chloride dihydrate** CaCl₂·2H₂O [K 8122, Special class]

**Calcium chloride for drying** CaCl₂ [K 8124, For drying]

**Calcium chloride for Karl Fischer method** CaCl₂ [K 8125, For water determination]

**Calcium chloride TS** Dissolve 7.5 g of calcium chloride dihydrate in water to make 100 mL (0.5 mol/L).

**Calcium gluconate for thin-layer chromatography** See calcium gluconate hydrate for thin-layer chromatography.

**Calcium gluconate hydrate for thin-layer chromatography** [Same as the monograph Calcium Gluconate Hydrate. When the test is performed as directed in the Identification (1) under Calcium Gluconate Hydrate, no spot other than the principal spot appears.]

**Calcium hydroxide** Ca(OH)₂ [K 8575, Special class]

**Calcium hydroxide for pH determination** Calcium hydroxide prepared for pH determination.

**Calcium hydroxide pH standard solution** See pH Determination <2.54>.

**Calcium hydroxide TS** To 3 g of calcium hydroxide add 1000 mL of cold distilled water, and occasionally shake the mixture vigorously for 1 hour. Allow to stand, and use the supernatant liquid (0.04 mol/L).

**Calcium nitrate** See calcium nitrate tetrahydrate.

**Calcium nitrate tetrahydrate** Ca(NO₃)₂·4H₂O [K 8549, Special class]

**Calcium oxide** CaO [K 8410, Special class]

**Calcium paraaminosalicylate hydrate for assay** C₇H₅CaNO₃·3½H₂O [Same as the monograph Calcium Paraaminosalicylate Hydrate. It contains not less than 99.0% of calcium paraaminosalicylate (C₇H₅CaNO₃), calculated on the anhydrous basis.]

**Camphor** C₈H₁₀O [Same as the monograph d-Camphor or dl-Camphor]
**d-Camphorsulfonic acid** $\text{C}_{10}\text{H}_{18}\text{O}_5\text{S}$ White crystals or crystalline powder, having a characteristic odor. Very soluble in water, and soluble in chloroform.

**Purity** Clarity and color of solution—Dissolve 1.0 g of d-camphorsulfonic acid in 10 mL of water: the solution is clear and colorless or pale yellow.

Loss on drying $<2.4\%$: not more than 2.0% (1 g, 105°C, 5 hours).

Content: not less than 99.0%, calculated on the dried basis. Assay—Weigh accurately about 4 g of d-camphorsulfonic acid, dissolve in 50 mL of water, and titrate $<2.50\%$ with 1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination in the same manner.

Each mL of 1 mol/L sodium hydroxide VS $= 232.3$ mg of $\text{C}_{10}\text{H}_{18}\text{O}_5\text{S}$

**Candesartan cilexetil** for assay $\text{C}_{33}\text{H}_{34}\text{N}_6\text{O}_6$ [Same as the monograph Candesartan Cilexetil. It contains not less than 99.5% of candesartan cilexetil ($\text{C}_{33}\text{H}_{34}\text{N}_6\text{O}_6$), calculated on the anhydrous basis, and when performed the test as directed in the Purity (2) under Candesartan Cilexetil, the total area of the peaks other than candesartan cilexetil obtained from the sample solution is not larger than 1/2 times the peak area of candesartan cilexetil from the standard solution.]

**Caprylic acid** $\text{CH}_{3}(\text{CH}_{2})_6\text{COOH}$ A clear and colorless oily liquid, having a slight unpleasant odor. Freely soluble in ethanol (95) and in chloroform, and very slightly soluble in water.

Refractive index $<2.45$ $n_\text{D}^\circ: 1.426 – 1.430$

Specific gravity $<2.56$ $d_\text{D}^\circ: 0.908 – 0.912$

Distilling range $<2.57$: 238 – 242°C, not less than 95 vol%.

**Capsaicin for assay** See (E)-capsaicin for assay.

(E)-**Capsaicin** for assay $\text{C}_{18}\text{H}_{27}\text{NO}_3$ Use (E)-capsaicin for thin-layer chromatography meeting the following additional specifications.

Absorbance $<2.48$: $E_{1\text{cm}}^{1\text{%
mL of this solution} is not larger than 1/2 times the peak area of capsaicin from the standard solution.

Operating conditions
Detector, column, column temperature, mobile phase, and flow rate: Proceed the operating conditions in the Assay under Capsicum.

Time span of measurement: About 3 times as long as the retention time of capsaicin beginning after the solvent peak.

System suitability
System performance, and system repeatability: Proceed the system suitability in the Assay under Capsicum.

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of capsaicin from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of capsaicin from 20 μL of the standard solution.

(E)-**Capsaicin for component determination** See (E)-capsaicin for assay.

**Capsaicin for thin-layer chromatography** See (E)-capsaicin for thin-layer chromatography.

(E)-**Capsaicin for thin-layer chromatography** $\text{C}_{18}\text{H}_{27}\text{NO}_3$ White crystals, having a strong irritative odor. Very soluble in methanol, freely soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Melting point $<2.60\%$: 65 – 70°C

Purity Related substances—Dissolve 20 mg of (E)-capsaicin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed in the Identification under Capsicum: any spot other than the principal spot (Rf value is about 0.5) from the sample solution is not more intense than the spot from the standard solution.

**Carbazochrome** $\text{C}_{10}\text{H}_{12}\text{N}_4\text{O}_3$ Yellow-red to red crystals or crystalline powder. Melting point: about 222°C (with decomposition).

Content: not less than 98.0%. Assay—Dissolve about 0.2 g of carbazochrome, previously weighed accurately, in 20 mL of acetic acid (100) by heating, add 80 mL of acetic anhydride, cool, and titrate $<2.50\%$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS $= 23.62$ mg of $\text{C}_{10}\text{H}_{12}\text{N}_4\text{O}_3$

**Carbazochrome sodium sulfonate for component determination** See carbazochrome sodium sulfonate trihydrate.

**Carbazochrome sodium sulfonate trihydrate** $\text{C}_{10}\text{H}_{14}\text{Na}_2\text{O}_5\text{S}_3\text{H}_2\text{O}$ [Same as the monograph Carbazochrome Sodium Sulfonate Hydrate. It contains not less than 99.0% of carbazochrome sodium sulfonate ($\text{C}_{10}\text{H}_{14}\text{Na}_2\text{O}_5\text{S}_3$), calculated on the anhydrous basis, and meets the following additional requirement.]

Water $<2.48\%$: 14.0 – 15.0%

**Carbazole** $\text{C}_{12}\text{H}_8\text{N}$ White to nearly white floccose or plate-like crystals or crystalline powder. Freely soluble in pyridine and in acetone, slightly soluble in ethanol (99.5), and practically insoluble in water. It readily sublimes when heated.

Melting point $<2.60\%$: 243 – 245°C

Purity Clarity and color of solution—To 0.5 g of carbazole add 20 mL of ethanol (99.5), and dissolve by warming: the solution is clear.

Residue on ignition: Not more than 0.1% (1 g).

**Carbazole TS** Dissolve 0.125 g of carbazole in ethanol (99.5) to make 100 mL.

0.1 mol/L Carbonate buffer solution, pH 9.6 Dissolve
3.18 g of anhydrous sodium carbonate and 5.88 g of sodium hydrogen carbonate in water to make 1000 mL.

Carbon dioxide CO₂ [Same as the namesake monograph]

Carbon disulfide CS₂ [K 8732, Special class]
Preserve in tightly stoppered containers in a dark, cold place, remote from fire.

Carbonic anhydrase White powder. Derived from bovine RBC. Molecular weight about 29,000.

Carbon monoxide CO A toxic, colorless gas. Prepare by passing the gas generated by reacting formic acid with sulfuric acid through a layer of sodium hydroxide TS. Carbon monoxide from a metal cylinder may be used.

Carbon tetrachloride CCl₄ [K 8459, Special class]

Carvedilol for assay C₁₈H₂₆N₂O₄ [Same as the monograph Carvedilol]

Casein, milk A white to light yellow powder or grain.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1650 cm⁻¹, 1540 cm⁻¹ and 1250 cm⁻¹.

Casein (milk origin) See casein, milk.

Casein peptone See peptone, casein.

Castor oil [Same as the namesake monograph]

Catechol C₆H₄(OH)₂ White crystals.

Melting point <2.60°: 104 – 107°C.

Preserve in a light-resistant tight container.

Cefadroxil C₁₆H₁₇N₃O₅S [Same as the namesake monograph]

Cefadroxil propylene glycolate

Cefadroxil for assay C₁₆H₁₇N₃O₅S [Same as the namesake monograph]

Cefepine pivoxil hydrochloride

Cefepine pivoxil hydrochloride hydrate

Cefepine pivoxil hydrochloride hydrate

Cefdinir lactam ring-cleavage lactones

Cefdinir lactam ring-cleavage lactones

Cetiostatin sodium C₁₄H₁₅N₅O₆S₂ A white to yellow powder. A mixture of diastereoisomers.

Identification—Determine the infrared absorption spectrum of cefdinir lactam ring-cleavage lactones as directed in the Paste method under Infrared Spectrophotometry of cefdinir lactam ring-cleavage lactones as directed in the Paste method under Infrared Spectrophotometry.

Content: not less than 95%. Assay—Dissolve about 5 mg of cefdinir lactam ring-cleavage lactones in 5 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Perform the test with 5 µL of the sample solution as directed in the operating conditions of the Purity (2) Related substances under Cefdinir, and calculate the areas of each peak by the automatic integration method. Determine the percent of the total peak area of 4 cefdinir lactam ring-cleavage lactones to the total area of all peaks.

Cell suspension solution for teclemoleukin Centrifuge for 5 minutes at 1000 r.p.m culture medium of NK-7 cells that have been cultured statically for 2 to 4 hours. Remove the supernatant by aspiration, and add culture medium for assay of teclemoleukin to a cell concentration of 2 to 4 x 10⁶ cells/mL.

Celmoleukin for liquid chromatography C_{693}H_{1118}N_{178}O_{203}S_{7} [Same as the monograph Celmoleukin (Genetical Recombination). However, contains 0.5 to 1.5 mg of protein per mL, polymers amount for 0.5% or less, and conforms to the following test].

Identification (1) When the amino acid sequence is investigated using the Edman technique and liquid chromatography, the amino acids are detected in the following sequence: alanine, proline, threonine, serine, serine, serine, threonine, lysine, lysine, threonine, glutamine, leucine, glutamine, leucine, and glutamic acid. Also, based on the results of the protein content determination test, place an amount of celmoleukin equivalent to about 0.3 mg in a hydrolysis tube, evaporate to dryness under vacuum, and then add 100 µL of hydrazine anhydride for amino acid sequence analysis. Reduce the internal pressure of the hydrolysis tube by heating for 6 hours at about 100°C. After evaporating to dryness under vacuum, add 250 µL of water to dissolve the residue. To this add 200 µL of benzaldehyde, shake occasionally, leave for one hour, centrifuge, and remove the aqueous layer. Add 250 µL of water to the benzaldehyde layer, shake, centrifuge, combine the aqueous layers, and evaporate to dryness under vacuum. Threonine is detected when amino acid analysis is conducted using the postcolumn technique with ninhydrin on a solution of the residue dissolved by adding 100 µL of 0.02 mol/L hydrochloric acid TS.

(2) Add 1 mL of protein digesting enzyme solution to 1 mL of celmoleukin, shake, and leave for 18 to 24 hours at 37°C. Pipet 1 mL of this solution and add 25 µL of trifluoroacetic acid (1 in 10). To another 1 mL, add 10 µL of 2-mercaptoethanol, leave for 30 minutes at 37°C, and then add 25 µL of trifluoroacetic acid (1 in 10). Perform Liquid Chromatography <2.07> on these two solutions separately under the conditions outlined in Celmoleukin (Genetical Recombination), Identification (4). Repeatedly pipet the celmoleukin derived peak fraction that elutes and when the test is performed according to Celmoleukin (Genetical Recombination), Identification (2), except for the lysines in positions 9 and 49 from the amino terminal amino acid, a peptide estimated from the complete primary structure is detected.

Cephaleine hydrobromate C_{29}H_{34}N_{2}O_{4}.2HBr.xH₂O A white or light-yellow crystalline powder.

Purity—Dissolve 10 mg in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed in the Assay under Ipecac: when measure the peak areas 2 times as long as the retention time of emetine, the total area of the peaks other than cephaleine is not larger than the peak area of cephaleine from the standard solution.

Ceric ammonium sulfate See cerium (IV) tetraammonium sulfate dihydrate.

Ceric ammonium sulfate-phosphoric acid TS See cerium
Powder. Very soluble in water, and freely soluble in ethanol.

Ceric ammonium sulfate TS See cerium (IV) tetraammonium sulfate TS.

Cerium (III) nitrate hexahydrate Ce(NO₃)₂.6H₂O A colorless or light yellow, crystalline powder. It dissolves in water.

Purity (1) Chloride <1.03%; not more than 0.036%.
(2) Sulfate <1.14%; not more than 0.120%.

Content: not less than 98.0%. Assay—To about 1.5 g of cerous nitrate, accurately weighed, add 5 mL of sulfuric acid, and heat until white fumes are evolved vigorously. After cooling, add 200 mL of water, 0.5 mL of 0.1 mol/L silver nitrate VS, dissolve 5 g of ammonium peroxodisulfate, dissolve, and boil it for 15 minutes. After cooling, add 2 drops of 1,10-phenanthroline TS, and titrate with 0.1 mol/L ferrous ammonium sulfate VS until the pale blue color of the solution changes to red.

Each mL of 0.1 mol/L ferrous ammonium sulfate VS = 43.42 mg of Ce(NO₃)₂.6H₂O

Cerium (III) nitrate TS Dissolve 0.44 g of cerium (III) nitrate hexahydrate in water to make 1000 mL.

Cerium (IV) diammonium nitrate Ce(NH₄)₂(NO₃)₆ [K 8556, Special class]

Cerium (IV) diammonium nitrate TS Dissolve 6.25 g of cerium (IV) diammonium nitrate in 160 mL of diluted dilute nitric acid (9 in 50). Use within 3 days.

Cerium (IV) sulfate tetrahydrate Ce(SO₄)₂·4H₂O [K 8976, Special class]

Cerium (IV) tetraammonium sulfate dihydrate Ce(SO₄)₂·2(NH₄)₂SO₄·2H₂O [K 8976, Special class]

Cerium (IV) tetraammonium sulfate-phosphoric acid TS Dissolve 0.1 g of cerium (IV) tetraammonium sulfate in diluted phosphoric acid (4 in 5) to make 100 mL.

Cerium (IV) tetrammonium sulfate-phosphoric acid TS Dissolve 0.44 g of cerium (IV) tetrammonium sulfate in diluted sulfuric acid (3 in 5) to make 100 mL.

Cerox nitrate See cerium (III) nitrate hexahydrate.

Cerox nitrate TS See cerium (III) nitrate TS.

Cesium chloride CsCl White crystals or crystalline powder. Very soluble in water, and freely soluble in ethanol (99.5).

Loss on drying <2.41%; Not more than 1.0% (1 g, 110°C, 2 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 0.5 g, previously dried, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution, add 30 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS = 16.84 mg of CsCl

Cesium chloride TS To about 0.5 g of cesium chloride add water to make 1000 mL.

Cetanol [Same as the namesake monograph]

Cetirizine hydrochloride for assay C₂₁H₂₂ClN₂O₃·2HCl [Same as the monograph Cetirizine Hydrochloride. When dried, it contains not less than 99.5% of cetirizine hydrochloride (C₁₂H₁₈ClN₂O₂·2HCl).

Cetirizine hydrochloride for assay C₂₁H₂₂ClN₂O₃·2HCl [Same as the monograph Cetirizine Hydrochloride. When dried, it contains not less than 99.5% of cetirizine hydrochloride (C₁₂H₁₈ClN₂O₂·2HCl).]
spot other than the principal spot at the Rf value of about 0.4 does not appear.

**Chlomotropic acid** See disodium chlomotropate dihydrate.

**Chlomotropic acid TS** Dissolve 0.05 g of disodium chlomotropate dihydrate in the solution prepared by cautiously adding 68 mL of sulfuric acid to 30 mL of water, cooling, then adding water to make 100 mL. Preserve in light-resistant containers.

**Chlomotropic acid TS, concentrated** Suspend 0.5 g of disodium chlomotropate dihydrate in 50 mL of sulfuric acid, centrifuge, and use the supernatant liquid. Prepare before use.

**Chloral hydrate** CCl₃CH(OH)₂ [Same as the namesake monograph]

**Chloral hydrate TS** Dissolve 5 g of chloral hydrate in 3 mL of water.

**Chloramine** See sodium toluensulfonchloramide trihydrate.

**Chloramphenicol** C₁₆H₁₄ClN₃O₂ [Same as the monograph Chloramphenicol]

**Chlorauric acid** See hydrogen tetrachloroaurate (III) tetrahydrate.

**Chlorauric acid TS** See hydrogen tetrachloroaurate (III) tetrahydrate TS.

**Chlordiazepoxide** C₁₆H₁₄ClN₃O₂ [Same as the namesake monograph]

**Chlordiazepoxide for assay** C₁₆H₁₄ClN₃O₂ [Same as the monograph Chlordiazepoxide. When dried, it contains not less than 99.0% of C₁₆H₁₄ClN₃O₂].

**Chlorhexidine hydrochloride** C₂₂H₃₀Cl₂N₁₀.2HCl [Same as the namesake monograph Chlorhexidine hydrochloride]

**Chlorinated lime** [Same as the namesake monograph]

**Chlorinated lime TS** Triturate 1 g of chlorinated lime with 9 mL of water, and filter. Prepare before use.

**Chlorine** Cl₂ A yellow-green gas, having a suffocating odor. It is heavier than air, and dissolves in water. Prepare from chlorinated lime with hydrochloric acid. Chlorine from a metal cylinder may be used.

**Chlorine TS** Use a saturated solution of chlorine in water. Preserve this solution in fully filled, light-resistant, glass-stoppered bottles, preferably in a cold place.

**Chloroacetic acid** C₂H₃ClO₂ [K 8899, Special class]

**p-Chloroaniline** See 4-chloroaniline

**4-Chloroaniline** H₂NC₆H₄Cl White crystals or crystalline powder. Freely soluble in ethanol (95) and in acetone, and soluble in hot water.

**Melting point** <2.60°: 70 – 72°C

**Residue on ignition** <2.44°: not more than 0.1% (1 g).

**4-Chlorobenzenediazonium TS** Dissolve 0.5 g of 4-chloroaniline in 1.5 mL of hydrochloric acid, and add water to make 100 mL. To 10 mL of this solution add 10 mL of sodium nitrite TS and 5 mL of acetone. Prepare before use.

**p-Chlorobenzene sulfonamide** See 4-chlorobenzene sulfonamide.

**4-Chlorobenzene sulfonamide** ClC₆H₄SO₂NH₂ White to pale yellow, odorless, crystalline powder. Dissolves in acetone.

**Purity** Related substances—Dissolve 0.60 g of 4-chlorobenzene sulfonamide in acetone to make exactly 300 mL, and perform the test with 5 μL of this solution as directed in the Purity (5) under Chlorproamidine: any spot other than the principal spot at the Rf value of about 0.5 does not appear.

**p-Chlorobenzoic acid** See 4-chlorobenzoic acid.

**4-Chlorobenzoic acid** C₆H₄COOH White crystals or powder. Sparingly soluble in ethanol (95), slightly soluble in chloroform, and practically insoluble in water.

**Melting point** <2.60°: 238 – 242°C

**Content** not less than 99.0%. Assay—Weigh accurately about 0.3 g of 4-chlorobenzoic acid, dissolve in 30 mL of neutralized ethanol, and titrate <2.50° with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Each mL of 0.1 mol/L sodium hydroxide VS

\[= 15.66 \text{ mg of C}_6\text{H}_4\text{ClO}_2\]

**1-Chlorobutane** CH₃(CH₂)₂Cl Clear and colorless liquid, miscible with ethanol (95) and with diethyl ether, practically insoluble in water.

**Refractive index** <2.45° n₂₀°: 1.401 – 1.045

**Specific gravity** <2.56° d₂₀°: 0.884 – 0.890

**Boiling point** <2.57°: about 78°C

**Chlorobutanol** C₆H₅ClO [Same as the namesake monograph]

**1-Chloro-2,4-dinitrobenzene** C₆H₃(NO₂)₂Cl Light yellow crystals or crystalline powder.

**Melting point** <2.60°: 50 – 54°C.

Preserve in a light-resistant tight container.

**3′-Chloro-3′-deoxythymidine for liquid chromatography** C₁₀H₁₃N₃O₄Cl Occurs as a white powder.

**Purity**—Dissolve 10 mg of 3′-chloro-3′-deoxythymidine for liquid chromatography in the mobile phase to make 100 mL. Perform the test with 10 μL of this solution as directed in the Purity (3) under Zidovudine: a peak is not observed at the retention time for zidovudine.

**(2-Chloroethyl) diethylamine hydrochloride** C₆H₁₄ClN.HCl White powder.

**Content** not less than 95.0%. Assay—Weigh accurately about 0.2 g of (2-chloroethyl)diethylamine hydrochloride, previously dried at 45°C for 3 hours under reduced pressure, and dissolve in 15 mL of acetic acid (100). To this solution add 10 mL of a mixture of acetic acid (100) and mercury (II) acetate TS for nonaqueous titration (5:3), and titrate <2.50° with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

\[= 17.21 \text{ mg of C}_6\text{H}_4\text{ClN.HCl}\]
Chloroform \( \text{CHCl}_3 \) [K 8322, Special class]

Chloroform, ethanol-free Mix 20 mL of chloroform with 20 mL of water, gently shake for 3 minutes, separate the chloroform layer, wash the layer again with two 20-mL portions of water, and filter it through dry filter paper. To the filtrate add 5 g of anhydrous sodium sulfate, shake for 5 minutes, allow the mixture to stand for 2 hours, and filter through dry filter paper. Prepare before use.

Chloroform for Karl Fischer method See Water Determination <2,48>.

Chlorogenic acid for thin-layer chromatography See (E)-chlorogenic acid for thin-layer chromatography.

(E)-Chlorogenic acid for thin-layer chromatography \( \text{C}_{16}\text{H}_{18}\text{O}_9 \) A white powder. Freely soluble in methanol and in ethanol (99.5), and sparingly soluble in water. Melting point: about 205°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of (E)-chlorogenic acid for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2,67>. Spot 10 \( \mu \)L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): no spot other than the principal spot at around \( R_f \) 0.5 appears.

\( p \)-Chlorophenol See 4-Chlorophenol.

4-Chlorophenol \( \text{ClC}_6\text{H}_4\text{OH} \) Colorless or pale red crystals or crystalline mass, having a characteristic odor. Very soluble in ethanol (95%), in chloroform, in diethyl ether and in glycerin, and sparingly soluble in water. Melting point: about 43°C.

Content: not less than 99.0%. Assay—Weigh accurately about 0.2 g of 4-chlorophenol, and dissolve in water to make 100 mL. Measure exactly 25 mL of this solution into an iodine flask, add exactly 20 mL of 0.05 mol/L bromine VS and then 5 mL of hydrochloric acid, stopper immediately, shake occasionally for 30 minutes, and allow to stand for 15 minutes. Add 5 mL of a solution of potassium iodide (1 in 5), stopper immediately, shake well, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS = 3.214 mg of \( \text{C}_6\text{H}_5\text{ClO} \)

Preserve in tight, light-resistant containers.

(2-Chlorophenyl)-diphenylmethanol for thin-layer chromatography \( \text{C}_{24}\text{H}_{40}\text{O}_5 \) To 5 g of clotrimazole add 300 mg of 4-chlorophenol, and dissolve in water to make exactly 250 mL. Proceed with 10 \( \mu \)L of this solution as directed in the Purity (7) under Clotrimazole: any spot other than the principal spot does not appear.

Chloroplatinic acid See hydrogen hexachloroplatinate (IV) hexahydrate.

Chloroplatinic acid-potassium iodide TS See hydrogen hexachloroplatinate (IV)-potassium iodide TS.

Chloroplatinic acid TS See hydrogen hexachloroplatinate (IV) TS.

Chlorophenesin carbamate for assay \( \text{C}_{16}\text{H}_{19}\text{ClN}_2\cdot\text{C}_2\text{H}_4\text{O}_4 \) [Same as the monograph Chlorphenesin Carbamate. When dried, it contains not less than 99.0% of chlorphenesin carbamate (\( \text{C}_{16}\text{H}_{17}\text{ClN}_2\text{O}_4\)).]

Chlorpheniramine maleate \( \text{C}_{16}\text{H}_{19}\text{ClN}_2\cdot\text{C}_2\text{H}_4\text{O}_4 \) [Same as the namesake monograph]

Chlorpromazine hydrochloride for assay \( \text{C}_{17}\text{H}_{14}\text{ClN}_2\text{S} \cdot \text{HCl} \) [Same as the monograph Chlorpromazine Hydrochloride]

Chlorpropamide for assay \( \text{C}_{16}\text{H}_{17}\text{ClN}_2\text{O}_4 \) [Same as the monograph Chlorpropamide. When dried, it contains not less than 99.0% of chlorpropamide (\( \text{C}_{16}\text{H}_{17}\text{ClN}_2\text{O}_4\)).]

Cholesterol \( \text{C}_{19}\text{H}_{36}\text{OH} \) [Same as the namesake monograph]

Cholesterol benzoate \( \text{C}_{15}\text{H}_{20}\text{O}_2 \) White crystalline powder.

Melting point <2.60>: 145 – 152°C

Cholic acid for thin-layer chromatography \( \text{C}_{28}\text{H}_{40}\text{O}_6 \) White, crystals or crystalline powder. Soluble in acetic acid (100), sparingly soluble in acetone and in ethanol (95), and very slightly soluble in water. Melting point: about 198°C.

Purity Related substances—Dissolve 25 mg in acetone to make exactly 250 mL. Proceed with 10 \( \mu \)L of this solution as directed in the Purity (7) under Ursodeoxycholic Acid: any spot other than the principal spot, having \( R_f \) about 0.1, does not appear.

Content: not less than 98.0%. Assay—Weigh accurately about 0.5 g, previously dried at 80°C for 4 hours (in vacuum, phosphorous (V) oxide), dissolve in 40 mL of neutralized ethanol and 20 mL of water, add 2 drops of phenolphthalein TS, and titrate with 0.1 mol/L sodium hydroxide VS until immediately before the end-point has been reached. Then add 100 mL of freshly boiled and cooled water, and continue the titration <2.50>. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 40.86 mg of \( \text{C}_{19}\text{H}_{36}\text{O}_6 \)

Choline chloride \( \left(\text{CH}_3\right)_3\text{NCH}_2\text{CH}_2\text{OH}\)Cl White crystalline powder.

Melting point <2.60>: 303 – 305°C (decomposition).

Water <2.48>: less than 0.1%.
Chromic acid-sulfuric acid TS
Saturate chromium (VI) trioxide in sulfuric acid.

Chromium trioxide See chromium (VI) trioxide.

Chromium trioxide TS See chromium (VI) trioxide TS.

Chromium (VI) trioxide CrO₃
A dark red-purple thin needle-shaped or inner prism-like crystals, or light masses.

Identification—To 5 mL of a solution (1 in 50) add 0.2 mL of lead (II) acetate TS: yellow precipitates appear which does not dissolve on the addition of acetic acid.

Chromium (VI) trioxide TS
Dissolve 3 g of chromium (VI) trioxide in water to make 100 mL.

Chromogenic synthetic substrate
Equal amount mixture of N-benzoyl-L-isoleucyl-L-glutamyl-glycyl-L-arginyl-p-nitroanilid hydrochloride and N-benzoyl-L-isoleucyl-p-methoxy glutamyl-glycyl-L-arginyl-p-nitroanilid hydrochloride. White or pale yellow masses or powder. It is slightly soluble in water.

Identification—Perform the test with the solution (1 in 30,000) as directed under Untraviolet-visible Spectrophotometry 2.24: the absorption maximum at about 316 nm is observed.

Purity
Free 4-nitroaniline: not more than 0.5%.

Loss on drying
<2.40: not more than 5% (0.2 g, reduced pressure (0.3 kPa), calcium chloride, between 30 and 40°C, 18 hours).

Content:
not less than 95% and not more than 105% of the label.

Chromophore TS for teceleukin
Mix 0.1 mL of diluted hydrogen peroxide (30) (1 in 20) with 10 mL of 0.2 mol/L citric acid buffer, pH 3.8, containing 0.2 mmol/L 3,3'-5,5'-tetramethylbenzidine dihydrochloride dehydrate, and use immediately.

Cibenzoline succinate for assay C₁₈H₁₈N₂C₄H₆O₄
Same as the monograph Cibenzoline Succinate. When dried, it contains not less than 99.0% of cibenzoline succinate (C₁₈H₁₈N₂C₄H₆O₄) and meets the following requirements.

Purity
Related substances—Dissolve 0.10 g of cibenzoline succinate in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. To exactly 1 mL of this solution add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.01. Spot 10 μL of each sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (20:3:2) to a distance of about 10 cm, air-dry the plate, and dry at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution. On standing the plate for 30 minutes in the tank saturated with iodine vapor, the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

Cilastatin ammonium for assay C₁₆H₂₉N₃O₅S: 375.48
A white crystalline powder.

Water
<2.40: not more than 0.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition
<2.40: not more than 0.5% (1 g).

Purity
Related substances—Dissolve 40 mg of the substance to be examined in 25 mL of water, and use this as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the each peak area by the automatic integration method. Separately, perform the test with 20 μL of water in the same manner to correct any variance of the peak area caused the variation of the baseline: the total area of the peaks other than cilastatin is not larger than 1/6 times the peak area of cilastatin from the standard solution.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: A mixture of dilute phosphoric acid (1 in 1000) and acetonitrile (7:3).

Mobile phase B: Diluted phosphoric acid (1 in 1000).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>15 → 100</td>
<td>85 → 0</td>
</tr>
<tr>
<td>30 – 40</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 2.0 mL per minute.

Time span of measurement: 40 minutes.

System suitability
Test for required detectability: To exactly 1 mL of the standard solution add water to make exactly 30 mL. Confirm that the peak area of cilastatin obtained with 20 μL of this solution is equivalent to 2.3 to 4.5% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions: the retention time of cilastatin is about 20 minutes, and the number of theoretical plates and the symmetry factor of the peak of cilastatin are not less than 10,000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilastatin is not more than 3.0%.

Residual solvent—Weigh accurately about 1 g, dissolve in water to make exactly 100 mL, and use this as the sample solution. Separately, weigh accurately about 0.10 g of ethanol (99.5), add water to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under
Gas Chromatography 2.02 according to the following conditions. Determine the peak areas, $A_T$ and $A_S$, of ethanol by the automatic integration method, and calculate the amount of ethanol ($C_2H_5OH$): not more than 0.5%.

$$\text{Amount} \times 100 \times M_1 \times M_S = M_2$$

$M_5$: Amount (mg) of ethanol (99.5)
$M_1$: Amount (mg) of the sample

Operating conditions

**Detector:** A hydrogen flame-ionization detector.

**Column:** A fused silica column 0.5 mm in inside diameter and 30 m in length, coated the inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 5 μm.

**Column temperature:** Inject the sample at a constant temperature of about 50°C, keep on for 150 seconds, then raise to 70°C at the rate of 8°C per minute, and keep this for 30 seconds.

**Carrier gas:** Helium.

**Flow rate:** Adjust so that the retention time of ethanol is about 1 minute.

**Sprint ratio:** 5:1

**System suitability**

Test for required detectability: To exactly 1 mL of the standard solution add water to make exactly 10 mL, and designate this the solution for system suitability test. To exactly 1 mL of the solution for system suitability test add water to make exactly 10 mL. Confirm that the peak area of ethanol obtained with 1 μL of this solution is equivalent to 7 to 13% of that with 1 μL of the solution for system suitability test.

**System performance:** When the procedure is run with 1 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ethanol are not less than 1500 and not more than 1500 and not more than 3.0, respectively.

**System repeatability:** When determine the peak area of methanol by repeating 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area is not more than 2.0%.

**Content:** not less than 99.0% of cilastatin ammonium ($C_{16}H_{29}N_3O_5S$), calculated on the anhydrous basis and corrected on the amount of ethanol. Assay—Weigh accurately about 0.5 g, dissolve in 30 mL of methanol, and add 5 mL of water. Adjust to pH 3.0 with 0.1 mol/L hydrochloric acid TS, and titrate 2.50 with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS

\[ = 14.72 \text{ mg of } C_{16}H_{29}N_3O_5 \]

**Cinchonidine** $C_{19}H_{22}N_2O$ White crystals or crystalline powder. Soluble in ethanol (95), in methanol and in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water. A solution of cinchonidine in ethanol (95) (1 in 100) is levorotatory. Melting point: about 207°C

**Content:** not less than 98.0%. Assay—Weigh accurately about 0.3 g of cinchonidine, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate 2.50 with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS

\[ = 14.72 \text{ mg of } C_{19}H_{22}N_2O \]

**Cinchonine** $C_{19}H_{22}N_2O$ White crystals or powder.

**Identification:** Dissolve 1 g in 20 mL of dilute hydrochloric acid (1 in 4), and add 2 mL of potassium hexacyanoferrate (II) TS: yellow precipitates appear, which are dissolved by heating, and crystals are formed after allowing to cool.

**Purity** Cinchonidine and quinine—To 1 g add 30 mL of water, add dilute hydrochloric acid (2 in 3) dropwise until the substance to be tested dissolves, and neutralize with ammonia TS. To this solution add 10 mL of a solution of sodium tartrate dihydrate (1 in 2), boil, and allow to stand for 1 hour: no precipitates appear.

**Content:** not less than 98.0%. Assay—Weigh accurately about 0.3 g, dissolve in 50 mL of acetic acid (100), and titrate 2.50 with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

\[ = 14.72 \text{ mg of } C_{19}H_{22}N_2O \]

**Cineol for assay** $C_{10}H_{18}O$ Clear and colorless liquid, having a characteristic aroma.

**Refractive index** 2.457 – 2.459

**Specific gravity** 2.50

**Purity** (1) Related substances (i)—Dissolve 0.20 g of cineol for assay in 10 mL of hexane and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography 2.02. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (9:1) to a distance of about 10 cm, and air-dry. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS, and heat at 105°C for 5 minutes: any spot other than the principal spot does not appear.

(2) Related substances (ii)—Dissolve 0.10 g of cineol for assay in 25 mL of hexane and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography 2.02 according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of cineol by the area percentage method: it is not less than 99.0%.

**Operating conditions**

Proceed the operating conditions in the Assay under Eucalyptus Oil except detection sensitivity and time span of measurement.

**Detection sensitivity:** Measure 1 mL of the sample solution and add hexane to make 100 mL. Adjust the detection sen-
sitivity so that the peak height of cineol obtained from 2 μL of this solution is 40% to 60% of the full scale.

Time span of measurement: About 3 times as long as the retention time of cineol beginning after the solvent peak.

**Cinnamaldehyde for thin-layer chromatography** See (E)-cinnamaldehyde for thin-layer chromatography.

**(E)-Cinnamaldehyde for thin-layer chromatography**

C_{9}H_{8}O \ A colorless or light yellow liquid, having a characteristic aromatic odor. Very soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Absorbance \ <2.24 \ \varepsilon_{273}^{1} (285 nm): \ 1679 – 1943 (5 mg, methanol, 2000 mL).

Purity Related substances—Dissolve 10 mg of (E)-cinnamaldehyde for thin-layer chromatography in 2 mL of methanol. Perform the test with 1 μL of this solution as directed in the Identification (3) under Kakkonto Extract: no spot other than the principal spot (Rf value is about 0.4) appears.

**Cinnamic acid** C_{9}H_{8}O_{2} White crystalline powder, having a characteristic odor.

Melting point \ <2.60: \ 132 – 135°C

**(E)-Cinnamic acid for assay** (E)-Cinnamic acid for thin-layer chromatography. It meets the following requirements.

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 10 mg in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \ <2.0: \ \varepsilon_{295}^{1} \ \text{according to the following conditions, and determine each peak area by the automatic integration method and calculate the amount of cinobufagin is about 7 minutes.} \ \text{the method: the total area of the peaks other than (E)-cinnamic acid and the solvent is not larger than the peak area of (E)-cinnamic acid obtained with the standard solution.}

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Ryokejutsukanto Extract.

Time span of measurement: About 6 times as long as the retention time of (E)-cinnamic acid.

System suitability

Test for required detectability: To exactly measured 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of (E)-cinnamic acid obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Ryokejutsukanto Extract.

**(E)-Cinnamic acid for thin-layer chromatography**

C_{9}H_{8}O_{2} White crystals or crystalline powder, having a characteristic aromatic odor. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point \ <2.60: \ 132 – 136°C

Absorbance \ <2.24 \ \varepsilon_{273}^{1} (273 nm): \ 1307 – 1547 (5 mg dried with silica gel for 24 hours, methanol, 1000 mL).

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 10 mg in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed the test with 10 μL each of the sample solution and standard solution as directed in the Identification (1) under Ryokejutsukanto Extract: the spot other than the principal spot of around Rf 0.5 is not more intense than the spot obtained with the standard solution.

**(E)-Cinnamic acid for component determination** See (E)-cinnamic acid for assay.

**Cinobufagin for assay** C_{22}H_{34}O_{5}xH_{2}O White crystalline odorless powder.

Absorbance \ <2.24 \ \varepsilon_{273}^{1} \text{(295 nm):} \ 125 – 127 (10 mg, methanol, 250 mL). Use the sample dried in a desiccator (silica gel) for 24 hours for the test.

Purity Related substances—Proceed with 40 mg of cinobufagin for assay as directed in the Purity under bufalin for component determination.

Content: Not less than 98.0%. Assay—Weigh accurately about 10 mg of cinobufagin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography \ <2.0: \ \varepsilon_{295}^{1} \ \text{according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of cinobufagin by the area percentage method.}

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 295 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of cinobufagin is about 7 minutes.

Selection of column: Dissolve 10 mg each of bufalin for assay, cinobufagin for assay and resibufogenin for assay in methanol to make 200 mL. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of bufalin, cinobufagin and resibufogenin in this order, and clearly dividing each peak.

Detection sensitivity: Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of cinobufagin obtained from 20 μL of the standard solution (2) can be measured by the automatic integration method, and the peak height of cinobufagin from 20 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of cinobufagin beginning after the solvent peak.

**Cinobufagin for component determination** See cinobufagin for assay.

**Cinnoxacin for assay** C_{13}H_{10}N_{2}O_{5} [Same as the mono-
graph Cinoxacin. When dried, it contains not less than 99.0% of cinoxacin (C₁₂H₁₀N₂O₅).

Cisplatin Cl₂H₂N₂Pt [Same as the namesake monograph]

Citic acid See citric acid monohydrate.

Citic acid-acetic acid TS To 1 g of citric acid monohydrate add 90 mL of acetic anhydride and 10 mL of acetic acid (100), and dissolve under shaking.

Citic acid-acetic anhydride TS To 1 g of citric acid monohydrate add 50 mL of acetic anhydride, and dissolve by heating. Prepare before use.

Citic acid monohydrate C₆H₈O₇·H₂O [K 8283, or same as the monograph Citric Acid Hydrate]

Citic acid-phosphate-acetonitrile TS Dissolve 2.1 g of citric acid monohydrate, 13.4 g of dipotassium hydrogen phosphate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3:1).

0.01 mol/L. Citric acid TS Dissolve 2.1 g of citric acid monohydrate in water to make 1000 mL.

1 mol/L. Citric acid TS for buffer solution Dissolve 210.14 g of citric acid monohydrate in water to make 1000 mL.

Clofibrate C₁₅H₁₃ClO₃ [Same as the namesake monograph]

Clozazolam C₁₇H₁₄Cl₂N₂O₂ [Same as the namesake monograph]

Cobalt (II) chloride-ethanol TS Dissolve 0.5 g of cobalt (II) chloride hexahydrate, previously dried at 105°C for 2 hours, in ethanol (99.5) to make 100 mL.

Cobalt (II) chloride hexahydrate CoCl₂·6H₂O [K 8129, Special class]

Cobalt (II) chloride TS Dissolve 2 g of cobalt (II) chloride hexahydrate in 1 mL of hydrochloric acid and water to make 100 mL (0.08 mol/L).

Cobalt (II) nitrate hexahydrate Co(NO₃)₂·6H₂O [K 8552, Special class]

Cobaltous chloride See cobalt (II) chloride hexahydrate.

Cobaltous nitrate See cobalt (II) nitrate hexahydrate.

Codeine phosphate for assay See codeine phosphate hydrate for assay.

Codeine phosphate hydrate for assay C₁₅H₁₂NO₃·H₃PO₄·½H₂O [Same as the monograph Codeine Phosphate Hydrate. It contains not less than 99.0% of codeine phosphate (C₁₅H₁₂NO₃·H₃PO₄), calculated on the anhydrous basis.]

Collodion Clear, colorless, viscous liquid, having a diethyl ether-like odor.

\[ \text{pH} < 2.5; 5.0 - 8.0 \]

Stir 5 g of collodion while warming, add 10 mL of water gradually, and dry at 110°C after evaporating to dryness: mass of the residue is 0.250–0.275 g.

Concentrated chromotropic acid TS See chromotropic acid, concentrated.

Concentrated diazobenzenesulfonic acid TS See diazobenzenesulfonic acid TS, concentrated.

Congo red C₁₂H₁₂N₂Na₂O₆S₂ [K 8532, Special class]

Congo red TS Dissolve 0.5 g of congo red in 100 mL of a mixture of ethanol (95) and water (1:9).

Control anti-interleukin-2 antiserum TS Anti-interleukin-2 antiserum is diluted with culture media for celmoleu-kin, so that the diluted antiserum solution neutralizes the same volume of about 800 unit/mL solution of Celmoleukin (Genetical Recombination).

Coomassie brilliant blue G-250 C₁₅H₁₈N₂O₅S [K 8552, Special class] A deep violet powder. A solution in ethanol (99.5) (1 in 100,000) exhibits an absorption maxima at a wavelength of 608 nm.


Content: not less than 50%.

Coomassie staining TS Dissolve 125 mg of Coomassie brilliant blue R-250 in 100 mL of a mixture of water, methanol and acetic acid (100) (5:4:1), and filter.

Copper Cu [K 8660, Special class]

Copper (II) acetate monohydrate Cu(CH₃COO)₂·H₂O Blue-green crystals or crystalline powder.

Identification—(1) Dissolve 1 g of copper (II) acetate monohydrate in 10 mL of diluted sulfuric acid (1 in 2), and heat: the odor of acetic acid is perceptible.

(2) Dissolve 0.1 g of copper (II) acetate monohydrate in 20 mL of water, and add 3 mL of ammonia solution (28): a dark blue color is developed.

Copper (II) acetate TS, strong Dissolve 13.3 g of copper (II) acetate monohydrate in a mixture of 195 mL of water and 5 mL of acetic acid.

Copper (II) chloride-acetone TS Dissolve 0.3 g of copper (II) chloride dihydrate in acetone to make 10 mL.

Copper (II) chloride dihydrate CuCl₂·2H₂O [K 8145, Special class]

Copper (II) disodium ethylenediamine tetraacetate tetrahydrate C₁₅₀H₁₂₀Cl₂N₂O₄·4H₂O A blue powder.

\[ \text{pH} < 2.5; 7.0 - 9.0 \]

Purity Clarity and color of solution—Add 0.10 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate to 10 mL of freshly boiled and cooled water: the solution is blue in color and clear.

Content: not less than 98.0%. Assay—Weigh accurately about 0.45 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate, and add water to make exactly 100 mL. Pipet 10 mL of this solution, adjust the pH of the mixture to about 1.5 by adding 100 mL of water and dilute nitric acid, then add 5 mL of a solution of 1,10-phenanthroline mono-
hydrate in methanol (1 in 20), and titrate $<2.50$ with 0.01 mol/L bismuth nitrate VS until the color of the solution changes from yellow to red (indicator: 2 drops of xylol orange TS).

Each mL of 0.01 mol/L bismuth nitrate VS = 4.698 mg of Cu$_{2}$H$_3$CuN$_2$Na$_2$O$_8$.4H$_2$O

Copper (II) hydroxide Cu(OH)$_2$. Light blue powder. Practically insoluble in water.

*Content*: not less than 95.0% as Cu(OH)$_2$. Assay—Weigh accurately about 0.6 g of Copper (II) hydroxide, and dissolve in 3 mL of hydrochloric acid and water to make exactly 500 mL. Pipet 25 mL of this solution, add 75 mL of water, 10 mL of a solution of ammonium chloride (3 in 50), 3 mL of diluted ammonia solution (28) (1 in 10) and 0.05 g of murexide-sodium chloride indicator, and titrate $<2.50$ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the liquid is changed from yellow-green to red-purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 0.9756 mg of Cu(OH)$_2$.

Copper (II) sulfate CuSO$_4$ [K 8984, First class]

Copper (II) sulfate pentahydrate CuSO$_4$.5H$_2$O [K 8983, Special class]

Copper (II) sulfate-pyridine TS Dissolve 4 g of copper (II) sulfate pentahydrate in 90 mL of water, then add 30 mL of pyridine. Prepare before use.

Copper (II) sulfate TS, alkaline Dissolve 150 g of potassium bicarbonate, 101.4 g of potassium carbonate and 6.93 g of copper (II) sulfate pentahydrate in water to make 1000 mL.

Copper (II) sulfate TS Dissolve 12.5 g of copper (II) sulfate pentahydrate in water to make 100 mL (0.5 mol/L).

Copper (standard reagent) Cu [K 8005, Standard reagent for qualitative analysis]

Coptisine chloride for thin-layer chromatography C$_{19}$H$_{14}$NO$_{4}$Cl Orange-red, crystals or crystalline powder. Slightly soluble in methanol, and very slightly soluble in water and in ethanol (99.5). Melting point: about 260°C (with decomposition).

Identification Determine the absorption spectrum of a solution (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $<2.24>$. It exhibits maxima between 237 nm and 241 nm, between 264 nm and 268 nm, between 354 nm and 358 nm, and between 452 nm and 462 nm.

Purity Related substances—Dissolve 1 mg in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.05>$. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of methanol, ammonium acetate solution (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spot other than the principal spot (Rf value is about 0.4) obtained from the sample solution is not more intense than the spot from the standard solution.

Corn oil [Same as the namesake monograph]

Cortisone acetate C$_{25}$H$_{35}$O$_{6}$ [Same as the namesake monograph]

Cottonseed oil A refined, nonvolatile fatty oil obtained from the seed of plants of Gossypium hirsutum Linné (Gossypium) or of other similar species. A pale yellow, odorless, oily liquid. Miscible with diethyl ether, and with hexane. Slightly soluble in ethanol (95).

Refractive index $<2.45> n^20_{D}$: 1.472 – 1.474

Specific gravity $<2.50> d^25_{20}$: 0.915 – 0.921

Acid value $<1.13>$: not more than 0.5.

Saponification value $<1.13>$: 190 – 198

Iodine value $<1.13>$: 103 – 116

Cresol CH$_3$C$_6$H$_4$(OH) [Same as the namesake monograph]

m-Cresol CH$_3$C$_6$H$_4$(OH) [K 8305, Special class]

m-Cresol purple See metacresol purple.

m-Cresol purple TS See metacresol purple TS.

p-Cresol CH$_3$OH [K 8306, Special class]

Cresol red C$_{22}$H$_{21}$O$_{6}$S [K 8308, Special class]

Cresol red TS Dissolve 0.1 g of cresol red in 100 mL of ethanol (95), and filter if necessary.

Crystalline trypsin To trypsin obtained from bovine pancreas gland add an appropriate amount of trichloroacetic acid to precipitate the trypsin, and recrystallize in ethanol (95). White to yellowish white crystals or powder. It is odorless. Freely soluble in water and in sodium tetraborate-calcium chloride buffer solution, pH 8.0.

*Content*: not less than 45 FIP Units of trypsin per mg. Assay—(i) Sample solution: Weigh accurately an appropriate amount of crystallized trypsin according to the labeled Units, dissolve in 0.001 mol/L hydrochloric acid TS to prepare a solution containing 50 FIP Units per mL, and use this solution as the sample solution. Prepare before use, and preserve in ice. (ii) Apparatus: Use a glass bottle as a reaction reservoir 20 mm in inside diameter and 50 mm in height, equipped with a rubber stopper for attachment to a glass/silver-silver chloride electrode for pH determination, nitrogen-induction tube and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature at 25 ± 0.1°C by means of a precise thermostimulator. (iii) Procedure: Pipet 1.0 mL of Na$_2$-benzoyl-L-arginine ethyl ester TS, transfer to the reaction reservoir, and add 9.0 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0. Allow to stand in the thermostat for 10 minutes to make the temperature of the contents reach to 25 ± 0.1°C, adjust the pH of the solution to 8.00 by adding dropwise 0.1 mol/L sodium hydroxide VS while stirring and passing a current of nitrogen, add exactly 0.05 mL of the sample solution previously allowed to stand at 25 ± 0.1°C, then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50 μL-micropipet (minimum graduation of 1 μL) while stirring to keep the reaction solution at pH 8.00, and read the amount of 0.1 mol/L sodium hydroxide VS consumed and the reaction time when the pH reached 8.00. Continue this procedure up to 8 minutes. Separately, transfer 10 mL of sodium
tetraborate-calcium chloride buffer solution, pH 8.0, and perform a blank determination in the same manner. (iv) Calculation: Plot the amount of consumption (μL) of 0.1 mol/L sodium hydroxide VS against the reaction time (minutes), select linear reaction times, \( t_1 \) and \( t_2 \), designate the corresponding consumption amount of 0.1 mol/L sodium hydroxide VS as \( v_1 \) and \( v_2 \), respectively, and designate \( f \) of sodium hydroxide consumed per minute as \( D \) (FIP Unit).

\[
D \text{ (μmol NaOH/min)} = \frac{v_2 - v_1}{t_2 - t_1} \times f \times \frac{1}{10}
\]

\( f \): Factor of 0.1 mol/L sodium hydroxide VS

FIP Units per mg of crystallized trypsin to be tested

\[
= \frac{(D_1 - D_0) \times T}{L \times M}
\]

\( D_1 \): μmol of sodium hydroxide consumed in 1 minute when the sample solution is used

\( D_0 \): μmol of sodium hydroxide consumed in 1 minute when the solution for blank determination is used

\( M \): Amount (mg) of crystallized trypsin sampled

\( L \): Amount (mL) of the sample solution put in the reaction reservoir

\( T \): Total volume (mL) of the sample solution prepared by dissolving in 0.001 mol/L hydrochloric acid TS

One FIP Unit is an amount of enzyme which decomposes 1 μmol of N-α-benzoyl-L-arginine ethyl ester per minute under the conditions described in the Assay.

Storage—Preserve in a cold place.

Crystalline trypsin for ulinastatin assay A proteolytic enzyme prepared from bovine pancreas. White to light yellow crystalline powder. Odorless. Sparingly soluble in water, and dissolves in 0.001 mol/L hydrochloric acid TS.

Content: not less than 3200 trypsin Units per mg. Assay—(i) Sample solution: Weigh accurately about 20 mg of crystalline trypsin for ulinastatin assay, and dissolve in 0.001 mol/L hydrochloric acid TS so that each mL of the solution contains about 3000 trypsin Units. Dilute this solution with 0.001 mol/L hydrochloric acid TS so that each mL of the solution contains about 40 trypsin Units, and use this solution as the sample solution. (ii) Diluent: Dissolve 4.54 g of potassium dihydrogen phosphate in water to make exactly 500 mL (Solution I). Dissolve 4.73 g of anhydrous disodium hydrogen phosphate in water to make exactly 500 mL (Solution II). To 80 mL of Solution II add a suitable amount of Solution I to adjust to pH 7.6. (iii) Substrate solution: Dissolve 85.7 mg of N-α-benzoyl-L-arginine ethyl ester hydrochloride in water to make exactly 100 mL, and use this solution as the substrate stock solution. Pipet 10 mL of the stock solution, add the diluent to make exactly 100 mL, and use this solution as the substrate solution. The absorbance of the substrate solution determined at 253 nm as directed under Ultra-violet-visible Spectrophotometry \( <2.24 > \) using water as the blank solution. Determine the difference of the absorbance change per minute, \( A \), when the difference has been constant for at least 3 minutes. (v) Calculation: Trypsin Units per mg is obtained by use of the following equation. One trypsin Unit is an amount of the enzyme which gives 0.003 change in absorbance per minute under the conditions described above.

\[
\text{Trypsin Units per mg} = \frac{A}{0.003 \times M}
\]

\( M \): Amount (mg) of the substance to be assayed in 0.2 mL of the sample solution

Storage—Preserve in a cold place.

Crystal violet C\(_2\)H\(_3\)N\(_3\)O\(_2\) [K 8289, Special class]

Crystal violet TS Dissolve 0.1 g of crystal violet 10 mL of acetic acid (100).

Culture medium for assay of teceleukin Add 100 mL of fetal calf serum to 1000 mL of medium for float culture. Store at 4°C.

Culture medium for celmoleukin Take a specified amount of RPMI-1640 powdered medium that contains glutamate but does not contain sodium hydrogen carbonate, add water to dissolve, and add N-2-hydroxyethylpiperidine-N-2-ethansulfonic acid as a buffering agent to a concentration of 0.025 mol/L. To 1000 mL of this solution add 0.1 g (potency) of streptomycin sulfate, 100,000 units of potassium benzylpenicillin, and 2 g of sodium hydrogen carbonate, adjust the pH to 7.1 to 7.2 with sodium hydroxide TS, and then sterilize by filtration. To this solution add fetal calf serum heated at 56°C for 30 minutes to 20 vol%.

Cu-PAN Prepare by mixing 1 g of 1-(2-pyridylazo)-2-naphthol (free acid) with 11.1 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate.

A grayish orange-yellow, grayish red-brown or light grayish purple powder.

Absorbance—Dissolve 0.50 g of Cu-PAN in diluted 1,4-dioxane (1 in 2) to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 100 mL. Read the absorbance of this solution at 470 nm as directed under Ultra-violet-visible Spectrophotometry \( <2.24 > \), using water as the blank solution: the absorbance is not less than 0.48.

Purity Clarity and color of solution—Dissolve 0.50 g of Cu-PAN in 50 mL of diluted 1,4-dioxane (1 in 2); the solution is clear and yellow-brown.

Cu-PAN TS Dissolve 1 g of Cu-PAN in 100 mL of diluted 1,4-dioxane (1 in 2).

Cupferron C\(_6\)H\(_4\)N\(_2\)O\(_2\) [K 8289, Special class]

Cupferron TS Dissolve 6 g of cupferron in water to make 100 mL. Prepare before use.

Cupric acetate See copper (II) acetate monohydrate.

Cupric acetate TS, strong See copper (II) acetate monohydrate TS, strong.

Cupric carbonate See cupric carbonate monohydrate.

Cupric carbonate monohydrate Cu\(_2\)CO\(_3\)·Cu(OH)\(_2\)·H\(_2\)O A blue to blue-green powder. It is insoluble in water, and dissolves annoyingly in dilute acid. It dissolves in ammonia TS and shows a deep blue color.

Purity (1) Chloride \(<1.0\%\>): not more than 0.036%.

...
(2) Sulfate \( <1.14\% \): not more than 0.120%.

(3) Iron—Dissolve 5.0 g of cupric carbonate monohydrate in excess ammonia TS and filter. Wash the residue with ammonia TS, dissolve in dilute hydrochloric acid, add excess ammonia TS and filter. Wash the residue with ammonia TS, and dry to constant mass: the residue is not more than 10 mg.

Cupric chloride See copper (II) chloride dihydrate.

Cupric chloride-acetone TS See copper (II) chloride-acetone TS.

Cupric sulfate See copper (II) sulfate pentahydrate.

Cupric sulfate, anhydrous See copper (II) sulfate (anhydrous).

Cupric sulfate-pyridine TS See copper (II) sulfate-pyridine TS.

Cupric sulfate solution, alkaline See copper (II) sulfate solution, alkaline.

Cupric sulfate TS See copper (II) sulfate TS.

1 mol/L Cupriethylenediamine TS Put 100 g of copper (II) hydroxide in a 1-L thick-walled bottle marked a 500-mL line, and add water to make 500 mL. Connect the bottle with a liquid introducing funnel, a introducing glass tube and a gas removing glass tube. Adjust so that the lower end of the bottom of the bottle. Introduce the nitrogen for about 3 hours to replace the inside gas by adjusting the pressure (about 14 kPa) to a mild bubbling. Then add gradually 160 mL of ethylenediamine TS through the funnel while introducing the nitrogen and cooling the bottle with the running water, and replace the funnel with a glass rod to close tightly. After introducing the nitrogen for further 10 minutes, replace the gas removing tube with a glass rod to close tightly. Keep the inside pressure with the nitrogen to about 14 kPa. After allowing the bottle to stand for about 16 hours while occasional shaking, filter the content if necessary using a glass-filter under reducing pressure, and reserve under nitrogen atmosphere. The concentration of copper (II) ion of this solution is about 1.3 mol/L. Determine the concentration of ethylenediamine of this solution using a titration method with 0.1 mol/L hydrochloric acid VS (pH Determination \( <2.5\% \)).

\[
X = \frac{N_1 \cdot a}{V_1}
\]

\( X \): Concentration of ethylenediamine (mol/L)
\( a \): Volume of 0.1 mol/L hydrochloric acid VS consumed for the titration (mL)
\( N_1 \): Concentration of 0.1 mol/L hydrochloric acid VS (mol/L)

(2) Copper (II) ion—Pipet 2 mL (\( V_2 \)) of the solution to be assayed, add 20 mL of water, about 3 g of potassium iodide and 50 mL of 2 mol/L sulfuric acid TS, shake for 5 minutes, and titrate \( <2.50\% \) the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution turns light yellow at near the end point add 3 mL of starch TS and 10 mL of a solution of ammonium thiocyanate (2 in 10), and then titrate until the blue color disappears.

\[
Y = \frac{N_2 \cdot b}{V_2}
\]

\( Y \): Concentration of copper (II) ion (mol/L)
\( b \): Volume of 0.1 mol/L sodium thiosulfate VS consumed for the titration (mL)
\( N_2 \): Concentration of 0.1 mol/L sodium thiosulfate VS (mol/L)

Curcumin \( C_{21}H_{20}O_6 \) A reddish yellow crystalline powder.

Melting point \( <2.60\% \): 180 – 183°C. Preserve in a light-resistant tight container.

Curcumin TS Dissolve 0.125 g of curcumin in acetic acid (100) to make 100 mL. Prepare this solution as the sample solution.

Curcumin for assay \( C_{21}H_{20}O_6 \) Yellow to orange crystalline powder. Slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water.

Absorbance \( <2.24\% \) \( E_{1\%}^{1cm} \) (422 nm): 1460 – 1700 (dried for 24 hours in a desiccator (in vacuum, silica gel), 2.5 mg, methanol, 1000 mL).

Melting point \( <2.60\% \): 180 – 184°C

Purity: Related substances—(1) Dissolve 4 mg of curcumin for assay in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography \( <2.0\% \). Spot 5 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at the Rt value of about 0.5 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of curcumin for assay in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.0\% \) according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of curcumin obtained from the sample solution is not larger than the peak area of curcumin from the standard solution. Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Turmeric.

Detector: A visible absorption photometer (wavelength: 422 nm).

Time span of measurement: About 4 times as long as the retention time of curcumin beginning after the solvent peak.

System suitability
Test for required detectability: Pipet 1 mL of the standard solution, add methanol to make exactly 20 mL. Confirm that the peak area of curcumin obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of curcumin from 10 μL of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Turmeric.

Curcumin for component determination See curcumin for assay.

Cyanoacetic acid C₂H₂NO₂ White to light yellow crystals. Very soluble in water.

Content: not less than 99%. Assay—Weigh accurately about 300 mg of cyanoacetic acid, add 25 mL of water and 25 mL of ethanol (95) to dissolve, and titrate \( <2.50 \) with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS \( = 85.06 \text{ mg of C}_2\text{H}_2\text{NO}_2 \)

Cyanocobalamin C₁₉₈H₅₀CoN₁₄O₁₁P \[ Same as the namesake monograph \]

Cyanogen bromide TS To 100 mL of ice-cold water add 1 mL of bromine, shake vigorously, and add ice-cold potassium cyanide TS dropwise until the color of bromine just disappears. Prepare this test solution in a hood before use.

On handling this solution, be careful not to inhale its vapors, which are very toxic.

1-Cyanoguanidine NH₂C(NH)NHCN A white crystalline powder. Freely soluble in water.

Melting point \( <2.60°: 209 – 212°C \)

Loss on drying \( <2.41°: \) not more than 0.1% (1 g, 105°C, 3 hours).

Nitrogen content \( <1.00°: 66.0 – 67.3% \) (after drying).

Cyanopropylmethylyphenylsilicone for gas chromatography Prepared for gas chromatography.

6% Cyanopropylphenyl-94% dimethyl silicone polymer for gas chromatography Prepared for gas chromatography.

6% Cyanopropyl-6% phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography.

7% Cyanopropyl-7% phenylmethylysilicone polymer for gas chromatography Prepared for gas chromatography.

Cycloartenyl ferulate for thin-layer chromatography C₃₀H₄₈O₆ A white to light brown, crystalline powder or powder. Soluble in acetone, slightly soluble in acetonitrile, and practically insoluble in water and in methanol. Melting point: about 155°C.

Identification (1) Determine the absorption spectrum of a solution in heptane (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24°: \) it exhibits maxima between 229 nm and 233 nm, between 289 nm and 293 nm, and between 313 nm and 317 nm.

(2) Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25°: \) it exhibits absorption at the wave numbers of about 2940 cm\(^{-1}\), 1691 cm\(^{-1}\), 1511 cm\(^{-1}\) and 1270 cm\(^{-1}\).

Purity Related substances—Dissolve 2.0 mg in 2 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed the test with 5 μL each of the sample solution and standard solution as directed in the Identification under Brown Rice: the spot other than the principle spot, having R\(_T\) value of about 0.4, obtained from the sample solution is not more intense than the spot from the standard solution.

Cyclohexane C₆H₁₂ \[ K 8464, Special class \]

Cyclohexylamine C₆H₅NH₂ A clear and colorless liquid, having a characteristic amine-like odor. Miscible with water, with \( N, N \)-dimethylformamide and with acetone.

Purity Related substances—Use cyclohexylamine as the sample solution. Separately, pipet 1 mL of cyclohexylamine, add hexane to make exactly 100 mL, and use this as the standard solution. Perform the test as directed in Thin-layer Chromatography \( <2.0°: \) Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol, ammonia water (28) and cyclohexane (6:2:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

Cyclohexylmethanol C₆H₁₂O A liquid having slight camphor odor. Soluble in ethanol (99.5).

Refractive index \( <2.45°: n_20^B: \) about 1.464

Biological point \( <2.57°: \) about 185°C

Cyclosporine U C₃₅H₅₆N₁₂O₁₂ White powder.

Optical rotation \( <2.49° \) \([α]_D^B: \) about \( −190°C \) (0.1 g, methanol, 20 mL 100 mm).

L-Cysteic acid C₇H₇NO₃S White powder.

Melting point \( <2.60°: \) about 260°C.

Optical rotation \( <2.49° \) \([α]_D^B: \) +7.5 – +9.0° (1.5 g, water, 20 mL, 100 mm).

L-Cysteine hydrochloride See L-cysteine hydrochloride monohydrate.

L-Cysteine hydrochloride monohydrate HSC₇H₇(CH(NH)₂COOH.HCl.H₂O \[ K 8470, Special class \]

L-Cystine HOOCCH(NH)₂SSCH₂CH(NH₂)COOH \[ K 9048, \( \lambda^{(-)}\)-Cystine, Special class \]

Cytochrome c An oxidase (molecular weight: 8000 – 13,000) derived from bovine cardiac muscle.

Absorbance \( <2.24° \) \( E_{cm}^{276} \): not less than 800 (after drying, 40 mg, 10,000 mL of 0.1 mol/L hydrochloric acid TS).

Dacuronium Bromide for thin-layer chromatography C₃₃H₃₁Br₂N₂O₆ White crystalline powder. Very soluble in water, freely soluble in ethanol (95), and practically insoluble in acetic anhydride. Hygrosopic.

Identification—Determine the infrared absorption spectrum of dacuronium bromide for thin-layer chromatography
according to the potassium bromide disk method under Infrared Spectrophotometry. It exhibits the absorptions at the wave numbers at about 2940 cm\(^{-1}\), 1737 cm\(^{-1}\), 1630 cm\(^{-1}\), 1373 cm\(^{-1}\), 1233 cm\(^{-1}\) and 1031 cm\(^{-1}\).

**Purity** Related substances—Dissolve 10 mg of dacuronic chloride for thin-layer chromatography in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 mL each of the sample solution and standard solution as directed in the Purity (2). Related substances under Pancuronium Bromide: the spots other than the principal spot from the sample solution do not show more intense color than the spot from the standard solution. Water \(<2.48\): not more than 1.0% (1 g, volumetric titration, direct titration).

**Content** not less than 98.0%, calculated on the dehydrated basis. Assay—Weigh accurately about 0.2 g of dacuronic chloride for thin-layer chromatography, dissolve in 50 mL of acetic anhydride by warming, and titrate \(<2.50\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 34.53 mg of C\(_3\)H\(_5\)BrN\(_2\)O\(_3\).

**p,p’-DDD** (2,2-Bis(4-chlorophenyl)-1,1-dichloroethane)

\[ C\(_{14}\)H\(_9\)Cl\(_5\) \]

**Melting point** \(<2.60\): 108 – 110°C

**Purity** Related substances—Dissolve 10 mg of p,p’-DDD in hexane for purity of crude drug to make exactly 100 mL, pipet 1 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1 mL each of the sample solution and standard solution (1) as directed under Gas Chromatography \(<2.02\) according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak area other than p,p’-DDD from the sample solution is not larger than the peak area of p,p’-DDD from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Purity (2) under Crude Drugs Test \(<5.01\) except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of p,p’-DDD obtained from 1 μL of the standard solution (2) can be measured by the automatic integration method, and the peak height of p,p’-DDD from 1 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of p,p’-DDD beginning after the solvent peak.

**p,p’-DDE** (2,2-Bis(4-chlorophenyl)-1,1-dichloroethylene)

\[ C\(_{14}\)H\(_9\)Cl\(_4\) \]

**Melting point** \(<2.60\): 88 – 90°C

**Purity** Related substances—Proceed as directed in the Purity of p,p’-DDD using the following standard solution (1).

Standard solution (1): Pipet 1 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

**o,p’-DDT** (1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane)

\[ C\(_{14}\)H\(_9\)Cl\(_3\) \]

**Melting point** \(<2.60\): 73 – 75°C

**Purity** Related substances—Proceed as directed in the Purity of p,p’-DDD.

**p,p’-DDT** (1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane)

\[ C\(_{14}\)H\(_9\)Cl\(_4\) \]

**Melting point** \(<2.60\): 108 – 110°C

**Purity** Related substances—Proceed as directed in the Purity of p,p’-DDD using the following standard solution (1).

Standard solution (1): Pipet 1 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

**n-Decyl trimethylammonium bromide** C\(_{15}\)H\(_{29}\)NBr

White powder. Melting point: about 232°C (with decomposition).

**Content** not less than 99%. Assay—Weigh accurately about 0.5 g of n-decyl trimethylammonium bromide, dissolve in 50 mL of water, and titrate \(<2.50\) with 0.1 mol/L silver nitrate VS (indicator: 1 mL of potassium chromate TS). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 28.03 mg of C\(_{15}\)H\(_{29}\)NBr.

**0.005 mol/L n-Decyl trimethylammonium bromide TS**

Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.40 g of n-decyl trimethylammonium bromide in water to make 1000 mL.

**Defibrinated blood of rabbit** Transfer 100 mL of blood obtained from rabbit to a flask, put in about 20 glass balls 8 mm in diameter, shake for 5 minutes gently, and filter through gauze. Prepare before use.

**Decolorized fuschin TS** Add 1 g of fuschin in 100 mL of water, heat at about 50°C, then cool with occasional shaking. After standing for 48 hours, mix and filter. To 4 mL of the filtration add 6 mL of hydrochloric acid and water to make 100 mL. Use after standing for at least 1 hour. Prepare before use.

**n-Decyl trimethylammonium bromide for assay** C\(_{15}\)H\(_{29}\)N\(_2\)O\(_7\)

Yellow, crystals or crystalline powder. It is sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

**Melting point**: about 240°C (with decomposition).

**Absorbance** \(<2.20\) E\(_{1%}\) (333 nm): 577 – 642 (3 mg, water, 500 mL). Use the sample dried in a desiccator (silica gel) for not less than 1 hour for the test.

**Purity** (1) Related substances 1—Dissolve 5.0 mg of dehydrocorydaline nitrate for assay in 1 mL of a mixture of
water and methanol (1:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop immediately with a mixture of methanol, a solution of ammonium acetate (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 30 cm, and air-dry the plate. Spray Dragendorff’s TS on the plate, air-dry, and spray sodium nitrite TS; the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(2) Related substances—Dissolve 5.0 mg of dehydrocorydaline nitrate for assay in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total area of peaks other than dehydrocorydaline from the sample solution is not larger than the peak area of dehydrocorydaline from the standard solution.

Operating conditions
Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Corydalis Tuber.
Detector: Ultraviolet absorption photometer (wavelength: 230 nm).
Time span of measurement: About 3 times as long as the retention time of dehydrocorydaline, beginning after the peak of nitric acid.
System suitability
System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Corydalis Tuber.
Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of dehydrocorydaline obtained from 10 μL of the solution is equivalent to 3.5 to 6.5% of that from 5 μL of the standard solution.

Dehydrocorydaline nitrate for component determination
See dehydrocorydaline nitrate for assay.

Demethoxycurcumin C20H18O5 Yellow to orange crystalline powder or powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 166 – 170°C.

Identification Determine the absorption spectrum of a solution of demethoxycurcumin in methanol (1 in 400,000) as directed under Ultraviolet-visible Spectrophotometry <2.2.4>: it exhibits a maximum between 415 nm and 420 nm.

Purity Related substances—(1) Dissolve 4 mg of demethoxycurcumin in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.01>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm); the spots other than the principal spot at the Rf value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of demethoxycurcumin in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of demethoxycurcumin obtained from the sample solution is not larger than the peak area of demethoxycurcumin from the standard solution.

Operating conditions
Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Turmeric.
Detector: A visible absorption photometer (wavelength: 422 nm).
Time span of measurement: About 4 times as long as the retention time of demethoxycurcumin beginning after the solvent peak.

System suitability
Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of demethoxycurcumin obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of demethoxycurcumin from 10 μL of the standard solution.
System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Turmeric.

N-Demethylerythromycin C36H65NO13 White pow- der.

N-Demethylroxithromycin C40H74N2O15 White pow- der.

Identification—Determine the infrared absorption spectrum of a solution of the substance to be tested in chloroform (1 in 20) as directed in the solution method under Infrared Spectrophotometry <2.25> using a 0.1-mm cell made of potassium bromide: it exhibits absorption at the wave numbers of about 3600 cm⁻¹, 3520 cm⁻¹, 3450 cm⁻¹, 3340 cm⁻¹, 1730 cm⁻¹ and 1627 cm⁻¹.

2'-Deoxyuridine for liquid chromatography C12H12N2O5 White, crystalline powder.

Melting point <2.60>: 162 – 166°C
Purity—Dissolve 3.0 mg of 2'-deoxyuridine for liquid chromatography in diluted methanol (1 in 25) to make 50 mL. Perform the test with 10 μL of this solution as directed under Liquid Chromatography <2.01> according to the operating conditions in the Purity under Idoxuridine Ophthalmic Solution. Measure each peak area by the automatic integration method to the range about twice the retention time of 2'-deoxyuridine, and calculate the amount of 2'-deoxyuridine by the area percentage method: it shows a purity of not less than 98.5%.
Deuterochloroform (CDCl₃), heavy water (D₂O), etc.

The maximum wavelength at about 262 nm.

Resonance spectroscopy

CD₃OD Prepared for nuclear magnetic resonance spectroscopy.

NMR solvents

Deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (CD₃)₂SO Prepared for nuclear magnetic resonance spectroscopy.

Deuterated formic acid for nuclear magnetic resonance spectroscopy DCOOD Prepared for nuclear magnetic resonance spectroscopy.

Deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy DCl Prepared for nuclear magnetic resonance spectroscopy.

Deuterated methanol for nuclear magnetic resonance spectroscopy CD₂OD Prepared for nuclear magnetic resonance spectroscopy.

Deuterated NMR solvents Prepared for nuclear magnetic resonance spectroscopy. For example: deuterated dimethylsulfoxide [CD₃]₂SO, deuterated pyridine (C₅D₅N), deuterochloroform (CDCl₃), heavy water (D₂O), etc.

Deuterated pyridine for nuclear magnetic resonance spectroscopy C₅D₅N Prepared for nuclear magnetic resonance spectroscopy.

Deuterochloroform for nuclear magnetic resonance spectroscopy CDCl₃ Prepared for nuclear magnetic resonance spectroscopy.

Devarda’s alloy [K 8653, For Nitrogen analysis]

Diacetyl CH₃COCOCH₃ A yellow to yellow-green, clear liquid, having a strong, pungent odor. Miscible with ethanol (95) and with diethyl ether, and freely soluble in water.

Congealing point <2.40>: −2.0 to −5.5°C

Refractive index <2.45> nD: 1.390 – 1.398

Specific gravity <2.56> d20°: 0.98 – 1.00

Boiling point <2.57>: 85 – 91°C

Purity Clarity of solution—Dissolve 1.0 g of diacetyl in 10 mL of water: the solution is clear.

Content: not less than 98.5%. Assay—Weigh accurately about 5 mg of 2′-deoxyuridine for liquid chromatography, previously dried in vacuum at 60°C for 3 hours, and dissolve in water to make exactly 250 mL. Pipet 10 mL of this solution, dilute with water to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine absorbance A at the maximum wavelength at about 262 nm.

Amount (mg) of deoxyuridine (C₉H₁₂N₂O₅)

\[ A = \frac{A}{447} \times 5000 \]

Dermatan sulfate Dermatan sulfate is mucopolysaccharide purified from the skin and small intestines of pigs by alkaline extraction, followed by digestion with protease and fractionation by alcohol. When cellulose acetate membrane electrophoresis of dermatan sulfate is performed and the membrane is stained in a toluidine blue O solution (1 in 200), a single band appears.

Operation conditions of cellulose acetate membrane electrophoresis—Cellulose acetate membrane: 6 cm in width and 10 cm in length.

Mobile phase: Dissolve 52.85 g of calcium acetate monohydrate in water to make 100 mL.

Run time: 3 hours (1.0 mA/cm)

Deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (CD₃)₂SO Prepared for nuclear magnetic resonance spectroscopy.

Deuterated pyridine for nuclear magnetic resonance spectroscopy DCOOD Prepared for nuclear magnetic resonance spectroscopy.

Deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy DCl Prepared for nuclear magnetic resonance spectroscopy.

Deuterated methanol for nuclear magnetic resonance spectroscopy CD₂OD Prepared for nuclear magnetic resonance spectroscopy.

Deuterated NMR solvents Prepared for nuclear magnetic resonance spectroscopy. For example: deuterated dimethylsulfoxide [CD₃]₂SO, deuterated pyridine (C₅D₅N), deuterochloroform (CDCl₃), heavy water (D₂O), etc.

Deuterated pyridine for nuclear magnetic resonance spectroscopy C₅D₅N Prepared for nuclear magnetic resonance spectroscopy.

Deuterochloroform for nuclear magnetic resonance spectroscopy CDCl₃ Prepared for nuclear magnetic resonance spectroscopy.

Devarda’s alloy [K 8653, For Nitrogen analysis]

Diacetyl CH₃COCOCH₃ A yellow to yellow-green, clear liquid, having a strong, pungent odor. Miscible with ethanol (95) and with diethyl ether, and freely soluble in water.

Congealing point <2.40>: −2.0 to −5.5°C

Refractive index <2.45> nD: 1.390 – 1.398

Specific gravity <2.56> d20°: 0.98 – 1.00

Boiling point <2.57>: 85 – 91°C

Purity Clarity of solution—Dissolve 1.0 g of diacetyl in 10 mL of water: the solution is clear.

Content: not less than 98.5%. Assay—Weigh accurately about 0.4 g of diacetyl, add exactly 75 mL of hydroxylamine TS, and heat on a water bath for 1 hour under a reflux condenser. After cooling, titrate <2.50> the excess hydroxylamine with 0.5 mol/L hydrochloric acid VS until the color of the solution changes from blue to yellow-green through green (indicator: 3 drops of bromophenol blue TS). Perform a blank determination in the same manner.

Each mL of 0.5 mol/L hydrochloric acid VS

\[ = 21.52 \text{ mg of C}_2\text{H}_4\text{O}_2 \]

Diacetyl TS Dissolve 1 mL of diacetyl in water to make 100 mL, and dilute 5 mL of this solution with water to make 100 mL. Prepare before use.

2,3-Diaminonaphthalene C₁₀H₁₀N₂ Light yellow-brown crystals or powder. Slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Melting point <2.60>: 193 – 198°C

Sensitivity—Pipe tet separately 40 mL each of the selenium standard solution and diluted nitric acid (1 in 60) as the blank solution into beakers, and to these solutions add ammonia solution (28) to adjust the pH to between 1.8 and 2.2. Dissolve 0.2 g of hydroxylaminommonium chloride in each of these solutions under gentle shaking, add 5 mL of 2,3-diaminonaphthalene TS, mix by shaking, and allow to stand for 100 minutes. Transfer these solutions to separators separately, rinse the beakers with 10 mL of water, add these rinsings to the separators, extract each with 5.0 mL of cyclohexane by thorough shaking for 2 minutes, and centrifuge the cyclohexane layers to remove moisture. When the absorbance at 378 nm of cyclohexane extract obtained from selenium standard solution is determined using the solution obtained from the blank solution as the reference solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, it is not less than 0.08.

Selenium standard solution—Weigh accurately 40 mg of selenium, dissolve in 100 mL of diluted nitric acid (1 in 2), by heating on water bath if necessary, and add water to make exactly 1000 mL. Pipet 5 mL of this solution, and add water to make exactly 200 mL. Pipet 2 mL of this solution, and add diluted nitric acid (1 in 60) to make exactly 50 mL. Prepare before use. This solution contains 0.04 µg of selenium (Se) per mL.

2,4-Diaminophenol dihydrochloride C₆H₇N₂O.2HCl Pale yellow-brown to grayish yellow crystalline powder. Freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Purity Clarity of solution—Dissolve 1.0 g of 2,4-diaminophenol hydrochloride in 20 mL of water: the solution is clear or a slight turbidity is produced.

Loss on drying <2.47>: not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44>: not more than 0.5% (1 g).

Content: not less than 98.0%. Assay—Weigh accurately about 0.2 g of 2,4-diaminophenol hydrochloride, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS

\[ = 9.853 \text{ mg of C}_6\text{H}_7\text{N}_2\text{O.2HCl} \]
2,4-Diaminophenol dihydrochloride TS
Dissolve 1 g of 2,4-diaminophenol hydrochloride and 20 g of sodium bisulfite in 100 mL of water, and filter, if necessary.

2,4-Diaminophenol See 2,4-diaminophenol dihydrochloride.

2,4-Diaminophenol hydrochloride TS
See 2,4-diaminophenol dihydrochloride TS.

Diammonium hydrogen citrate C$_6$H$_8$N$_2$O$_7$ • H$_2$O [K 8284, Special class]

Diammonium hydrogen phosphate (NH$_4$)$_2$HPO$_4$ [K 9016, Special class]

Diazepam for assay C$_{16}$H$_{13}$ClN$_2$O [Same as the monograph, Diazepam. When dried, it contains not less than 99.0% of diazepam (C$_{16}$H$_{15}$ClN$_2$O), and meets the additional following requirement.]

Purity Related substance—Dissolve 50 mg in 10 mL of water, add methanol to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than diazepam from the sample solution is not larger than the peak area of diazepam from the standard solution.

Operating conditions
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Diazepam Tablets.

Time span of measurement: About 4.5 times as long as the retention time of diazepam, beginning after the solvent peak.

System suitability
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of diazepam are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diazepam is not more than 2.0%.

Diazobenzenesulfonylic acid TS
Weigh 0.9 g of sulfanilic acid, previously dried at 105°C for 3 hours, dissolve it in 10 mL of dilute hydrochloric acid by heating, and add water to make 100 mL. Pipet 3.0 mL of this solution, add 2.5 mL of sodium nitrite TS, and allow to stand for 5 minutes while cooling with ice. Then add 5 mL of sodium nitrite TS and water to make 100 mL, and allow to stand in ice water for 15 minutes. Prepare before use.

Diazobenzenesulfonylic acid TS, concentrated
Weigh 0.2 g of sulfanilic acid, previously dried at 105°C for 3 hours, dissolve it in 20 mL of 1 mol/L hydrochloric acid TS by warming. Cool this solution with ice, and add 2.2 mL of a solution of sodium nitrite (1 in 25) dropwise under stirring. Allow to stand in ice water for 10 minutes, and add 1 mL of a solution of sulfaminic acid (1 in 20). Prepare before use.

Diazobenzenesulfonylic acid TS, concentrated
Weigh accurately 0.9 g of sulfanilic acid, add 0.9 mL of hydrochloric acid and 20 mL of water, and dissolve by heating. After cooling, filter, and dilute the filtrate with water to make exactly 100 mL. Pipet 1.5 mL of this solution, cool in an ice bath, and add exactly 1 mL of sodium nitrite solution (1 in 20) dropwise, while shaking. Cool in an ice bath for 10 minutes, add cold water to make exactly 50 mL. Store in a cold place, and use within 8 hours.

Dibasic ammonium phosphate See diammominhydrogen phosphate.

Dibasic potassium phosphate See dipotassium hydrogen phosphate.

Dibasic potassium phosphate-citric acid buffer solution, pH 5.3
See dipotassium hydrogen phosphate-citric acid buffer solution, pH 5.3.

1 mol/L Dibasic potassium phosphate TS for buffer solution
See 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

Dibasic sodium ammonium phosphate See ammonium sodium hydrogen phosphate tetrahydrate.

Dibasic sodium phosphate See disodium hydrogen phosphate dodecahydrate.

Dibasic sodium phosphate, anhydrous See disodium hydrogen phosphate.

Dibasic sodium phosphate, anhydrous, for pH determination See disodium hydrogen phosphate for pH determination.

Dibasic sodium phosphate-citric acid buffer solution, pH 4.5
See disodium hydrogen phosphate-citric acid buffer solution, pH 4.5.

Dibasic sodium phosphate-citric acid buffer solution, pH 5.4
See disodium hydrogen phosphate-citric acid buffer solution, pH 5.4.

Dibasic sodium phosphate-citric acid buffer solution, pH 6.0
See disodium hydrogen phosphate-citric acid buffer solution, pH 6.0.

Dibasic sodium phosphate TS
See disodium hydrogen phosphate TS.

0.05 mol/L Dibasic sodium phosphate TS
See 0.05 mol/L disodium hydrogen phosphate TS.

0.5 mol/L Dibasic sodium phosphate TS
See 0.5 mol/L disodium hydrogen phosphate TS.

Dibekacin sulfate C$_{18}$H$_{37}$N$_5$O$_8$.H$_2$SO$_4$
[Same as the namesake monograph]

Dibenz[a,h]anthracene C$_{22}$H$_{14}$ Very pale yellow to green-yellow crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 265–270°C.

Identification Perform the test with dibenz[a,h]anthracene as directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak (m/z 278) and a fragment ion peak (m/z 139).

Purity Related substances—Dissolve 3.0 mg of dibenz[a,h]anthracene in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 1
1.02 g of disodium hydrogen phosphate, anhydrous and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 8 mg of acetic acid (100), add 25 mL of methanol, and add the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 20 mL, and use this solution as the control solution. Perform the test with exactly 20 mL of each of the sample solution and control solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. After making correction for the peak areas based on the baseline and the peak of acetic acid on the chromatogram obtained with the sample solution, calculate the amount of \( N,N' \)-dibenzylethylenediamine by the area percentage method.

### System suitability

- **Detector:** An ultraviolet absorption photometer (wavelength: 220 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecytrimethylsilane silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).
- **Column temperature:** A constant temperature of about 40\({}^\circ\)C.
- **Mobile phase:** A mixture of water, methanol and 0.25 mol/L potassium dihydrogen phosphate TS, pH 3.5 (11:7:2).
- **Flow rate:** Adjust the flow rate so that the retention time of \( N,N' \)-dibenzylethylenediamine is about 4 minutes.
- **Time span of measurement:** About 5 times as long as the retention time of \( N,N' \)-dibenzylethylenediamine.

### Related substances

- **System suitability**
  - **System performance:** Dissolve an amount of Benzylpenicillin Benzyathine, equivalent to about 85,000 Units, in 25 mL of methanol, add a solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL. When the procedure is run with 20 mL of this solution under the above operating conditions, \( N,N' \)-dibenzylethylenediamine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 20.
  - **System repeatability:** When the test is repeated 6 times with 20 mL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of \( N,N' \)-dibenzylethylenediamine is not more than 2.0%.

### 2.6-Dibromo-N-chloro-1,4-benzoquinone monoimine

- **CAS number:** \( \text{C}_8\text{H}_6\text{Br}_2\text{CINO} \) [K 8491, Special class]
- **Dissolve** 0.5 g of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine TS
monoxide in methanol to make 100 mL.

2,6-Dibromo-N-chloro-1,4-benzoquinone monoxide TS, 
dilute Dissolve 0.2 g of 2,6-dibromo-N-chloro-1,4-benzo-
quione monoxide in methanol to make 100 mL.

2,6-Dibromoquinone chlorimide See 2,6-dibromo-N-
chloro-1,4-benzoquinone monoxide.

2,6-Dibromoquinone chlorimide TS See 2,6-dibromo-N-
chloro-1,4-benzoquinone monoxide TS.

Dibucaine hydrochloride C_{20}H_{29}N_{3}O_{2}HCl [Same as the namesake monograph]

Dibucaine hydrochloride Monograph Ascorbic Acid Powder.

Dichloro-1,4-benzoquinone monoimine TS, 
dilute Dissolve 0.2 g of dichloro-1,4-benzoquinone monoimine in methanol to make 100 mL.

2,6-Dichloroindophenol sodium-sodium acetate TS Mix equal volumes of 2,6-dichloroindophenol sodium dihydrate solution (1 in 20) and acetic acid-sodium acetate TS, pH 7.0. Prepare before use.

Dichloromethane CH_{2}Cl_{2} [K 8161, Special class]

2,6-Dichlorophenol C_{6}H_{4}Cl_{2}O White to purplish white 
crystals.

2,6-Dichlorophenol-indophenol sodium TS See 2,6-
dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium TS for titration
See 2,6-dichloroindophenol sodium TS for titration.

2,6-Dichlorophenol-indophenol sodium-sodium acetate TS
See 2,6-dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium-sodium acetate TS
See 2,6-dichloroindophenol sodium dihydrate.

Dichlorofluorescein TS
See Dichlorofluorescein Monograph Ascorbic Acid Powder.

2,6-Dichloroindophenol sodium-sodium acetate TS Mix equal volumes of 2,6-dichloroindophenol sodium dihydrate solution (1 in 20) and acetic acid-sodium acetate TS, pH 7.0. Prepare before use.

Dichloromethane CH_{2}Cl_{2} [K 8161, Special class]

2,6-Dichlorophenol C_{6}H_{4}Cl_{2}O White to purplish white 
crystals.

2,6-Dichlorophenol-indophenol sodium TS See 2,6-
dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium TS for titration
See 2,6-dichloroindophenol sodium TS for titration.

2,6-Dichlorophenol-indophenol sodium-sodium acetate TS
See 2,6-dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium-sodium acetate TS
See 2,6-dichloroindophenol sodium dihydrate.

Dichlorofluorescein TS
See Dichlorofluorescein Monograph Ascorbic Acid Powder.

2,6-Dichloroindophenol sodium-sodium acetate TS Mix equal volumes of 2,6-dichloroindophenol sodium dihydrate solution (1 in 20) and acetic acid-sodium acetate TS, pH 7.0. Prepare before use.

Dichloromethane CH_{2}Cl_{2} [K 8161, Special class]

2,6-Dichlorophenol C_{6}H_{4}Cl_{2}O White to purplish white 
crystals.

2,6-Dichlorophenol-indophenol sodium TS See 2,6-
dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium TS for titration
See 2,6-dichloroindophenol sodium TS for titration.

2,6-Dichlorophenol-indophenol sodium-sodium acetate TS
See 2,6-dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium-sodium acetate TS
See 2,6-dichloroindophenol sodium dihydrate.

Dichlorofluorescein TS
See Dichlorofluorescein Monograph Ascorbic Acid Powder.

2,6-Dichloroindophenol sodium-sodium acetate TS Mix equal volumes of 2,6-dichloroindophenol sodium dihydrate solution (1 in 20) and acetic acid-sodium acetate TS, pH 7.0. Prepare before use.

Dichloromethane CH_{2}Cl_{2} [K 8161, Special class]

2,6-Dichlorophenol C_{6}H_{4}Cl_{2}O White to purplish white 
crystals.

2,6-Dichlorophenol-indophenol sodium TS See 2,6-
dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium TS for titration
See 2,6-dichloroindophenol sodium TS for titration.

2,6-Dichlorophenol-indophenol sodium-sodium acetate TS
See 2,6-dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium-sodium acetate TS
See 2,6-dichloroindophenol sodium dihydrate.

Dichlorofluorescein TS
See Dichlorofluorescein Monograph Ascorbic Acid Powder.

2,6-Dichloroindophenol sodium-sodium acetate TS Mix equal volumes of 2,6-dichloroindophenol sodium dihydrate solution (1 in 20) and acetic acid-sodium acetate TS, pH 7.0. Prepare before use.

Dichloromethane CH_{2}Cl_{2} [K 8161, Special class]

2,6-Dichlorophenol C_{6}H_{4}Cl_{2}O White to purplish white 
crystals.

2,6-Dichlorophenol-indophenol sodium TS See 2,6-
dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium TS for titration
See 2,6-dichloroindophenol sodium TS for titration.

2,6-Dichlorophenol-indophenol sodium-sodium acetate TS
See 2,6-dichloroindophenol sodium dihydrate.
standard class. The water content is not more than 0.01

2-(2-ethoxyethoxy)ethanol Clear, colorless liquid, of which the boiling point is about 203

water is alkaline, and readily absorbs carbon dioxide in air.

Specific gravity <2.50> d^20_1: 0.702 – 0.708

Diethanolamine hydrochloride See 2,2’-iminodiethanol hydrochloride.

Diethylamine (C_2H_5)NH A clear, colorless liquid, having an amine-like odor. Miscible with water and with ethanol (95). The solution in water is alkaline, and readily absorbs carbon dioxide in air.

Specific gravity <2.50> d^20_1: 1.118 – 1.120

Diethylylm glycol adipinate for gas chromatography Prepared for gas chromatography.

Diethylene glycol monoethyl ether (C_2H_5OCH_2CH_2)OH Clear and colorless liquid, miscible with water.

Specific gravity <2.50> d^20_1: 0.940 – 0.950

Distilling range <2.50>: 158 – 160°C, not less than 95 vol%.

Diethylene glycol monoethyl ether for water determination See Water Determination <2.40>.

Diethylene glycol succinate ester for gas chromatography Prepared for gas chromatography.

Diethylene glycol succinate polyester for gas chromatography Prepared for gas chromatography.

Diethylym ether C_2H_5OC_2H_5 [K 8103, Special class]

Diethyl ether, dehydrated C_2H_5OC_2H_5 [K 8103, Special class. The water content is not more than 0.01%].

Diethyl ether for purity of crude drug C_2H_5OC_2H_5 [K 8103, Special class]. Use diethyl ether meeting the following additional specification. Evaporate 300.0 mL of diethyl ether for purity of crude drug in vacuum at a temperature not higher than 40°C, add the diethyl ether to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of γ-BHC in hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane for purity of crude drug to make exactly 100 mL. Pipet 2 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution I. Perform the test with 1 μL each of the sample solution and standard solution I as directed under Gas Chromatography <2.02> according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the solvent peak from the sample solution is not larger than the peak area of γ-BHC from the standard solution I.

Operating conditions
Proceed the operating conditions in the Purity (2) under the Crude Drugs Test <5.02> except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution I, add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution II. Adjust the detection sensitivity so that the peak area of γ-BHC obtained from 1 μL of the standard solution II can be measured by the automatic integration method, and the peak height of γ-BHC from 1 μL of the standard solution I is about 20% of the full scale.

Time span of measurement: About three times as long as the retention time of γ-BHC beginning after the peak of solvent.

N,N-Diethyl-N’-1-naphthylethylenediamine oxalate C_18H_32N_4O_4 A white crystalline powder.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3340 cm⁻¹, 2940 cm⁻¹, 1581 cm⁻¹, 1536 cm⁻¹, 1412 cm⁻¹, 789 cm⁻¹, 774 cm⁻¹ and 721 cm⁻¹.

Purity Clarity of solution—To 0.1 g add 20 mL of water, and dissolve by warming: the solution is clear.

N,N-Diethyl-N’-1-naphthylethylenediamine oxalate-ace tone TS Dissolve 1 g of N,N-diethyl-N’-1-naphthylethylenediamine oxalate in 100 mL of a mixture of acetone and water (1:1). Prepare before use.

N,N-Diethyl-N’-1-naphthylethylenediamine oxalate TS Dissolve 1 g of N,N-diethyl-N’-1-naphthylethylenediamine oxalate in water to make 1000 mL.

Diethyl phthalate C_8H_6(COOC_2H_5)_2 A colorless, clear liquid.

Refractive index <2.45> n^20_2: 1.500 – 1.505

Purity Related substances—To 1 mL of diethyl phthalate add a solution of tetra-n-heptylammonium bromide in a mixture of water, acetonitrile and methanol (137:80:23) (2 in 625) to make 100 mL. To 6 mL of this solution add a solution of tetra-n-heptylammonium bromide in a mixture of water, acetonitrile and methanol (137:80:23) (2 in 625) to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed in the Assay under Cefetamet Pivoxil Hydrochloride: any peaks other than peaks of diethyl phthalate and the solvent are not observed.
Diethyl terephthalate \( \text{C}_2\text{H}_4\text{(COOC}_2\text{H}_5\text{)}_2 \) White to pale brownish white, crystalline or mass.  
*Melting point* \( <2.60^\circ \text{C} \); \( >44 - 46^\circ \text{C} \)
*Content*: not less than \( 99\% \). Assay—Dissolve 100 mg of diethyl terephthalate in 10 mL of methanol. Perform the test with \( 2 \mu\text{L} \) of this solution as directed under Gas Chromatography \( <2.02> \) according to the following conditions, and determine the area of each peak by the automatic integration method.

\[
\text{Content (\%)} = \frac{\text{peak area of diethyl terephthalate}}{\text{total of all peak areas}} \times 100
\]

*Operating conditions*
- **Detector**: Hydrogen flame-ionization detector.
- **Column**: A glass tube 4 mm in inside diameter and 2 m in length, packed with Shimalted W(AW, DMCS) coated with SE-30 in 10% (177 – 250 \( \mu\text{m} \) in particle diameter).
- **Column temperature**: A constant temperature of about \( 200^\circ \text{C} \).
- **Carrier gas**: Helium.
- **Flow rate**: Adjust the flow rate so that the retention time of diethyl terephthalate is between 6 and 7 minutes.
- **Time span of measurement**: About 5 times as long as the retention time of diethyl terephthalate beginning after the solvent peak.

**Difenidol hydrochloride** \( \text{C}_9\text{H}_{12}\text{NO}_4\text{HCl} \) [Same as the namesake monograph]

**Digitonin** \( \text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_4\text{HCl} \) [Same as the monograph Dihydrocodeine Phosphate. It contains not less than \( 99.0 \% \) of dihydrocodeine phosphate \( \text{C}_{18}\text{H}_{23}\text{NO}_3\text{H}_3\text{PO}_4 \), calculated on the dried basis.]

**Dihydrocodeine phosphate for assay** \( \text{C}_{18}\text{H}_{23}\text{NO}_3\text{H}_3\text{PO}_4 \) [Same as the namesake monograph]

**Dihydroergocristine mesilate for thin-layer chromatography** \( \text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_4 \) A pale yellowish white powder.  
*Purity* Related substances—Dissolve 6 mg of dihydroergocristine mesilate for thin-layer chromatography in exact 100 mL of a mixture of chloroform and methanol (9:1), and perform the test with 5 \( \mu\text{L} \) of this solution as directed in the Purity (3) under Zidovudine: spots other than the principal spot with an RF value of about 0.23 are not observed.

**3,4-Dihydroxy-6-hydroxy-2(1H)-quinolinone** \( \text{C}_7\text{H}_3\text{NO}_2 \) A white to light brown powder or granule. Melting point: about \( 240^\circ \text{C} \) (with decomposition).

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25> \); it exhibits absorption at the wave numbers of about 3210 cm\(^{-1}\), 1649 cm\(^{-1}\), 1502 cm\(^{-1}\), 1252 cm\(^{-1}\), and 816 cm\(^{-1}\).

**2,4-Dihydroxybenzoic acid** \( \text{C}_8\text{H}_4\text{(COOC}_2\text{H}_5\text{)}_2 \) [Same as the namesake monograph]

**1,3-Dihydroxynaphthalene** \( \text{C}_9\text{H}_4\text{(OH)}_2 \) Purple-brown, crystals or powder. Freely soluble in water and in ethanol (95).

*Melting point* \( <2.60^\circ \text{C} \); about \( 125^\circ \text{C} \)

**2,7-Dihydroxynaphthalene** \( \text{C}_9\text{H}_4\text{(OH)}_2 \)

*Purity* not less than \( 97.0\% \).

**2,7-Dihydroxynaphthalene TS** Dissolve 0.10 g of 2,7-dihydroxynaphthalene in 1000 mL of sulfuric acid, and allow to stand until the yellow color initially developed disappears. If the solution is blackened notably, prepare freshly.

**Diisopropylamine** \( [(\text{CH}_3)_2\text{CH}]_2\text{NH} \) Colorless, clear liquid, having an amine-like odor. Miscible with water and with ethanol (95). The solution in water is alkaline.

*Refractive index* \( <2.45> \) \( n_2^\circ \): 1.391 – 1.394  
*Specific gravity* \( <2.56> \) \( d_2^\circ \): 0.715 – 0.722

**Diltiazem hydrochloride** \( \text{C}_2\text{H}_{18}\text{N}_2\text{O}_4\text{S.HCl} \) [Same as the namesake monograph]

**Dilute acetic acid** See acetic acid, dilute.

**Dilute bismuth subnitrate-potassium iodide TS for spray** Dissolve 10 g of \( \text{L-tartaric acid} \) in 50 mL of water, and add 5 mL of bismuth subnitrate TS.

**Dilute bromophenol blue TS** See bromophenol blue TS, dilute.

**Dilute p-dimethylaminobenzaldehyde-ferric chloride TS** See 4-dimethylaminobenzaldehyde-ferric chloride TS, dilute.

**Dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS** See 4-dimethylaminobenzaldehyde-iron (III) chloride TS, dilute.

**Diluted ethanol** See ethanol, diluted.

**Dilute ethanol** See ethanol, dilute.

**Dilute ferric ammonium sulfate TS** See ammonium iron...
Dilute ferric chloride TS See iron (III) chloride TS, dilute.

Dilute hydrochloric acid See hydrochloric acid, dilute.

Dilute hydrogen peroxide TS See hydrogen peroxide TS, dilute.

Dilute iodine TS See iodine TS, dilute.

Dilute iron-phenol TS See iron-phenol TS, dilute.

Dilute lead subacetate TS See lead subacetate TS, dilute.

Dilute methyl red TS See methyl red TS, dilute.

Dilute nitric acid See nitric acid, dilute.

Dilute potassium hydroxide-ethanol TS See potassium hydroxide-ethanol TS, dilute.

Dilute sodium hydroxide TS See sodium hydroxide TS, dilute.

Dilute sulfuric acid See sulfuric acid, dilute.

Dilute thymol blue TS See thymol blue TS, dilute.

Dilute vanadium pentoxide TS See vanadium (V) oxide TS, dilute.

Dimedon C6H12O2 White to pale yellow, crystalline powder. Melting point <2.60°: 145 – 149°C

Dimenhydrinate for assay C17H21NO.C7H7ClN4O2 [Same as the monograph Dimenhydrinate. When dried, it contains not less than 53.8% and not more than 54.9% of diphenhydramine (C17H21NO) and not more than 46.1% of 8-chlorotheophylline (C12H17N2O).]

Dimethoxymethane C3H8O2 Colorless, clear and volatile liquid. Miscible with methanol, with ethanol (95) and with diethyl ether. N,N-Dimethylacetamide CH2CON(CH3)2 Clear and colorless liquid. Specific gravity <2.50°: d: 0.938 – 0.945 (Method 3). Boiling point <2.57°: 163 – 165°C Water <2.48°: not more than 0.2% (0.1 g, Coulometric titration). Purity—Perform the test with 3 μL of N,N-dimethylacetamide as directed under Gas Chromatography <2.02> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of N,N-dimethylacetamide by the area percentage method: not less than 98.0%.

Operating conditions Detector: A hydrogen flame-ionization detector. Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated the inside surface 0.5 μm in thickness with polyethylene glycol 20 M for gas chromatography.

Column temperature: The sample is injected at a constant temperature of about 70°C, keep this temperature for 1 minute, then raise to 200°C in a rate of 10°C per minute, and keep 200°C for 3 minutes.


Time span of measurement: About 2 times as long as the retention time of N,N-dimethylacetamide.

System suitability Test for required detection: To exactly 1.0 g of N,N-dimethylacetamide add acetone to make exactly 100 mL. Pipet 5 mL of this solution, and add acetone to make exactly 50 mL. Confirm that the peak area of N,N-dimethylacetamide obtained from 3 μL of this solution is equivalent to 40 to 60% of the full-scale.

System repeatability: When the test is repeated with 3 μL of N,N-dimethylacetamide under the above operating conditions, the relative standard deviation of the peak area of N,N-dimethylacetamide is not more than 2.0%.

Dimethylyamine (CH3)2NH Colorless, clear liquid, having amine-like, characteristic odor. It is miscible with water and with ethanol (99.5). It is alkaline. Specific gravity <2.50°: d: 0.85 – 0.93 Content: 38.0 – 45.0%. Assay—Weigh accurately about 1 g of dimethylyamine, transfer to a flask containing exactly 20 mL of 0.5 mol/L sulfuric acid VS, and titrate <2.50> the excess sulfuric acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of methyl red TS). Perform a blank determination in the same manner.

Each mL of 0.5 mol/L sulfuric acid VS = 45.08 mg of (CH3)2NH

4-Dimethylaminooantipyrine C13H17N3O Colorless or white crystals, or a white crystalline powder. Purity Related substances—Proceed the test with 5 μL of a solution of 4-dimethylaminooantipyrine (1 in 2000) as directed in the Assay under Cefpime Sodium, determine each peak area in a range of about 2 times as long as the retention time of 4-dimethylaminooantipyrine after the solvent peak by the automatic integration method, and calculate the total amount of the peaks other than 4-dimethylaminooantipyrine by the area percentage method: not more than 1.8%.

(Dimethylamino)azo(benzensulfonyl chloride C14H10ClN2O5 Prepared for amino acid analysis or biochemistry.

p-Dimethylaminobenzaldehyde See 4-dimethylaminobenzaldehyde.

4-Dimethylaminobenzaldehyde (CH3)2NC6H4CHO [K 8496, p-Dimethylaminobenzaldehyde, Special class]

p-Dimethylaminobenzaldehyde-ferric chloride TS See 4-dimethylaminobenzaldehyde-ferric chloride TS, dilute

p-Dimethylaminobenzaldehyde-hydrochloric acid TS See 4-dimethylaminobenzaldehyde-hydrochloric acid TS.
4-Dimethylaminobenzaldehyde-hydrochloric acid TS  
Dissolve 1.0 g of 4-dimethylaminobenzaldehyde in 50 mL of hydrochloric acid while cooling, and add 50 mL of ethanol (95).

4-Dimethylaminobenzaldehyde-iron (III) chloride TS  
Dissolve 0.125 g of 4-dimethylaminobenzaldehyde in a cold mixture of 65 mL of sulfuric acid and 35 mL of water, then add 0.05 mL of iron (III) chloride TS. Use within 7 days.

4-Dimethylaminobenzaldehyde-iron (III) chloride TS, dilute  
To 80 mL of water add carefully 100 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS and 0.15 mL of iron (III) chloride TS, while cooling with ice.

\( p \)-Dimethylaminobenzaldehyde TS  
See 4-dimethylaminobenzaldehyde TS.

4-Dimethylaminobenzaldehyde TS  
Dissolve 10 g of 4-dimethylaminobenzaldehyde in a cold mixture of 90 mL of sulfuric acid and 10 mL of water. Prepare before use.

\( p \)-Dimethylaminobenzaldehyde TS for spraying  
See 4-dimethylaminobenzaldehyde TS for spraying.

4-Dimethylaminobenzaldehyde TS for spraying  
Dissolve 1.0 g of 4-dimethylaminobenzaldehyde in 20 mL of dilute sulfuric acid. Prepare before use.

\( p \)-Dimethylaminobenzylidene rhodanine  
See 4-dimethylaminobenzylidene rhodanine.

4-Dimethylaminobenzylidene rhodanine  
\( C_{12}H_{12}N_2O_2 \) [K 8495, Special class]

\( p \)-Dimethylaminobenzylidene rhodanine TS  
See 4-dimethylaminobenzylidene rhodanine TS.

4-Dimethylaminobenzylidene rhodanine TS  
Dissolve 20 mg of 4-dimethylaminobenzylidene rhodanine in acetone to make 100 mL.

\( p \)-Dimethylaminocinnamaldehyde  
See 4-dimethylaminocinnamaldehyde.

4-Dimethylaminocinnamaldehyde  
\( C_9H_7NO \) Orange crystals or crystalline powder, having a characteristic odor. Freely soluble in dilute hydrochloric acid, sparingly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Melting point \(<2.60\%>: 140 – 142^\circ\text{C}\)

Purity  
Clarity of solution—Dissolve 0.2 g of 4-dimethylaminocinnamaldehyde in 20 mL of ethanol (95): the solution is clear.

Loss on drying \(<2.41\%>: 0.5\%\) (1 g, 105°C, 2 hours).

Residue on ignition \(<2.44\%>: 0.1\%\) (1 g).

Nitrogen content \(<1.08\%>: 7.8 – 8.1\%\) (105°C, 2 hours, after drying).

\( p \)-Dimethylaminocinnamaldehyde TS  
See 4-dimethylaminocinnamaldehyde TS.

4-Dimethylaminocinnamaldehyde TS  
Before use, add 1 mL of acetic acid (100) to 10 mL of a solution of 4-dimethylaminocinnamaldehyde in ethanol (95) (1 in 2000).

Dimethylaminophenol  
\( (CH_3)_2NC_6H_4OH \) Dark purple, crystals or crystalline mass.

Melting point \(<2.60\%>: 85^\circ\text{C}\)

Dimethylamine  
See \( N,N \)-dimethylamine.

\( N,N \)-Dimethylamine  
\( C_9H_{12}N(CH_3)_2 \) Colorless or light yellow liquid, having a characteristic odor.

Specific gravity \(<2.56> d^2_20: 0.955 – 0.960\)

Distilling range \(<2.57\>: 192 – 195^\circ\text{C}, not less than 95 vol\%.

Dimethylformamide  
See \( N,N \)-dimethylformamide.

\( N,N \)-Dimethylformamide  
\( \text{HCON(CH}_3\text{)}_2 \) [K 8500, Special class]  
Read absorbance as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\) (in a 1-cm cell, using water as the blank): the absorbance is not more than 0.60 at 270 nm, not more than 0.15 at 280 nm, and not more than 0.05 at 300 nm.

Dimethylglyoxime  
\( C_9H_{12}O_2 \) [K 8498, Special class]

Dimethylglyoxime-thiosemicarbazide TS  
Solution A: Dissolve 0.5 g of dimethylglyoxime in hydrochloric acid to make 100 mL. Prepare before use. Solution B: Dissolve 0.1 g of thiosemicarbazide in 50 mL of water with the acid of warming if necessary, and add diluted hydrochloric acid (1 in 2) to make 100 mL. Prepare before use.

Mix 10 mL of each solution A and solution B, add diluted hydrochloric acid (1 in 2) to make 100 mL, and allow the mixture to stand for 1 hour. Use within 24 hours.

Dimethylglyoxime TS  
Dissolve 1 g of dimethylglyoxime in ethanol (95) to make 100 mL.

Dimethyl malonate  
\( C_9H_{12}O_2 \) Clear, colorless or pale yellow liquid.

Specific gravity \(<2.48\%>: 1.152 – 1.162\)

Water \(<2.48\%>: 0.3\%\).

Residue on ignition \(<2.44\%>: 0.1\%\).

\( N,N \)-Dimethyl-n-octylamine  
\( C_{11}H_{24}N \) Colorless liquid.

Refractive index \(<2.45\%>: n^D_20: 1.424\)

\( N,N \)-Dimethyl-p-phenylenediammonium dichloride  
\( C_{12}H_{14}N_2Cl_2 \) [K 8193, \( N,N \)-Dimethyl-p-phenylenediammonium dichloride, Special class]

\( N,N \)-Dimethyl-p-phenylenediammonium hydrochloride  
See \( N,N \)-dimethyl-p-phenylenediamine dichloride.

Dimethyl phthalate  
\( C_9H_{10}O_4 \) Colorless, clear liquid, having a slight aroma.

Refractive index \(<2.45\%>: n^D_20: 1.491 – 1.493\)

Purity—To 6.0 mL of a solution of Dimethyl phthalate in isooctane (1 in 100) add a solution of n-amyl alcohol in hexane (3 in 1000) to make 50 mL, and perform the test with 10 mL of this solution as directed under Liquid Chromatography \(<2.01\>\) according to the conditions described in the Assay under Ergocalciferol or Cholecalciferol: any peak other than the principal peak does not appear.

Dimethylsulfoxide  
\( (CH_3)_2SO \) [K 9702, Special class]

Dimethylsulfoxide for ultraviolet-visible spectrophotometry  
\( (CH_3)_2SO \) Colorless crystals or clear colorless liquid, having a characteristic odor. It is highly hygroscopic.

Congealing point \(<2.42\>\): not less than 18.3°C.

Purity—Read absorbance of dimethylsulfoxide for ultra-
violet-visible spectrophotometry, immediately after saturating with nitrogen, using water as the blank as directed under Ultraviolet-visible Spectrophotometry \(2.26\); its value is not more than 0.20 at 270 nm, not more than 0.09 at 275 nm, not more than 0.06 at 280 nm, and not more than 0.015 at 300 nm. It exhibits no characteristic absorption between 260 nm and 350 nm.

\(\text{Water } < 2.45; \text{ not more than 0.1\%}.\)

2,6-Dimethyl-4(2-nitrosoaryl)3,5-pyridinedicarboxylic acid dimethyl ester for thin-layer chromatography

\(\text{C}_{17}\text{H}_{17}\text{O}_{4}\) Irradiate xenon light at 50,000 lx of illumination for 8 hours to a methanol solution of nifedipine (1 in 100), and evaporate the methanol on a water bath. Recrystallize the residue 4 times from 1-propanol, and dry in a desiccator (in vacuum, phosphorus pentoxide). Pale blue crystals. Very soluble in chloroform, freely soluble in acetone, and practically insoluble in water.

\(\text{Melting point } < 2.60; 93 - 95^\circ\text{C}\)

Content: not less than 99.0%. Assay—Weigh accurately about 0.4 g of 2,6-dimethyl-4(2-nitrosoaryl)-3,5-pyridinedicarboxylic acid dimethyl ester for thin-layer chromatography, dissolve in 70 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS = 32.83 mg of \(\text{C}_{17}\text{H}_{17}\text{O}_{4}\).

3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide \(\text{C}_{16}\text{H}_{16}\text{BrN}_{5}\) Yellow crystals. Melting point: about 195\(^\circ\text{C}\) (with decomposition).

3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide TS Dissolve 5 g of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide in phosphate-buffered sodium chloride TS to make 1000 mL.

Dimorpholamine for assay [Same as the monograph Dimorpholamine. When dried, it contains not less than 99.0% of dimorpholamine (\(\text{C}_{20}\text{H}_{38}\text{N}_{4}\text{O}_{4}\)).

\(\text{m-Dinitrobenezene } \text{See 1,3-dinitrobenezene.}\)

1,2-Dinitrobenezene \(\text{C}_{6}\text{H}_{4}(\text{NO}_{2})_{2}\) Occurs as yellowish white to brownish yellow crystals or a crystalline powder.

Identification—Determine the infrared absorption spectrum of 1,2-dinitrobenezene as directed in the paste method under Infrared Spectrophotometry \(2.25\); it exhibits absorption at the wave numbers of about 3100 cm\(^{-1}\), 1585 cm\(^{-1}\), 1526 cm\(^{-1}\), 1352 cm\(^{-1}\), and 793 cm\(^{-1}\).

\(\text{Melting point } < 2.60; 116 - 119^\circ\text{C}\)

1,3-Dinitrobenezene \(\text{C}_{6}\text{H}_{5}(\text{NO}_{2})_{2}\) Light yellow to reddish-yellow crystals or crystalline powder.

\(\text{Melting point } < 2.60; 88 - 92^\circ\text{C}\).

Preserve in a light-resistant tight container.

\(\text{m-Dinitrobenezene TS } \text{See 1,3-dinitrobenezene TS.}\)

1,3-Dinitrobenezene TS Dissolve 1 g of 1,3-dinitrobenezene in 100 mL of ethanol (95%). Prepare before use.

\(\text{m-Dinitrobenezene TS, alkaline } \text{See 1,3-dinitrobenezene TS, alkaline.}\)

1,3-Dinitrobenezene TS, alkaline Mix 1 mL of tetramethylammonium hydroxide and 140 mL of ethanol (99.5), titrate a part of the mixture with 0.01 mol/L hydrochloric acid VS, and dilute the remainder with ethanol (99.5) to give a 0.008 mol/L solution. Before use, mix 40 mL of this solution with 60 mL of a solution of 1,3-dinitrobenezene in benzene (1 in 20).

2,4-Dinitrochlorobenezene See 1-chloro-2, 4-dinitrobenzene.

2,4-Dinitrofluorobenzene See 1-fluoro-2, 4-dinitrobenzene.

2,4-Dinitrophenol \(\text{C}_{6}\text{H}_{4}\text{OH(NO}_{2})_{2}\) Yellow crystals or crystalline powder.

\(\text{Melting point } < 2.60; 110 - 114^\circ\text{C}\)

2,4-Dinitrophenol TS Dissolve 0.5 g of 2,4-dinitrophenol in 100 mL of ethanol (95).

2,4-Dinitrophenylhydrazine \((\text{NO}_{2})_{2}\text{C}_{6}\text{H}_{3}\text{NHNH}_{2}\) [K 8460, Special class]

2,4-Dinitrophenylhydrazine-diethylene glycol dimethyl ether TS Dissolve 3 g of 2,4-dinitrophenylhydrazine in 100 mL of diethylene glycol dimethyl ether while heating, cool, and filter if necessary.

2,4-Dinitrophenylhydrazine-ethanol TS Dissolve 1.5 g of 2,4-dinitrophenylhydrazine in a cold mixture of 10 mL of sulfuric acid and 10 mL of water, then add a mixture of 1 volume of aldehyde-free ethanol and 3 volumes of water to make 100 mL, and filter if necessary.

2,4-Dinitrophenylhydrazine TS Dissolve 1.5 g of 2,4-dinitrophenylhydrazine in a cold mixture of 10 mL of sulfuric acid and 10 mL of water, then add water to make 100 mL, and filter if necessary.

Dinonyl phthalate \(\text{C}_{20}\text{H}_{38}\text{O}_{4}\) Colorless to pale yellow, clear liquid.

\(\text{Specific gravity } < 2.50; 0.967 - 0.987\)

\(\text{Acid value } < 1.13\); not more than 2.

Dioxane See 1,4-dioxane.

1,4-Dioxane \(\text{C}_{4}\text{H}_{8}\text{O}_{2}\) [K 8461, Special class]

Diphenhydramine \(\text{C}_{17}\text{H}_{19}\text{NO}\) [Same as the namesake monograph]

Diphenhydramine tannate [Same as the namesake monograph]

Diphenyl \(\text{C}_{12}\text{H}_{10}\) White crystals or crystalline powder, having a characteristic odor. Freely soluble in acetone and in diethyl ether, soluble in ethanol (95), and practically insoluble in water.

\(\text{Melting point } < 2.60; 68 - 72^\circ\text{C}\)

\(\text{Purity} \text{—Dissolve } 0.1 \text{ g of diphenyl in } 5 \text{ mL of acetone and use this solution as the sample solution. Perform the test with } 2 \mu\text{L of this solution as directed under Gas Chromatography } <2.02> \text{ according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of diphenyl by the area percentage method; it shows the purity of not less than 98.0\%.}\)

Operating conditions

Detector: Hydrogen flame-ionization detector.

Column: A glass tube about 3 mm in inside diameter and about 2 m in length, packed with 150 to 180 \(\mu\text{m}\) mesh siliceous earth for gas chromatography coated with 10\% of
polyethylene glycol 20 M for thin-layer chromatography.

Column temperature: A constant temperature of about 180°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of diphenyl is about 8 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of diphenyl obtained from 2 μL of the solution prepared by adding acetone to 1.0 mL of the sample solution to make 100 mL is 5% to 15% of the full scale.

Time span of measurement: About 3 times as long as the retention time of diphenyl beginning after the solvent peak.

Diphenylamine (C₆H₅)₂NH [K 8487, Special class]

Diphenylamine-acetic acid TS Dissolve 1.5 g of diphenylamine in 1.5 mL of sulfuric acid and acetic acid (100) to make 100 mL.

Diphenylamine-acetic acid (100) TS See diphenylamine-acetic acid TS.

Diphenylamine TS Dissolve 1 g of diphenylamine in 100 mL of sulfuric acid. Use the colorless solution.

9,10-Diphenylanthracene C₂₆H₁₈ Yellow crystalline powder. Soluble in diethyl ether, and practically insoluble in water.

Melting point <2.60°: about 248°C

1,4-Diphenylbenzene C₂₆H₂₀ White scaly crystals, having a slight aromatic odor. It is freely soluble in ethanol (99.5), and slightly soluble in water.

Identification—Determine the infrared absorption spectrum of 1,4-diphenylbenzene as directed in the potassium bromide disk method as di-bromide disk method under Infrared Spectrophotometry 〈2,25〉: it exhibits absorption at the wave numbers of about 3050 cm⁻¹, 3030 cm⁻¹, 2960 cm⁻¹, 1449 cm⁻¹, 1302 cm⁻¹, 1267 cm⁻¹, 1124 cm⁻¹, 995 cm⁻¹, 834 cm⁻¹, 740 cm⁻¹ and 680 cm⁻¹.

Diphenylcarbazide See 1,5-diphenylcarbonohydrazide.

Diphenylcarbazide TS See 1,5-diphenylcarbonohydrazide TS.

Diphenylcarbazone C₁₈H₁₄N₂O [K 8488, Special class]

1,5-Diphenylcarbonohydrazide C₁₃H₁₄N₄O [K 8487, Special class]

1,5-Diphenylcarbonohydrazide TS Dissolve 0.2 g of 1,5-diphenylcarbonohydrazide in 100 mL of a mixture of ethanol (95) and acetic acid (100) (9:1).

5% Diphenyl-95% dimethylpolysiloxane for gas chromatography Prepared for gas chromatography.

Diphenyl ether C₆H₄O Colorless crystals, having a geranium-like aroma. Dissolves in alcohol (95) and in diethyl ether, and practically insoluble in water.

Specific gravity <2.56> d₄° = 1.072 – 1.075

Boiling point <2.57>: 254 – 259°C

Melting point <2.60°>: 28°C

Diphenyl imidazole C₆H₅N₂ White crystals or crystaline powder, freely soluble in acetic acid (100), and sparingly soluble in methanol.

Melting point <2.60°>: 234 – 238°C

Loss on drying <2.41>: not more than 0.5% (0.5 g, 105°C, 3 hours).

Content: not less than 99.0%. Assay—Dissolve about 0.3 g of diphenyl imidazole, previously dried and weighed accurately, in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS).

Each mL of 0.1 mol/L perchloric acid VS = 22.03 mg of C₆H₅N₂

Diphenyl phthalate C₄H₆(COOC₆H₅)₂ White crystalline powder.

Melting point <2.60°>: 71 – 76°C

Purity Related substances—Dissolve 60 mg of diphenyl phthalate in 50 mL of chloroform and use this solution as the sample solution. Proceed with 10 μL of the sample solution as directed in the Assay under Tolnaftate Solution: any peak other than the principal peak at the retention time of about 8 minutes and the peak of the solvent does not appear. Adjust the detection sensitivity so that the peak height of diphenyl phthalate obtained from 10 μL of the sample solution is 50 to 100% of the full scale, and the time span of measurement is about twice as long as the retention time of diphenyl phthalate after the solvent peak.

1,1-Diphenyl-4-pyridinino-1-butene hydrochloride for thin-layer chromatography C₂₁H₂₅N.HCl To 1 g of diphenidole hydrochloride add 30 mL of 1 mol/L hydrochloric acid TS, and heat under a reflux condenser for 1 hour. After cooling, extract twice with 30 mL-portions of chloroform, combine the chloroform extracts, wash twice with 10 mL portions of water, and evaporate chloroform under reduced pressure. Recrystallize the residue from a mixture of diethyl ether and ethanol (95) (3:1), and dry in a desiccator (in vacuum, silica gel) for 2 hours. White crystals or crystalline powder.

Absorbance <2.24> E₅₅₆ cm⁻¹ (250 nm): 386 – 446 (10 mg, water, 1000 mL).

Melting point <2.60°>: 176 – 180°C

Content: not less than 99.0%. Assay—Dissolve about 0.2 g of 1,1-diphenyl-4-pyridinino-1-butene hydrochloride for thin-layer chromatography, previously weighed accurately, in 20 mL of acetic acid (100), add 20 mL of acetic anhydride, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 16.39 mg of C₂₁H₂₅N.HCl

Dipicolinic acid C₆H₅NO₂ White powder.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry 〈2.25〉: it exhibits absorption at the wave numbers of about 2630 cm⁻¹, 1701 cm⁻¹, 1576 cm⁻¹, 1416 cm⁻¹, 1300 cm⁻¹ and 1267 cm⁻¹.
Purity: Clarity and color of solution—Dissolve by warming 0.5 g in 20 mL of ethanol (99.5), and cool; a clear, colorless liquid.

Content: Not less than 98.0%. Assay—Weigh accurately about 0.1 g, add 25 mL of ethanol (99.5), dissolve by warming, cool, then titrate with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 8.356 mg of C₈H₇O₄N

Dipotassium hydrogen phosphate K₂HPO₄ [K 9017, Special class]

Disodium hydrogen phosphate-citric acid buffer solution, pH 5.3 Mix 100 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution and 38 mL of 1 mol/L citric acid TS for buffer solution, and add water to make 200 mL.

1 mol/L Disodium hydrogen phosphate TS for buffer solution Dissolve 174.18 g of diopotassium hydrogen phosphate in water to make 1000 mL.

Diprophyline C₆H₅N₂O₂ A white, powder or grain. Freely soluble in water, and slightly soluble in ethanol (95).

Identification—Determine the infrared absorption spectrum of the substance to be examined, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry (<2.25): it exhibits absorption at the wave numbers of about 3460 cm⁻¹, 3330 cm⁻¹, 1651 cm⁻¹, 1242 cm⁻¹, 1059 cm⁻¹, and 1035 cm⁻¹.

α,α'-Dipyridyl See 2,2'-bipyridyl.

Disodium chloromotropate dihydrate C₁₀H₁₄N₄O₄ [K 8316, Special class] Preserve in light-resistant containers.

Disodium hydrogen ethylenediamine tetaacetate dihydrate C₁₀H₁₂N₂Na₂O₈.2H₂O [K 9020, Special class]

0.1 mol/L Disodium hydrogen ethylenediamine tetaacetate TS Dissolve 37.2 g of disodium hydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL.

0.04 mol/L Disodium hydrogen ethylenediamine tetaacetate TS Dissolve 14.890 g of disodium hydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL.

0.4 mol/L Disodium hydrogen ethylenediamine tetaacetate TS, pH 8.5 Dissolve 148.9 g of disodium hydrogen ethylenediamine tetraacetate dihydrate in about 800 mL of water, adjust to pH 8.5 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Disodium ethylenediaminetetraacetate See disodium hydrogen ethylenediamine tetraacetate dihydrate.

Disodium ethylenediaminetetraacetate copper See copper (II) disodium ethylenediamine tetraacetate tetrahydrate.

0.1 mol/L Disodium ethylenediaminetetraacetate TS See 0.1 mol/L disodium hydrogen ethylenediamine tetaacetate TS.

Disodium hydrogen phosphate, anhydrous Na₂HPO₄ [K 9020, Special class]

Disodium hydrogen phosphate-citric acid buffer solution, pH 3.0 Dissolve 35.8 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL. To this solution add a solution of citric acid monohydrate (21 in 1000) to adjust the pH to 3.0.

Disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 Dissolve 21.02 g of citric acid monohydrate in water to make 1000 mL, and adjust the pH to 4.5 with a solution prepared by dissolving 35.82 g of disodium hydrogen phosphate 12-water in water to make 1000 mL.

Disodium hydrogen phosphate-citric acid buffer solution, pH 5.0 Dissolve 7.1 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL, and adjust to pH 5.0 with a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL.

Disodium hydrogen phosphate-citric acid buffer solution, pH 5.4 Dissolve 1.05 g of citric acid monohydrate and 2.92 g of disodium hydrogen phosphate dodecahydrate in 200 mL of water, and adjust the pH with phosphoric acid or sodium hydroxide TS, if necessary.

Disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 Dissolve 71.6 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To this solution add a solution, prepared by dissolving 21.0 g of citric acid monohydrate in water to make 1000 mL, until the pH becomes 6.0 (ratio of volume: about 63:37).

Disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 To 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS add a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL to adjust pH 6.0.

Disodium hydrogen phosphate-citric acid buffer solution, pH 7.2 Dissolve 7.1 g of disodium hydrogen phosphate in water to make 1000 mL. Adjust this solution to pH 7.2 with a solution prepared by dissolving 5.3 g of citric acid monohydrate in water to make 1000 mL.

Disodium hydrogen phosphate-citric acid buffer solution, pH 7.5 Dissolve 5.25 g of citric acid monohydrate in water to make 1000 mL. Add this solution to 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS to adjust the pH to 7.5.

Disodium hydrogen phosphate-citric acid buffer solution for penicillium origin β-galactosidase, pH 4.5 Dissolve 71.6 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL, and adjust the pH to 4.5 with a solution prepared by dissolving 21.0 g of citric acid monohydrate in water to make 1000 mL (volume ratio: about 44:56).

Disodium hydrogen phosphate dodecahydrate Na₂HPO₄.12H₂O [K 9019, Special class]

Disodium hydrogen phosphate for pH determination Na₂HPO₄ [K 9020, for pH determination]

Disodium hydrogen phosphate TS Dissolve 12 g of disodium hydrogen phosphate dodecahydrate in water to make 100 mL (0.3 mol/L).
0.05 mol/L Disodium hydrogen phosphate TS Dissolve 7.0982 g of disodium hydrogen phosphate in water to make 1000 mL.

0.5 mol/L Disodium hydrogen phosphate TS Dissolve 70.982 g of disodium hydrogen phosphate in water to make 1000 mL.

Disodium 1-nitroso-2-naphthol-3,6-disulfonate

C_{10}H_{5}NNa_{2}O_{8}S_{2} Yellow crystals or crystalline powder. Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry 2.255: it exhibits absorption at the wave numbers of about 3400 cm\(^{-1}\), 1639 cm\(^{-1}\), 1451 cm\(^{-1}\), 1270 cm\(^{-1}\), 1231 cm\(^{-1}\), 1173 cm\(^{-1}\), 1049 cm\(^{-1}\), 848 cm\(^{-1}\) and 662 cm\(^{-1}\).

Preserve in a light-resistant tight container.

Dissolved acetylene C_{2}H_{2} [K 1902]

Distigmine bromide for assay C_{22}H_{32}Br_{2}N_{4}O_{4} [Same as the monograph Distigmine Bromide. It contains not less than 99.0% of distigmine bromide (C_{22}H_{32}Br_{2}N_{4}O_{4}), calculated on the anhydrous basis.]

Distilled water for injection [Use the water prescribed by the monographs of Water for Injection or Sterile Water for Injection in Containers. Prepared by distillation. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of relevant test.]

2,6-Di-tert-butylcresol [(CH\(_{3}\))\(_{2}\)C\(_{6}\)H\(_{4}\)(CH\(_{3}\))OH A white, crystalline powder. Freely soluble in ethanol (95). Melting point 2.60: 69 - 71°C

Residue on ignition 2.44: not more than 0.05%.

2,6-Di-tert-butylcresol TS Dissolve 0.1 g of 2,6-di-tert-butylcresol in ethanol (95) to make 10 mL.

2,6-Di-tert-butyl-p-cresol See 2,6-di-tert-butylicresol.

2,6-Di-tert-butyl-p-cresol TS See 2,6-di-tert-butylcresol TS.

1,3-Di (4-pyridyl) propane C\(_{10}\)H\(_{14}\)N\(_{2}\) A pale yellow powder.

Melting point 2.60: 61 - 62°C

Water 2.48: less than 0.1%.

1,1'-[3,3'-Dithiobis(2-methyl-1-oxopropyl)]-1-diproline C\(_{18}\)H\(_{28}\)N\(_{2}\)O\(_{2}\)S\(_{2}\) White, crystals or crystalline powder. Sparingly soluble in methanol, and practically insoluble in water. Identification—Determine the infrared absorption spectrum of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-1-diproline according to potassium bromide disk method under Infrared Spectrophotometry 2.255: it exhibits absorption at the wave numbers of about 2960 cm\(^{-1}\), 1750 cm\(^{-1}\), 1720 cm\(^{-1}\), 1600 cm\(^{-1}\), 1480 cm\(^{-1}\), 1450 cm\(^{-1}\) and 1185 cm\(^{-1}\).

Purity Related substances—Dissolve 0.10 g of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-1-diproline in exactly 10 mL of methanol. Perform the test with this solution as directed in the Purity (3) under Captopril: any spot other than the principal spot at the RF value of about 0.2 does not appear.

Content: not less than 99.0%. Assay—Weigh accurately about 0.3 g of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-1-diproline, dissolve in 20 mL of methanol, add 50 mL of water, and titrate 2.50 with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through bluish green to blue (indicator: 3 drops of bromothymol blue TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 21.63 mg of C\(_{22}\)H\(_{32}\)N\(_{2}\)O\(_{4}\)

Dithiodiglycolic acid C\(_{4}\)H\(_{6}\)O\(_{2}\)S Prepared for amino acid analysis or biochemistry.

Dithiodipropionic acid C\(_{4}\)H\(_{8}\)O\(_{2}\) Prepared for amino acid analysis or biochemistry.

Dithiothreitol C\(_{4}\)H\(_{6}\)O\(_{2}\)S Crystals.

Melting point 2.60: about 42°C

Dithizone C\(_{4}\)H\(_{9}\)NHNHC\(_{6}\)N\(_{2}\)H\(_{2}\) [K 8490, Special class]

Dithizone solution for extraction Dissolve 30 mg of dithizone in 1000 mL of chloroform, and add 5 mL of ethanol (95). Store in a cold place. Before use, shake a suitable volume of the solution with one-half of its volume of diluted nitric acid (1 in 100), and use the chloroform layer after discarding the water layer.

Dithizone TS Dissolve 25 mg of dithizone in ethanol (95) to make 100 mL. Prepare before use.

Dopamine hydrochloride for assay C\(_{9}\)H\(_{11}\)NO\(_{2}\)HCl [Same as the monograph Dopamine hydrochloride. When dried, it contains not less than 99.0% of dopamine hydrochloride (C\(_{9}\)H\(_{11}\)NO\(_{2}\)HCl)].

Doxifluridine C\(_{8}\)H\(_{11}\)FN\(_{2}\)O\(_{3}\) [Same as the namesake monograph]

Doxifluridine for assay C\(_{8}\)H\(_{11}\)FN\(_{2}\)O\(_{3}\) [Same as the monograph Doxifluridine. When dried, it contains not less than 99.5% of dioxifluridine (C\(_{8}\)H\(_{11}\)FN\(_{2}\)O\(_{3}\)).]

Dragendorff’s TS Dissolve 0.85 g of bismuth subnitrate in 10 mL of acetic acid (100) and 40 mL of water with vigorous shaking (solution A). Dissolve 8 g of potassium iodide in 20 mL of water (solution B). Immediately before use, mix equal volumes of solution A, solution B and acetic acid (100). Store solution A and solution B in light-resistant containers.

Dragendorff’s TS for spraying Add 20 mL of diluted acetic acid (31) (1 in 5) to 4 mL of a mixture of equal volumes of solution A and solution B of Dragendorff’s TS. Prepare before use.

Dried human normal plasma powder Freeze-dried normal plasma obtained from healthy human.

Dried sodium carbonate Na\(_{2}\)CO\(_{3}\) [Same as the namesake monograph]

Droxidopa for assay C\(_{9}\)H\(_{12}\)NO\(_{3}\) [Same as the monograph Droxidopa. When dried, it contains not less than 99.5% of droxidopa (C\(_{9}\)H\(_{12}\)NO\(_{3}\)).]

Dydrogesterone for assay C\(_{21}\)H\(_{28}\)O\(_{2}\) [Same as the monograph Dydrogesterone. When dried, it contains not less than 99.5% of C\(_{21}\)H\(_{28}\)O\(_{2}\)].

Ebastine for assay C\(_{32}\)H\(_{39}\)NO\(_{2}\) [Same as the monograph

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Ebastine. When dried, it contains not less than 99.5% of ebastine (C₂₂H₂₉NO₅).

Ebacet sodium hydrate for assay C₂₀H₂₇NaO₅S.5H₂O
[Same as the monograph Ebacet Sodium Hydrate. It contains not less than 99.5% of ebacet sodium (C₂₀H₂₇NaO₅S), calculated on the anhydrous basis.]

E. coli protein Process E. coli cells (E. coli N4830/pTB281) retaining a plasmid deficient in the celmoleukin gene according to the celmoleukin purification process in the following order; (i) extraction, (ii) butylated vinyl polymer hydrophobic chromatography, (iii) carboxymethylated vinyl polymer ion-exchange column chromatography, and (iv) sulfopropyl-polymer ion-exchange chromatography, and during process (iv) collect the fractions corresponding to the celmoleukin elution position. Dialyze the fractions obtained in (iv) against 0.01 mol/L acetate buffer solution, pH 5.0, and take the dialysis solution as E. coli protein.

Identification—Clear and colorless solution.

Protein content: When determining the protein content using the Assay (I) Total protein content under Celmoleukin (Genetical Recombination), the protein content per mL is 0.1 to 0.5 mg.

E. coli protein stock solution A solution obtained by culturing a bacteria that contains a plasmid lacking the teceleukin gene but is otherwise exactly identical to the teceleukin-producing E. coli strain in every function except teceleukin production, and then purified using a purification technique that is more simple than that for teceleukin. Determine the amount of protein by Brodford method using bovine serum albumin as the standard substance. Store shielded from light at -70°C.

Eleutherose B for liquid chromatography C₁₇H₂₀O₄.H₂O A white crystalline powder. Sparingly soluble in methanol, slightly soluble in water, and very slightly soluble in ethanol (99.5). Melting point: 190 – 194°C.

Identification—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 261 nm and 265 nm.

Purity Related substances—Dissolve 1.0 mg in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the total area of peaks other than emetine from the sample solution is not larger than the peak of emetine from the standard solution (1).

Operating conditions
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Identification under Eleutherococcus Senticosus Rhizome.

Time span of measurement: About 3 times as long as the retention time of eleutherose B beginning after the solvent peak.

System suitability
Test for required detectability: To exactly 1 mL of the standard solution add methanol to make exactly 20 mL. Confirm that the peak area of eleutherose B obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Identification under Eleutherococcus Senticosus Rhizome.

EMB plate medium Melt eosin methylene blue agar medium by heating, and cool to about 50°C. Transfer about 20 mL of this medium to a Petri dish, and solidify horizontally. Place the dish with the cover slightly opened in the incubator to evaporate the inner vapor and water on the plate.

Emetine hydrochloride for assay C₂₉H₂₁N₂O₄.2HCl.xH₂O A white or light-yellow crystalline powder. Soluble in water.

Melting point <2.60>; about 250°C [with decomposition, after drying in a desiccator (reduced pressure below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours].

Absorbance <2.24> E₁cm(283 nm): 116 – 127 (10 mg, diluted methanol (1 in 2), 400 mL) [after drying in a desiccator (reduced pressure below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours.]

Purity Related substances—Dissolve 10 mg of emetine hydrochloride for assay in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μL each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following operating conditions. Determine the peak areas from both solutions by the automatic integration method: the total area of peaks other than emetine from the sample solution is not larger than the peak of emetine from the standard solution (1).

Operating conditions
Proceed the operating conditions in the Assay under Ipecac except the detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the sensitivity so that the peak area of emetine obtained from 10 μL of the standard solution (2) can be measured by the automatic integration method, and the peak height of emetine obtained from 10 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of emetine beginning after the solvent peak.

Emetine for component determination See emetine for assay.

Emorfonyl for assay C₁₁H₁₅N₃O₃ [Same as the monograph Emorfonyl. When dried, it contains not less than 99.0% of emorfonyl (C₁₁H₁₅N₃O₃).]

Enalapril maleate C₂₀H₂₉N₂O₄,C₆H₄O₄ [Same as the namesake monograph]
dium by heating in a water bath, and adjust the pH to between 7.5 and 7.8. Add 10 g of lactose monohydrate previously dissolved in a small quantity of water, mix thoroughly, and add 1 mL of fuchsin-ethanol (95 TS). After cooling to about 50°C, add dropwise a freshly prepared solution of sodium bisulfite (1 in 10) until a light red color develops owing to reducing fuchsin, requiring about 10 to 15 mL of a solution of sodium sulfite heptahydrate (1 in 10). Dispense the mixture, and sterilize fractionally on each of three successive days for 15 minutes at 100°C.

**Endo’s plate medium** Melt Endo’s medium by heating, and cool to about 50°C. Transfer about 20 mL of this medium to a Petri dish, and solidify horizontally. Place the dishes with the cover slightly opened in the incubator to evaporate the inner vapor and water on the surface of the agar.

**Enflurane** C₇H₆ClF₂O [Same as the namesake monograph]

**Enzyme TS** The supernatant liquid is obtained as follows: To 0.3 g of an enzyme preparation potent in amylolytic and phosphorolytic activities, obtained from Aspergillus oryzae, add 10 mL of water and 0.5 mL of 0.1 mol/L hydrochloric acid TS, mix vigorously for a few minutes, and centrifuge. Prepare before use.

**Eosin** See eosin Y.

**Eosin Y** C₉H₈Br₃Na₂O₅ Red, masses or powder.

**Identification**—To 10 mL of a solution (1 in 1000) add 1 drop of hydrochloric acid: yellow-red precipitates appear.

**Eosin methyl blue agar medium** Dissolve by boiling 10 g of casein peptone, 2 g of dipotassium hydrogenphosphate and 25 to 30 g of agar in about 900 mL of water. To this mixture add 10 g of lactose monohydrate, 20 mL of a solution of eosin Y (1 in 50), 13 mL of a solution of sodium bisulfite (1 in 10) until a light red color develops owing to reducing fuchsin, requiring about 10 to 15 mL of a solution of sodium sulfite heptahydrate (1 in 10). Dispense the mixture, and sterilize fractionally on each of three successive days for 15 minutes at 100°C.

**Ephedrine hydrochloride** C₁₀H₁₅NO.HCl [Same as the namesake monograph]

**Ephedrine hydrochloride for assay** See ephedrine hydrochloride.

**6-Epideoxytetracycline hydrochloride** C₂₀H₂₁NO₇.HCl Yellow to dark yellow, crystals or crystalline powder.

**Purity** Related substances—Dissolve 20 mg of 6-epideoxytetracycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20 μL of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than 4-epoxytetracycline is not more than 10%.

**Eriochrome black T** C₉H₁₂N₃NaO₆S [K 8736, Special class]

**Eriochrome black T-sodium chloride indicator** Mix 0.1 g of eriochrome black T and 10 g of sodium chloride, and triturate until the mixture becomes homogeneous.

**Eriochrome black T TS** Dissolve 0.3 g of eriochrome black T and 2 g of hydroxyamphetamine chloride in methanol to make 50 mL. Use within 1 week. Preserve in light-resistant containers.

**Erythromycin B** C₃₇H₆₇NO₁₂ White to light yellowish white powder.

**Purity** Related substances—Dissolve 10 mg of erythromycin B in 1 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 20 mL, and use this solution as the standard solution. Proceed with exactly 100 μL each of the sample solution and standard solution as directed in the Purity (3) Related substances under Erythromycin, and determine each peak area from the solutions by the automatic integration method: the total of areas of the peaks other than erythromycin B from the sample solution is not more than the peak area of erythromycin B from the standard solution.

**Erythromycin C** C₃₆H₆₅NO₁₃ White to light yellowish white powder.

**Purity** Related substances—Dissolve 10 mg of erythromycin C in 1 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 20 mL, and use this solution as the standard solution. Proceed with exactly 100 μL each of the sample solution and standard solution as directed in the Purity (3) Related substances under Erythromycin, and determine each peak area from the solutions by the automatic integration method: the total of areas of the peaks other than erythromycin C from the sample solution is not more than the peak area of erythromycin C from the standard solution.

**Essential oil** Same as the essential oil under the monograph.

**Etacrylic acid for assay** [Same as the monograph Etacrylic acid. When dried, it contains not less than 99.0% of etacrylic acid (C₁₂H₁₂Cl₂O₄)₃.]

**Ethanol** See ethanol (95).

**Ethanol, aldehyde-free** Transfer 1000 mL of ethanol (95) to a glass-stoppered bottle, add the solution prepared by dissolving 2.5 g of lead (II) acetate trihydrate in 5 mL of water, and mix thoroughly. In a separate container, dissolve 5 g of potassium hydroxide in 25 mL of warm ethanol (95), cool, and add this solution gently, without stirring, to the first so-
lution. After 1 hour, shake this mixture vigorously, allow to stand overnight, decant the supernatant liquid, and distil the ethanol.

**Ethanol, dehydrated** See ethanol (99.5).

**Ethanol, dilute** To 1 volume of ethanol (95) add 1 volume of water. It contains 47.45 to 50.00 vol% of C₂H₅OH.

**Ethanol, diluted** Prepare by diluting ethanol (99.5).

**Ethanol for alcohol number determination** See Alcohol Number Determination <1.01>.

**Ethanol for disinfection** [Same as the namesake monograph]

**Ethanol for gas chromatography** Use ethanol prepared by distilling ethanol (99.5) with iron (II) sulfate heptahydrate. Preserve in containers, in which the air has been displaced with nitrogen, in a dark, cold place.

**Ethanol-free chloroform** See chloroform, ethanol-free.

**Ethanol-isotonic sodium chloride solution** To 1 volume of ethanol (95) add 19 volumes of isotonic sodium chloride solution.

**Ethanol, methanol-free** See ethanol (95), methanol-free.

**Ethanol, neutralized** To a suitable quantity of ethanol (95) add 2 to 3 drops of phenolphthalein TS, then add 0.01 mol/L or 0.1 mol/L sodium hydroxide VS until a light red color develops. Prepare before use.

**Ethanol (95)** C₂H₅OH [K 8102, Special class]

**Ethenzamide** C₉H₁₁NO₂ [Same as the namesake monograph]

**Ether** See diethyl ether.

**Ether, anesthetic** C₆H₅OC₂H₅ [Same as the namesake monograph]

**Ether, dehydrated** See diethyl ether, dehydrated.

**Ether for purity of crude drug** See diethyl ether for purity of crude drug.

**Ethynylestradiol** C₂₀H₂₄O₂ [Same as the namesake monograph]

**3-Ethoxy-4-hydroxybenzaldehyde** C₉H₁₀O₃ White to pale yellowish white crystalline. Freely soluble in ethanol (95), and slightly soluble in water.

**Melting point** <2.60°: 76 – 78°C

**Content:** not less than 98.0%. Assay—Weigh accurately about 0.3 g of 3-ethoxy-4-hydroxybenzaldehyde, previously dried in a desiccator (phosphorous (V) oxide) for 4 hours, dissolve in 50 mL of N,N-dimethylacetamide, and titrate <2.50° with 0.1 mol/L sodium methoxide VS (indicator: thymol blue TS).

Each mL of 0.1 mol/L sodium methoxide VS = 16.62 mg of C₉H₁₀O₃

**p-Ethoxyphenol** See 4-ethoxyphenol.

**4-Ethoxyphenol** C₈H₁₀O₂ White to light yellow-brown crystals or crystalline powder. Freely soluble in ethanol (95), and very slightly soluble in water.

**Melting point** <2.60°: 62 – 68°C

**Purity**—Dissolve 0.5 g of 4-Ethoxyphenol in 5 mL of ethanol (95), and use this solution as the sample solution. Perform the test as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of substance other than 4-ethoxyphenol by the area percentage method: it is not more than 2.0%.

**Operating conditions**

Detector: Thermal conductivity detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with 180- to 250-μm siliceous earth for gas chromatography coated with methyl-silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 150°C.

Carrier gas: Herium.

Flow rate: Adjust the flow rate so that the retention time of 4-ethoxyphenol is about 5 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 4-ethoxyphenol obtained from 1 μL of the sample solution is not less than 50% of the full scale.

Time span of measurement: 3 times as long as the retention time of 4-ethoxyphenol beginning after the solvent peak.

**Ethyl acetate** CH₃COOC₂H₅ [K 8361, Special class]

**Ethyl aminobenzoate** C₉H₁₀O₂ [Same as the namesake monograph]

**Ethylbenzene** C₆H₅C₂H₅ A colorless liquid. Freely soluble in ethanol (99.5) and in acetone, and practically insoluble in water.

**Specific gravity** <2.56°: d₂₀°: 0.862 – 0.872

**Boiling point** <2.57°: about 135°C

**Ethyl benzoate** C₆H₅COOC₂H₅ Clear, colorless liquid.

**Refractive index** <2.45°: n₂₀°: 1.502 – 1.507

**Specific gravity** <2.56°: d₂₀°: 1.045 – 1.053

**Ethyl n-caprylate** C₁₀H₂₀O₂ Clear and colorless to almost colorless liquid.

**Specific gravity** <2.56°: d₂₀°: 0.864 – 0.871

**Purity** Related substances—Dissolve 0.1 g of ethyl n-caprylate in 10 mL of dioxane and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 5 μL of each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak areas other than ethyl n-caprylate from the sample solution is not larger than the peak area of ethyl n-caprylate from the standard solution (1).

**Operating conditions**

Proceed the operating conditions in the Assay under Men-
Ethyl parahydroxybenzoate \( \text{HOC}_2\text{H}_3\text{COOC}_2\text{H}_3 \) [Same as the namesake monograph]

2-Ethyl-2-phenylmalondiamide \( \text{C}_1\text{H}_3\text{O}_2\text{N}_2 \) White, odorless crystals. Soluble in ethanol (95), and very slightly soluble in water. Melting point: about 120°C (with decomposition).

Purity Related substances—To 5.0 mg of 2-ethyl-2-phenylmalondiamide add 4 mL of pyridine and 1 mL of bis-trimethylsilylaceton, shake thoroughly, and heat at 100°C for 5 minutes. After cooling, add pyridine to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 2 \( \mu \)L of the sample solution as directed under Gas Chromatography (2.02) according to the conditions in the Purity (3) under Primidone: any peak other than the peaks of 2-ethyl-2-phenylmalondiamide and the solvent does not appear. Adjust the detection sensitivity so that the peak height of 2-ethyl-2-phenylmalondiamide obtained from 2 \( \mu \)L of the sample solution is about 80% of the full scale, and the time range of measurement is about twice as long as the retention time of 2-ethyl-2-phenylmalondiamide beginning after the solvent peak.

Ethyl propionate \( \text{CH}_3\text{CH}_2\text{COOC}_2\text{H}_5 \) Colorless, clear liquid.

Specific gravity \( d^25_0 \): 0.890 – 0.892

Etidronate disodium for assay \( \text{C}_10\text{H}_15\text{NO}_2\cdot\text{P}_2 \) [Same as the monograph Ethidronate Disodium. When dried, it contains not less than 99.0% of \( \text{C}_10\text{H}_15\text{NO}_2\cdot\text{P}_2 \).]

Etilerfrine hydrochloride \( \text{C}_{10}\text{H}_{15}\text{NO}_2\cdot\text{HCl} \) [Same as the namesake monograph]

Etilerfrine hydrochloride for assay \( \text{C}_{10}\text{H}_{15}\text{NO}_2\cdot\text{HCl} \) [Same as the monograph Etilerfrine Hydrochloride. When dried, it contains not less than 99.0% of etilerfrine hydrochloride (\( \text{C}_{10}\text{H}_{15}\text{NO}_2\cdot\text{HCl} \)).]

Etizolam for assay \( \text{C}_{17}\text{H}_{15}\text{ClN}_4\text{S} \) [Same as the monograph Etizolam. When dried, it contains not less than 99.0% of etizolam (\( \text{C}_{17}\text{H}_{15}\text{ClN}_4\text{S} \)).]

Factor Xa It is prepared from lyophilization of Factor Xa which has been prepared from bovine plasma. White or pale yellow masses or powder.

Purity Clarity and color of solution—Dissolve 71 \( \text{nkat,} \text{2222} \) of it in 10 mL water; the solution is clear and colorless or pale yellow.

Content: not less than 75% and not more than 125% of the label.

Factor Xa TS Dissolve 71 \( \text{nkat,} \text{2222} \) of factor Xa in 10 mL of water.

Famotidine for assay \( \text{C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3 \) [Same as the monograph Famotidine. When dried, it contains not less than 99.0% of famotidine (\( \text{C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3 \)), and when proceed as directed in the Purity (3), the total related substance is not more than 0.4%.]

Fatty oil Same as the fatty oil under the monograph.

Fehling’s TS The copper solution—Dissolve 34.66 g of copper (II) sulfate pentahydrate in water to make 500 mL. Keep this solution in a glass-stoppered bottles in well-filled. The alkaline tartrate solution—Dissolve 173 g of potassium sodium tartrate tetrahydrate and 50 g of sodium hy-
droxide in water to make 500 mL. Preserve this solution in a polyethylene container.

Before use, mix equal volumes of both solutions.

**Fehling’s TS for amylolytic activity test** The copper solution—Dissolve 34.660 g of copper (II) sulfate pentahydrate, accurately weighed, in water to make exactly 500 mL. Preserve this solution in well-filled, glass-stoppered bottles.

The alkaline tartrate solution—Dissolve 173 g of potassium sodium tartrate tetrahydrate and 50 g of sodium hydroxide in water to make exactly 500 mL. Preserve this solution in polyethylene containers.

Before use, mix exactly equal volumes of both solutions.

**Ferric ammonium citrate** See ammonium iron (III) citrate.

**Ferric ammonium sulfate** See ammonium iron (III) sulfate dodecahydrate.

**Ferric ammonium sulfate TS** See ammonium iron (III) sulfate TS.

**Ferric ammonium sulfate TS, dilute** See ammonium iron (III) sulfate TS, dilute.

**Ferric chloride** See iron (III) chloride hexahydrate.

**Ferric chloride-acetic acid TS** See iron (III) chloride-acetic acid TS.

**Ferric chloride-iodine TS** See iron (III) chloride-iodine TS.

**Ferric chloride-methanol TS** See iron (III) chloride-methanol TS.

**Ferric chloride-pyridine TS, anhydrous** See iron (III) chloride-pyridine TS, anhydrous.

**Ferric chloride TS** See iron (III) chloride TS.

**Ferric chloride TS, acidic** See iron (III) chloride TS, acidic.

**Ferric chloride TS, dilute** See iron (III) chloride TS, dilute.

**Ferric nitrate** See iron (III) nitrate TS.

**Ferric nitrate TS** See iron (III) nitrate TS.

**Ferric perchlorate** See iron (III) perchlorate hexahydrate.

**Ferric perchlorate-dehydrated ethanol TS** See iron (III) perchlorate-dehydrated ethanol TS.

**Ferric salicylate TS** See iron salicylate TS.

**Ferric sulfate** See iron (III) sulfate n-hydrate.

**Ferric sulfate TS** See iron (III) sulfate TS.

**Ferrous ammonium sulfate** See ammonium iron (II) sulfatehexahydrate.

**Ferrous sulfate** See iron (II) sulfate heptahydrate.

**Ferrous sulfate TS** See iron (II) sulfate TS.

**Ferrous sulfide** See iron (II) sulfide.

**Ferrous tartrate TS** See iron (II) tartrate TS.

**Ferrous thiocyanate TS** See iron (II) thiocyanate TS.

**Ferrous trisodium pentacyanoamine TS** See iron (II) trisodium pentacyanoamine TS.

**(E)-Ferulic acid** C_{16}H_{18}O_{4} White to light yellow, crystals or crystalline powder. Freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water.

Melting point: 173 – 176°C.

**Identification**—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry. It exhibits maxima between 215 nm and 219 nm, between 231 nm and 235 nm, and between 318 nm and 322 nm.

**Purity** Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 2 µL of this solution as directed in the Identification (II) under Hochuekkito Extract: no spot other than the principle spot of around Rf 0.6 appears.

**Fetal calf serum** Serum obtained from fetal calves. Interleukin-2 dependent cell growth suppression substance is removed by heat at 56°C for 30 min before use.

**Fibrinogen** Fibrinogen is prepared from human or bovine blood by fractional precipitation with ethanol or ammonium sulfate. It may contain citrate, oxalate and sodium chloride. A white, amorphous solid. Add 1 mL of isotonic sodium chloride solution to 10 mg of fibrinogen. It, when warmed to 37°C, dissolves with a slight turbidity, and clots on the subsequent addition of 1 unit of thrombin.

**1st Fluid for disintegration test** See 1st fluid for dissolution test.

**1st Fluid for dissolution test** Dissolve 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid and water to make 1000 mL. It is clear and colorless, and has a pH of about 1.2.

**Fixed oil** Same as the vegetable oils under the monograph.

**Flecainide acetate** C_{17}H_{20}F_{6}N_{2}O_{3}.C_{2}H_{4}O_{2} [Same as the namesake monograph]

**Flecainide acetate for assay** C_{17}H_{20}F_{6}N_{2}O_{3}.C_{2}H_{4}O_{2} [Same as the monograph Flecainide Acetate. When dried, it contains not less than 99.0% of flecainide acetate (C_{17}H_{20}F_{6}N_{2}O_{3}.C_{2}H_{4}O_{2}). Additionally, when perform the test as directed in the Purity (3), the sample solution does not show the spot corresponding to the spot obtained from the standard solution, and when perform the test as directed in the Purity (4), the total area of the peaks other than flecainide is not larger than the peak area of flecainide from the standard solution.]

9-Fluorenylmethyl chloroformate C_{13}H_{11}ClO_{2} White crystals or crystalline powder. Melting point <2.60º: 60 – 63ºC

**Flopropione** C_{8}H_{16}O_{4} [Same as the namesake monograph]

**Flopropione for assay** C_{8}H_{16}O_{4} [Same as the monograph Flopropione. It contains not less than 99.0% of flopropione (C_{8}H_{16}O_{4}: 182.17), calculated on the dehydrated basis.]

**Fluid thioglycolate medium** See the Sterility Test <4.06>.
Fluocinolone acetonide C_{24}H_{30}F_{2}O_{6} [Same as the namesake monograph]

9-Fluorenymethyl chloroformate C_{12}H_{11}ClO_{2} Prepared for amino acid analysis or biochemistry.

Fluorescein C_{2}H_{5}O_{3} An yellowish red powder.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 1597 cm~^{-1}, 1466 cm~^{-1}, 1389 cm~^{-1}, 1317 cm~^{-1}, 1246 cm~^{-1}, 1247 cm~^{-1}, 1213 cm~^{-1}, 1114 cm~^{-1} and 849 cm~^{-1}.

Fluorescein sodium C_{20}H_{10}Na_{2}O_{5} [Same as the namesake monograph].

Fluorescein sodium TS Dissolve 0.2 g of fluorescein sodium in water to make 100 mL.

4-Fluorobenzoic acid C_{6}H_{5}FO_{2} White, crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 1684 cm~^{-1}, 1606 cm~^{-1} and 1231 cm~^{-1}.

**Melting point** 182 – 188°C

1-Fluoro-2,4-dinitrobenzene C_{6}H_{3}(NO_{2})_{2}F Light yellow liquid or crystalline masses. Melting point: about 25°C.

**Identification**—Determine the infrared absorption spectrum as directed in the liquid film method as directed under Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 3110 cm~^{-1}, 1743 cm~^{-1}, 1546 cm~^{-1}, 1538 cm~^{-1}, 1365 cm~^{-1}, 1334 cm~^{-1}, 1262 cm~^{-1} and 743 cm~^{-1}.

Preserve in a light-resistant tight container.

7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole C_{19}H_{13}ClF_{3}O_{7} Prepared for amino acid analysis or biochemistry.

Flurazepam for assay C_{21}H_{18}ClF_{5}N_{3}O Prepared as the monograph Flurazepam. When dried, it contains not less than 99.0% of flurazepam (C_{21}H_{18}ClF_{5}N_{3}O).

Flutoprazepam for assay C_{19}H_{16}ClF_{5}N_{2}O Prepared as the monograph Flutoprazepam. When dried, it contains not less than 99.5% of flutoprazepam (C_{19}H_{16}ClF_{5}N_{2}O).

Folic acid C_{19}H_{19}N_{7}O_{6} [Same as the namesake monograph]

Folin’s TS Place 20 g of sodium tungstate (VI) dihydrate, 5 g of sodium molybdate (VI) dihydrate and about 140 mL of water in a 300-mL volumetric flask, add 10 mL of diluted phosphoric acid (17 in 20) and 20 mL of hydrochloric acid, and boil gently using a reflux condenser with ground-glass joints for 10 hours. To the mixture add 30 g of lithium sulfate monohydrate and 10 mL of water, and then add a very small quantity of bromine to change the deep green color of the solution to yellow. Remove the excess bromine by boiling for 15 minutes without a condenser, and cool. Add water to make 200 mL, and filter through a glass filter. Store it free from dust. Use this solution as the stock solution, and dilute with water to the directed concentration before use.

Folin’s TS, dilute Titrate 2.50 Folin’s TS with 0.1 mol/L sodium hydroxide VS (indicator: phenolphthalein TS), and determine the acid concentration. Prepare by adding water to Folin’s TS so the acid concentration is 1 mol/L.

**Formaldehyde solution** HCHO [K 8872, Special class]

**Formaldehyde solution-sulfuric acid TS** Add 1 drop of formaldehyde solution to 1 mL of sulfuric acid. Prepare before use.

**Formaldehyde solution TS** To 0.5 mL of formaldehyde solution add water to make 100 mL.

**Formaldehyde TS, dilute** See Test Methods for Plastic Containers <7.02>.

**Formalin** See formaldehyde solution.

**Formalin TS** See formaldehyde solution TS.

**Formalin-sulfuric acid TS** See formaldehyde solution-sulfuric acid TS.

**Formamide** HCONH_{2} [K 8873, Special class]

**Formamide for Karl Fischer method** HCONH_{2} [K 8873, Special class; water content per g of formamide for Karl Fischer method should be not more than 1 mg.]

**Formic acid** HCOOH [K 8824, Special class, specific gravity: not less than 1.21].

2-Formylbenzoic acid CHOC_{6}H_{4}COOH White crystals. Melting point: 97 – 99°C

**Content**: not less than 99.0%. Assay—Weigh accurately about 0.3 g of 2-formylbenzoic acid, previously dried (in vacuum, phosphorus (V) oxide, 3 hours), dissolve in 50 mL of freshly boiled and cooled water, and titrate 2.50 with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenol red TS).

Each mL of 0.1 mol/L sodium hydroxide VS = 15.01 mg of C_{6}H_{4}O_{2}

**Freund’s complete adjuvant** A suspension of 5 mg of mycobacteria of Corynebacterium butyricum, killed by heating, in 10 mL of a mixture of mineral oil and aracel A (17:3).

**Fruuctose** C_{6}H_{12}O_{6} [Same as the namesake monograph]

**Fuchsin** A lustrous, green, crystalline powder or mass, slightly soluble in water and in ethanol (95). **Loss on drying** 2.41: 17.5 – 20.0% (1 g, 105°C, 4 hours). **Residue on ignition** 2.44: not more than 0.1% (1 g).

**Fuchsin-ethanol TS** Dissolve 11 g of fuchsin in 100 mL of ethanol (95).

**Fuchsin-sulfuric acid TS** Dissolve 0.2 g of fuchsin in 120 mL of hot water, and allow the solution to cool. Add a solution prepared by dissolving 2 g of anhydrous sodium sulfate in 20 mL of water, then add 2 mL of hydrochloric acid and water to make 200 mL, and allow to stand for at least 1 hour. Prepare before use.

**Fumaric acid for thin-layer chromatography** C_{4}H_{4}O_{4} White, crystalline powder, odorless, and has a characteristic acid taste.

**Purity**—Perform the test as directed in the Identification (5) under Clemastine Fumarate: any spot other than the principal spot at the RT value of about 0.8 does not appear.
Fuming nitric acid  See nitric acid, fuming.

Fuming sulfuric acid  See sulfuric acid, fuming.

Furfural  C₅H₄O₂  A clear, colorless liquid.

Specific gravity  1.256  d₅₁₀:  1.600 – 1.165

Distillation range  257°  160 – 163°C, not less than 95 vol%.

D-Galactosamine hydrochloride  C₁₇H₂₄O₁₀·HCl  White powder. Melting point: about 180°C (with decomposition).

Optical rotation  <2.49° [α]D: +90 – +97° (1 g, water, 100 mL, 100 mm).

Galactose  See D-galactose.

D-Galactose  C₆H₁₂O₆  White crystals, granules or powder.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25: it exhibits absorption at the wave numbers of about 3390 cm⁻¹, 3210 cm⁻¹, 3140 cm⁻¹, 1151 cm⁻¹, 1068 cm⁻¹, 956 cm⁻¹, 836 cm⁻¹, 765 cm⁻¹ and 660 cm⁻¹.

Optical rotation  <2.49° [α]D: +79 – +82° (desiccator (silica gel), 2.5 g after drying for 18 hours, diluted ammonia solution (28) (1 in 300), 25 mL, 100 mm).

Gallic acid  See gallic acid monohydrate.

Gallic acid monohydrate  C₆H₄(OH)₂COOH·H₂O  White to pale yellowish white, crystals or powder.

Melting point  <2.60°: about 260°C (with decomposition).

Gelatin  [Same as the namesake monograph]

Gelatin, acid-treated  [Same as the monograph Gelatin. Its isoelectric point is at pH between 7.0 and 9.0]

Gelatin peptone  See peptone, gelatin.

Gelatin-phosphate buffer solution  pH 7.0  Dissolve 1.15 g of sodium dihydrogen phosphate dihydrate and 5.96 g of disodium hydrogen phosphate dodecahydrate and 5.4 g of sodium chloride in 500 mL of water. Dissolve 1.2 g of gelatin to this solution by heating, and after cooling add water to make 600 mL.

Gelatin-phosphate buffer solution, pH 7.4  To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution add 39.50 mL of 0.2 mol/L sodium hydroxide VS and 50 mL of water. Dissolve 0.2 g of gelatin to this solution by heating, then after cooling adjust to pH 7.4 with 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL.

Gelatin-phosphate buffer solution, pH 8.0  Dissolve 40 g of 2-amino-2-hydroxyethyl-1,3-propanediol and 5.4 g of sodium chloride in 500 mL of water. Add 1.2 g of gelatin to dissolve by heating, adjust to pH 8.0 with dilute hydrochloric acid after cooling, and add water to make 600 mL.

Gelatin TS  Dissolve 1 g of gelatin in 50 mL of water by gentle heating, and filter if necessary. Prepare before use.

Geniposide for assay  Use geniposide for thin-layer chromatography meeting the following additional specifications.

Absorbance  <2.24° E₁cm (240 nm): 249 – 269 [10 mg dried in a desiccator (reduced pressure of not exceeding 0.67 kPa, phosphorus (V) oxide) for 24 hours, diluted methanol (1 in 2), 500 mL].

Purity  Related substances—Dissolve 5 mg of geniposide for assay in 50 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 µL each of the sample solution and standard solution (1) as directed under Liquid Chromatography 2.01 according to the following conditions, and measure each peak area of the both solutions by the automatic integration method: the total area of the peaks other than geniposide from the sample solution is not larger than the peak area of geniposide from the standard solution (1). Operating conditions

Proceed as directed in the Assay under Gardenia Fruit except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of geniposide obtained from 10 µL of the standard solution (2) can be measured by the automatic integration method and the peak height of geniposide obtained from 10 µL of the standard solution (1) shows about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of geniposide beginning after the solvent peak.

Geniposide for component determination  See geniposide for assay.

Geniposide for thin-layer chromatography  C₁₇H₂₅O₁₀  White crystals or crystalline powder. Melting point: 159 – 163°C.

Purity  Related substances—Dissolve 1.0 mg of geniposide for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with 20 µL of this solution as directed in the Identification (2) under Gardenia Fruit: any spot other than the principal spot at the Rf value of about 0.3 does not appear.

Gentamicin B  C₁₉H₃₂N₄O₁₀  White to pale yellowish white powder. Very soluble in water, and practically insoluble in ethanol (95).

Content:  not less than 80.0%.  Assay—Dissolve a suitable amount of gentamicin B in 0.05 mol/L sulfuric acid TS to make the solution containing 0.1 mg of gentamicin B per mL, and use this solution as the sample solution. Perform the test with 5 µL of the sample solution as directed under Liquid Chromatography 2.01 according to the following conditions.
conditions, and measure each peak area by the automatic integration method. Calculate the amount of gentamicin B by the area percentage method.

Operating conditions

Apparatus, detector, column, column temperature, reaction coil, mobile phase, reagent, reaction temperature, flow rate of the mobile phase, and flow rate of the reagent: Proceed the operating conditions in the Assay under Isepamicin Sulfate.

Time span of measurement: About 3 times as long as the retention time of gentamicin B.

System suitability

Procede the system suitability in the Assay under Isepamicin Sulfate.

**Gentiopicroside for thin-layer chromatography**

C_{48}H_{82}O_{18} A white powder. Freely soluble in water and in methanol, and practically insoluble in diethyl ether. Melting point: about 110°C (with decomposition).

**Purity** Related substances—Dissolve 10 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed in the Identification (2) under Gentian: the spots other than the principal spot at the Rf value of about 0.4 from the sample solution are not more intense than the spot from the standard solution.

**Giemsa’s TS**

Dissolve 3 g of azure II-eosin Y and 0.8 g of azure II in 250 g of glycerin by warming to 60°C. After cooling, add 250 g of methanol, and mix well. Allow to stand for 24 hours, and filter. Store in tightly stoppered bottles.

Azure II-eosin Y is prepared by coupling eosin Y to azure II. Azure II is the mixture of equal quantities of methylene azure (azure I), prepared by oxidizing methylene blue, and methylene blue.

**Giemsa’s TS, dilute** See Test Methods for Plastic Containers <7.02>.

**[6]-Gingerol for assay**

[6]-Gingerol for thin-layer chromatography. However, it meets the following requirements:

\[ \text{Absorbance } \lt 2.24 \times e_{\text{281 nm}}^{1 \text{cm}} (281 \text{ nm}) : 101 - 112 \text{ mg} (7 \text{ mg, ethanol (99.5), 200 mL}) \]

**Purity** Related substances—Dissolve 5 mg of [6]-gingerol for assay in 5 mL of methanol, and use this as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than [6]-gingerol from the sample solution is not larger than the peak area of [6]-gingerol from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Hangekobokuto Extract.

Time span of measurement: About 6 times as long as the retention time of [6]-gingerol.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of [6]-gingerol obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (3) under Hangekobokuto Extract.

**[6]-Gingerol for component determination** See [6]-gingerol for assay.

**[6]-Gingerol for thin-layer chromatography**

C_{16}H_{16}O_{4} A yellow-white to yellow, liquid or solid. Freely soluble in methanol, in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

**Identification**—Determine the absorption spectrum of a solution of [6]-gingerol for thin-layer chromatography in ethanol (99.5) (7 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits a maximum between 279 nm and 283 nm.

**Purity** Related substances—Dissolve 1.0 mg of [6]-gingerol for thin-layer chromatography in exactly 2 mL of methanol. Perform the test with 10 μL of this solution as directed in the Identification under Ginger: any spot other than the principal spot at the Rf value of about 0.3 does not appear.

**Ginsenoside Re**

C_{48}H_{70}O_{27}.xH_{2}O A white crystalline powder. It is odorless.

**Purity**—Dissolve 1 mg in diluted methanol (3 in 5) to make 10 mL. Perform the test with 10 μL of this solution as directed under Liquid Chromatography <2.01> according to the conditions directed in the Assay under Ginseng: the total area of the peak other than ginsenoside Re and solvent peak is not more than 1/10 times the total peak area excluding the peak area of the solvent.

**Ginsenoside Re**

C_{68}H_{82}O_{27}.xH_{2}O A white crystalline powder. It is odorless.

**Purity**—Dissolve 1 mg in diluted methanol (3 in 5) to make 10 mL. Perform the test with 10 μL of this solution as directed under Liquid Chromatography <2.01> according to the conditions directed in the Assay under Ginseng: the total area of the peak other than ginsenoside Re and solvent peak is not more than 1/10 times the total peak area excluding the peak area of the solvent.

**Ginsenoside Rg2 for thin-layer chromatography**

C_{42}H_{73}O_{14} White, crystalline powder, having a slight, bitter taste. Freely soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether and in chloroform.

**Melting point** <2.60>: 194 – 196.5°C

**Purity** Related substances—Dissolve 1 mg of ginsenoside Rg2 for thin layer chromatography in 1 mL of methanol, and perform the test with 20 μL of this solution as directed in the Identification (2) under Ginseng: any spot other than the principal spot at the Rf value of about 0.4 does not appear.

**Glacial acetic acid** See acetic acid (100).

**Glacial acetic acid for nonaqueous titration** See acetic acid for nonaqueous titration.

**Glacial acetic acid-sulfuric acid TS** See acetic acid (100)-sulfuric acid TS.
γ-Globulin  A plasma protein obtained from human serum as Cohn’s II and III fractions. White crystalline powder. It contains not less than 98% of γ-globulin in the total protein.

δ-Glucosamine hydrochloride C6H11NO4.HCl White crystals or crystalline powder. 

Content: not less than 98%. Assay—Dissolve about 0.4 g of δ-glucosamine hydrochloride, accurately weighed, in 50 mL of water, add 5 mL of diluted nitric acid (1 in 3), and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS = 21.56 mg of C6H11NO4.HCl

Glucose C6H12O6 [Same as the namesake monograph]

Glucose detection TS Dissolve 1600 units of glucose oxidase, 16 mg of 4-aminopyridine, 145 units of peroxidase and 0.27 g of p-hydroxybenzoic acid in acetic acid (100) in 1 mL of the sample solution, add dilute ethanol to make exactly 200 mL.

Glucose detection TS for penicillin origin β-galactosidase Dissolve glucose oxidase (not less than 500 units), peroxidase (not less than 50 units), 0.01 g of 4-aminopyridine, and 0.1 g of phenol in phosphate buffer, pH 7.2 to make 100 mL.

Glucose oxidase Obtained from Aspergillus niger. White powder. It is freely soluble in water. It contains about 200 Units per mg. One unit indicates an amount of the enzyme which produces 1 μmol of δ-glucono-δ-lactone in 1 minute at 25°C and pH 7.0 from glucose used as the substrate.

Glucose-peptone medium for sterility test See soybean-casein digest medium

Glucose TS Dissolve 30 g of glucose in water to make 100 mL. Prepare as directed under Injections.

4-O-Glucosyl-5-O-methylvisamminol for thin-layer chromatography C23H30N6O7 White crystals or crystalline powder. It is freely soluble in acetic acid (100), sparingly soluble in dimethylsulfoxide, and practically insoluble in water.

Absorbance 2.24  E1%1cm (325 nm): 310 – 350 [2 mg, diluted acetic acid (100) (1 in 500), 200 mL].

Optical rotation 2.49 [α]D = 50 – 60° [0.1 g, diluted acetic acid (100) (1 in 2), 10 mL, 100 mm].

Purity Related substances—Prepare the sample solution by dissolving 5 mg of 7-(glutarylglycyl-L-arginylalanyl)-4-methylcoumarin in 0.5 mL of acetic acid (100), and perform the test as directed under Thin-layer Chromatography 2.03. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (15:12:10:3) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, allow the plate to stand for 30 minutes in a box filled with iodine vapors: any observable spot other than the principal spot at the Rf value of about 0.6 does not appear.

7-(Glutarylglycyl-L-arginylalanyl)-4-methylcoumarin TS Dissolve 5 mg of 7-(glutarylglycyl-L-arginylalanyl)-4-methylcoumarin in 0.5 to 1 mL of acetic acid (100), lyophilize, dissolve this in 1 mL of dimethylsulfoxide, and use this solution as the standard solution as solution A. Dissolve 30.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.6 g of sodium chloride in 400 mL of water, adjust the pH to 8.5 with dilute hydrochloric acid, add water to make 500 mL, and use this solution as solution B. Mix 1 mL of the solution A and 500 mL of the solution B before use.

Glutathione C10H17N3O6S [Same as the namesake monograph]

Glycerin C3H8O3 [Same as the monograph Concentrated Glycerin]

85% Glycerin C3H8O3 [Same as the monograph Glycerin]

Glycine C2H4NO2 [K 8291, Special class]

Glycolic acid C2H3O2

Glycerrhizinic acid for thin-layer chromatography C42H62O16 Colorless or white, sweet, crystalline powder. Freely soluble in hot water and in ethanol (95%), and practically insoluble in diethyl ether. Melting point: 213 – 218°C (with decomposition).

Purity Related substances—Dissolve 10 mg of glycerrhizinic acid for thin-layer chromatography in 5 mL of dilute ethanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed in the Identification under Glycerrhiza: the spots other than the principal spot at the Rf value of about 0.3 from the sample solution are not more intense than the spot from the standard solution.

Goat anti-ECP antibody Combine 1 volume of ECP standard substance (equivalent to about 1 mg of protein) and 1 volume of Freund’s complete adjuvant, and immunize goats subcutaneously in the back region with this solution 5 times at 2 week intervals. Harvest blood on the 10th day after completing the immunization to obtain goat antiserum. Goat anti-ECP antibody is obtained by preparing an immobilized ECP column in which ECP standard substance is bound to
spharose 4B and then purifying by affinity column chromatography.

**Description:** Clear and colorless solution.

**Identification:** When sodium lauryl sulfate-supplemented polyacrylamide gel electrophoresis is conducted under non-reducing conditions, the molecular weight of the major band is within the range of $1.30 \times 10^5$ to $1.70 \times 10^5$.

**Protein content:** When determining the protein content using Assay (1) under Celmoleukin (Genetical Recombination), the protein content per mL is 0.2 to 1.0 mg.

**Goat anti-ECP antibody TS** Dilute goat anti-ECP antibody with 0.1 mol/L carbonate buffer solution, pH 9.6 to prepare a solution containing 50 μg protein per mL.

**Griess-Romijn's nitric acid reagent** Triturate thoroughly 1 g of 1-naphthylamine, 10 g of sulfanilic acid and 1.5 g of zinc dust in a mortar.

**Storage**—Preserve in tight, light-resistant containers.

**Griess-Romijn's nitrous acid reagent** Triturate thoroughly 1 g of 1-naphthylamine, 10 g of sulfanilic acid and 89 g of tartaric acid in a mortar.

**Storage**—Preserve in tight, light-resistant containers.

**Guaiacol** CH$_3$OC$_6$H$_4$OH Clear, colorless to yellow liquid or colorless crystals, having a characteristic aroma. Sparingly soluble in water, and miscible with ethanol (95), with diethyl ether and with chloroform. Melting point: about 28°C.

**Purity**—Perform the test with 0.5 μL of guaiacol as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of guaiacol by the area percentage method: It showed the purity of not less than 99.0%.

**Operating conditions**
- **Detector:** Hydrogen flame-ionization detector
- **Column:** A glass column about 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography, 150- to 180-μm in particle diameter, coated with polyethylene glycol 20 M at the ratio of 20%.
- **Column temperature:** A constant temperature of about 200°C.
- **Carrier gas:** Nitrogen.
- **Flow rate:** Adjust the flow rate so that the retention time of guaiacol begins after the solvent peak.
- **Detection sensitivity:** Adjust the detection sensitivity so that the peak height of guaiacol obtained from 0.5 μL of guaiacol is 4 to 6 minutes.
- **Detection sensitivity:** Adjust the detection sensitivity so that the peak height of guaiacol obtained from 0.5 μL of guaiacol is 90% of the full scale.
- **Time span of measurement:** About 3 times as long as the retention time of guaiacol beginning after the solvent peak.

**Guaiacol for assay** C$_7$H$_8$O$_2$ Colorless to yellow clear liquid or colorless crystals with a characteristic, aromatic odor. Miscible with methanol and with ethanol (99.5), and sparingly soluble in water. Congealing point: 25 – 30°C.

**Identification**—Determine the infrared absorption spectrum of guaiacol for assay as directed in the ATR method <2.25>: it exhibits absorption at the wave numbers of about 1595 cm$^{-1}$, 1497 cm$^{-1}$, 1443 cm$^{-1}$, 1358 cm$^{-1}$, 1255 cm$^{-1}$, 1205 cm$^{-1}$, 1108 cm$^{-1}$, 1037 cm$^{-1}$, 1020 cm$^{-1}$, 916 cm$^{-1}$, 833 cm$^{-1}$, and 738 cm$^{-1}$.

**Purity**—Related substances—Perform the test with 0.5 μL of guaiacol for assay as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than the peak of guaiacol is not more than 2.0%.

**Operating conditions**
- **Detector:** A hydrogen flame-ionization detector.
- **Column:** A fused silica column 0.25 mm in inside diameter and 60 m in length, coated inside with polyethyleneoxide for gas chromatography in 0.25 to 0.5 μm in thickness.
- **Column temperature:** Raise the temperature from 100°C to 130°C at a rate of 5°C per minute, raise to 140°C at a rate of 2°C per minute, raise to 200°C at a rate of 15°C per minute, and maintain at 200°C for 2 minutes.
- **Injection port temperature:** 200°C.
- **Detector temperature:** 250°C.
- **Carrier gas:** Helium.
- **Flow rate:** Adjust the flow rate so that the retention time of guaiacol is about 8 minutes.
- **Split ratio:** 1:50.

**System suitability**
- **Test for required detectability:** Weigh accurately about 70 mg of guaiacol for assay, add methanol to make exactly 100 mL, and use this solution for the solution for system suitability test. Confirm that the peak area of guaiacol obtained from 1 μL of the solution for system suitability test is equivalent to 0.08 to 0.16% of that of guaiacol obtained when 0.5 μL of guaiacol for assay is injected.

**System performance:** When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of guaiacol are not less than 200,000 and not more than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of guaiacol is not more than 2.0%.

**Guaifenesin** C$_{10}$H$_{14}$O$_4$ [Same as the namesake monograph]

**Guanine** C$_5$H$_5$N$_5$O White to pale yellowish white powder.

**Absorbance** <2.24> Weigh accurately about 10 mg of guanine, dissolve in 20 mL of dilute sodium hydroxide TS, and add 2 mL of 1 mol/L hydrochloric acid TS and 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL. Determine the absorbances, $E_{1\%}$, of this solution at 248 nm and 273 nm: they are between 710 and 770, and between 460 and 500, respectively.

**Loss on drying** <2.41>: Not more than 1.5% (0.5 g, 105°C, 4 hours).

**Haloperidol for assay** C$_{19}$H$_{23}$ClFNO$_2$ [Same as the monograph Haloperidol]

**Hanus' TS** Dissolve 20 g of iodine monobromide in 1000 mL of acetic acid (100). Preserve in light-resistant, glass-stopped bottles, in a cold place.

**Heart infusion agar medium** Prepared for biochemical tests.

**Heavy hydrogenated solvent for nuclear magnetic resonance spectroscopy** Prepared for nuclear magnetic resonance spectroscopy. Heavy hydrogenated chloroform (CDCl$_3$), heavy hydrogenated dimethyl sulfoxide [(CD$_3$)$_2$O]}
with silica gel for 24 hours, methanol, 50 mL, 100 mm). Larger than the peak area of hesperidin obtained with the automatic integration method: the total area of the peaks hydroxyethylpiperazine-
<
under Liquid Chromatography each of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry.

After 24 hours, mix these two prepared solutions. Allow to stand for 8 hours in a wide-mouthed bottle without using a stopper, and filter.

Related substances—Dissolve 1 mg in 2 mL of diluted sulfuric acid (3 in 20) to make 40 mL. Prepare a desiccator (silica gel) for 24 hours, methanol, 500 mL.

Purity Related substances—Dissolve 1 mg in 2 mL of methanol. Proceed the test with 20 μL of this solution as directed in the Identification (6) under Hochuekkito Extract: no spot other than the principle spot of around Rf 0.3 appears.

Hexaammonium heptamolybdate-cerium (IV) sulfate TS Dissolve 2.5 g of hexaammonium heptamolybdate tetrhydrate and 1.0 g of cerium (IV) sulfate tetrahydrate in diluted sulfuric acid (3 in 50) to make 100 mL. Prepare before use.

Hexaammonium heptamolybdate-sulfuric acid TS Dissolve 1.0 g of hexaammonium heptamolybdate tetrhydrate in diluted sulfuric acid (3 in 20) to make 40 mL. Prepare before use.

Hexaammonium heptamolybdate tetrhydrate (NH₄)₂Mo₇O₂₄·4H₂O [K 8905, Special class]

Hexaammonium heptamolybdate TS dissolve 21.2 g of hexaammonium heptamolybdate tetrhydrate in water to make 200 mL (10%). Prepare before use.

Hexamethylenetetramine (CH₂)₄N₄ [K 8847, Special class]

Hexamethylenetetramine TS Dissolve 2.5 g of hexamethylenetetramine in 25 mL of water.

Hexane C₆H₁₄ [K 8848, Special class]

Hexane for liquid chromatography CH₃(CH₂)₅CH₃ Colorless, clear liquid. Miscible with ethanol (95), with diethyl ether, with chloroform and with benzene. Boiling point < 2.57>: about 69°C

Purity (1) Ultraviolet absorbive substances—Read the absorbances of hexane for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: not more than 0.3 at the wave-
length of 210 nm, and not more than 0.01 between 250 nm and 400 nm.

(2) Peroxide—To a mixture of 100 mL of water and 25 mL of dilute sulfuric acid add 25 mL of a solution of potassium iodide (1 in 10) and 20 g of hexane for liquid chromatography. Stopper tightly, shake, and allow to stand in a dark place for 15 minutes. Titrate \( < 2.50 \) this solution, while shaking well, with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

**n-Hexane for liquid chromatography** See hexane for liquid chromatography.

**Hexane for purity of crude drug** [K 8848, Special class] Use hexane meeting the following additional specification. Evaporate 300.0 mL of hexane for purity of crude drug in vacuum at a temperature not higher than 40°C, add the hexane to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of \( \gamma \)-BHC in hexane to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane to make exactly 100 mL. Further pipet 2 mL of this solution, add hexane to make exactly 100 mL, and use this solution as the standard solution I. Perform the test with exactly 1 \( \mu L \) each of the sample solution and standard solution I as directed under Gas Chromatography \( < 2.02 \) according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peak other than the solvent peak from the sample solution is not larger than the peak area of \( \gamma \)-BHC from the standard solution I.

**Operating conditions** Proceed the operating conditions in the Purity (2) under Crude Drugs Test \( < 5.01 \), except detection sensitivity and time span of measurement.

**Detection sensitivity:** Pipet 1 mL of the standard solution I, add hexane to make exactly 20 mL, and use this solution as the standard solution II. Adjust the detection sensitivity so that the peak area of \( \gamma \)-BHC obtained from 1 \( \mu L \) of the standard solution II can be measured by the automatic integration method, and the peak height of \( \gamma \)-BHC from 1 \( \mu L \) of the standard solution I is about 20% of the full scale.

**Time span of measurement:** About three times as long as the retention time of \( \gamma \)-BHC beginning after the solvent peak.

**Hexane for ultraviolet-visible spectrophotometry** [K 8848, Special class]. When determining the absorbance of hexane for ultraviolet-visible spectrophotometry as directed under Ultraviolet-visible Spectrophotometry \( < 2.24 \), using water as the blank solution, its value is not more than 0.10 at 220 nm and not more than 0.02 at 260 nm, and it has no characteristic absorption between 260 nm and 350 nm.

**n-Hexane for ultraviolet-visible spectrophotometry** See hexane for ultraviolet-visible spectrophotometry.

**Hexyl parahydroxybenzoate** \( C_{13}H_{18}O_3 \) White crystals or crystalline powder.

**Melting point** \( < 2.60 \): 49 – 53°C

**Content:** Not less than 98.0%. Assay—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS = 222.3 mg of \( C_{13}H_{18}O_3 \)

**Hirsutine** See hirsutine for thin-layer chromatography.

**Hirsutine for assay** \( C_{22}H_{28}N_2O_3 \) Hirsutine for thin-layer chromatography. It meets the following requirements. \( \text{Absorbance} \ < 2.24\% \): \( E_1^1cm \) (245 nm): 354 – 389 (5 mg calculated on the anhydrous basis, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

**Purity** Related substances—Dissolve 5 mg of hirsutine for assay in 100 mL of a mixture of methanol and dilute acetic acid (7:3), use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu L \) each of the sample solution and standard solution as directed under Liquid-chromatography \( < 2.02 \) according to the following conditions. Determine the peak area of each solution by the automatic integration method: the total area of the peaks other than hirsutine obtained from the sample solution is not larger than the peak area of hirsutine from the standard solution.

**Operating conditions**

**Detector, column, column temperature, mobile phase, and flow rate:** Proceed as directed in the operating conditions in the Assay under Uncaria Hook.

**Time span of measurement:** About 1.5 times as long as the retention time of hirsutine, beginning after the solvent peak.

**System suitability** Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 20 mL. Confirm that the peak area of hirsutine obtained with 20 \( \mu L \) of this solution is equivalent to 3.5 to 6.5% of that with 20 \( \mu L \) of the standard solution.

**System performance:** Proceed as directed in the system suitability in the Assay under Uncaria Hook.

**System repeatability:** When the test is repeated 6 times with 20 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hirsutine is not more than 1.5%.

**Hirsutine for thin-layer chromatography** \( C_{22}H_{28}N_2O_3 \) A white or light orange crystalline powder or powder. Very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water. Melting point: about 105°C.

**Identification**—Determine the absorption spectrum of a solution of hirsutine for thin-layer chromatography in a mixture of methanol and dilute acetic acid (7:3) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( < 2.24 \): it exhibits a maximum between 287 nm and 291 nm.

**Purity** Related substances—Dissolve 1.0 mg of hirsutine for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography \( < 2.60 \). Spot 10 \( \mu L \) of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); no spot other than the principal spot at around \( R_f \) 0.55 appears.

**L-Histidine** \( C_6H_9N_3O_2 \) [Same as the namesake monograph]

**L-Histidine hydrochloride** See L-histidine hydrochloride monohydrate.
1-Histidine hydrochloride monohydrate
\[ C_6H_7NO_2 \cdot HCl \cdot H_2O \quad [K \, 9050, \text{Special class}] \]

Homatropine hydrobromide \[ C_{16}H_{23}NO_3 \cdot HBr \quad [\text{Same as the namesake monograph}] \]

Honokiol \[ C_{19}H_{18}O_2 \cdot xH_2O \quad \text{Odorless white, crystals or crystalline powder.} \]

Purity—Dissolve 1 mg of honokiol in the mobile phase to make 10 mL, and use this solution as the sample solution. Perform the Liquid Chromatography \( <2.0D \) with 10 mL of the sample solution as directed in the Assay under Magnolia Bark: when measure the peak areas 2 times as long as the retention time of magnolone, the total area of peaks other than honokiol is not larger than 1/10 times the total area of the peaks other than the solvent peak.

Horseradish peroxidase An oxidase (Molecular weight: about 40,000) derived from horseradish.

Horse serum Collect the blood from horse in a flask, coagulate, and allow to stand at room temperature until the serum is separated. Transfer the separated serum in glass containers, and preserve at \(-20^\circ\text{C}\).

Human antithrombin III Serine protease inhibition factor obtained from normal plasma of human. It is a protein, which inhibits the activities of thrombin and activated blood coagulation factor X. It contains not less than 300 Units per mg protein. One unit indicates an amount of the antithrombin III which inhibits 1 unit of thrombin at 25°C under the existence of heparin.

Human chorionic gonadotrophin TS Weigh accurately a suitable amount of Human Chorionic Gonadotrophin according to the labeled amount, and dissolve in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2 so that each mL contains 80 human chorionic gonadotrophin Units.

Human insulin desamide substance-containing TS Dissolve 1.5 mg of Insulin Human in 1 mL of 0.01 mol/L hydrochloric acid TS, allow to stand at 25°C for 3 days, and when the procedure is run with this solution according to the conditions as directed in the Purity (1) under Insulin Human (Genetical Recombination), the solution contains about 5% of the desamide substance.

Human insulin dimer-containing TS Allow to stand Insulin Human (Genetical Recombination) at 25°C for 10 days or more, and dissolve 4 mg of this in 1 mL of 0.01 mol/L hydrochloric acid TS.

Human normal plasma Dissolve an amount of dried human normal plasma powder, equivalent to 1 mL of the normal plasma of human, in 1 mL of water. Store between 2 and 10°C, and use within one week.

Human serum albumin for assay White to pale-yellow powder. Albumin content is at least 99%. Convert to the dehydrate using the following water determination method.

Water content \( <2.4D \) \( (0.2 \text{ g, volumetric titration, direct titration}) \). However, in a dehydration solvent, use a mixture of pyridine for water determination and ethylene glycol for water determination \( (5:1) \).

Hydralazine hydrochloride \[ C_6H_7N_2 \cdot HCl \quad [\text{Same as the namesake monograph}] \]

Hydrazine hydrochloride for assay \[ C_6H_7N_2 \cdot HCl \quad [\text{Same as the monograph Hydralazine Hydrochloride. When dried, it contains not less than 99.0% of hydralazine hydrochloride (C_6H_7N_2 \cdot HCl).}] \]

Hydrazine monohydrate \[ NH_2NH_2 \cdot H_2O \quad \text{Colorless liquid, having a characteristic odor.} \]

Hydrazine sulfate See hydrazinium sulfate.

Hydrazinium sulfate \[ NH_2H_4SO_4 \quad [K \, 8992, \text{Special class}] \]

Hydrazinium sulfate TS Dissolve 1.0 g of hydrazinium sulfate in water to make 100 mL.

Hydrobromic acid \[ HBr \quad [K \, 8509, \text{Special class}] \]

Hydrochloric acid \[ HCl \quad [K \, 8180, \text{Special class}] \]

Hydrochloric acid-ammonium acetate buffer solution, pH 3.5 Dissolve 25 g of ammonium acetate in 45 mL of 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

Hydrochloric acid, dilute Dilute 23.6 mL of hydrochloric acid with water to make 100 mL (10%).

Hydrochloric acid-ethanol TS See hydrochloric acid-ethanol (95) TS.

Hydrochloric acid-ethanol (95) TS Dilute 23.6 mL of hydrochloric acid with ethanol to make 100 mL.

0.01 mol/L Hydrochloric acid-methanol TS To 20 mL of 0.5 mol/L hydrochloric acid TS add methanol to make 1000 mL.

0.05 mol/L Hydrochloric acid-methanol TS To 100 mL of 0.5 mol/L hydrochloric acid TS add methanol to make 1000 mL.

Hydrochloric acid-2-propanol TS Add 0.33 mL of hydrochloric acid to 100 mL of 2-propanol, mix, and store in a dark and cool place.

Hydrochloric acid-potassium chloride buffer solution, pH 2.0 To 10.0 mL of 0.2 mol/L hydrochloric acid VS add 88.0 mL of 0.2 mol/L potassium chloride TS, adjust the pH to 2.0 ± 0.1 with 0.2 mol/L hydrochloric acid VS or 0.2 mol/L potassium chloride TS, then add water to make 200 mL.

Hydrochloric acid, purified Add 0.3 g of potassium permanganate to 1000 mL of diluted hydrochloric acid (1 in 2), distill, discard the first 250 mL of the distillate, and collect the following 500 mL of the distillate.

0.001 mol/L Hydrochloric acid TS Dilute 10 mL of 0.1 mol/L hydrochloric acid TS with water to make 1000 mL.

0.01 mol/L Hydrochloric acid TS Dilute 100 mL of 0.1 mol/L hydrochloric acid TS with water to make 1000 mL.

0.02 mol/L Hydrochloric acid TS Dilute 100 mL of 0.2 mol/L hydrochloric acid TS with water to make 1000 mL.

0.05 mol/L Hydrochloric acid TS Dilute 100 mL of 0.5 mol/L hydrochloric acid TS with water to make 1000 mL.

0.1 mol/L Hydrochloric acid TS Dilute 100 mL of 1 mol/L hydrochloric acid TS with water to make 1000 mL.

0.2 mol/L Hydrochloric acid TS Dilute 18 mL of hydro-
chloric acid with water to make 1000 mL.

0.5 mol/L Hydrochloric acid TS Dilute 45 mL of hydrochloric acid with water to make 1000 mL.

1 mol/L Hydrochloric acid TS Dilute 90 mL of hydrochloric acid with water to make 1000 mL.

2 mol/L Hydrochloric acid TS Dilute 180 mL of hydrochloric acid with water to make 1000 mL.

3 mol/L Hydrochloric acid TS Dilute 270 mL of hydrochloric acid with water to make 1000 mL.

5 mol/L Hydrochloric acid TS Dilute 450 mL of hydrochloric acid with water to make 1000 mL.

6 mol/L Hydrochloric acid TS Dilute 540 mL of hydrochloric acid with water to make 1000 mL.

7.5 mol/L Hydrochloric acid TS Dilute 675 mL of hydrochloric acid with water to make 1000 mL.

10 mol/L Hydrochloric acid TS Dilute 1000 mL of hydrochloric acid with water to make 1000 mL.

Hydrocotarnine C21H30O5 [Same as the namesake Hydrocortisone

Hydrocotarnine hydrochloride hydrate for assay See hydrocotarnine hydrochloride hydrate for assay.

Hydrocortamine hydrochloride hydrate for assay C12H15NO3.HCl.H2O [Same as the monograph Hydro-...
tals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum according to the paste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3300 cm⁻¹, 1690 cm⁻¹, 1600 cm⁻¹, 1307 cm⁻¹, 1232 cm⁻¹ and 760 cm⁻¹.

**Melting point** <2.60>: 203 – 206°C

**Purity** Clarity—Dissolve 1 g of 3-hydroxybenzoic acid in 20 mL of methanol; the solution is clear.

**Content:** not less than 99.0%. Assay—Weigh accurately about 0.2 g of 3-hydroxybenzoic acid, dissolve in 20 mL of diluted ethanol (95) (1 in 2), and titrate <2.50 with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of cresol red TS) until the color of the solution changes from yellow to dark orange-red. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 13.81 mg of C₇H₆O₃

**p-Hydroxybenzoic acid** See parahydroxybenzoic acid.

**10-Hydroxy-2-(E)-decanoic acid for assay** 10-hydroxy-2-(E)-decanoic acid for thin-layer chromatography meeting the following additional specifications.

**Purity** Related substances—Dissolve 10 mg of 10-hydroxy-2-(E)-decanoic acid for assay in 100 mL of methanol, and use this solution as the standard solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of 10-hydroxy-2-(E)-decanoic acid from sample solution is not larger than the peak area of 10-hydroxy-2-(E)-decanoic acid from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Royal Jelly.

Time span of measurement: About 4 times as long as the retention time of 10-hydroxy-2-(E)-decanoic acid beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of 10-hydroxy-2-(E)-decanoic acid obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of 10-hydroxy-2-(E)-decanoic acid from 10 μL of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Component determination under Royal Jelly.

**10-Hydroxy-2-(E)-decanoic acid for component determination** See 10-hydroxy-2-(E)-decanoic acid for assay

**10-Hydroxy-2-(E)-decanoic acid for thin-layer chromatography** C₁₀H₁₈O₃ White crystalline powder. Very soluble in methanol, freely soluble in ethanol (99.5), soluble in diethyl ether, and slightly soluble in water.

**Identification**—Determine the absorption spectrum of a solution of 10-hydroxy-2-(E)-decanoic acid for thin-layer chromatography in ethanol (99.5) (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 206 nm and 210 nm.

**Melting point** <2.60>: 63 – 66°C

**Purity** Related substances—Dissolve 5.0 mg of 10-hydroxy-2-(E)-decanoic acid for thin-layer chromatography in 1 mL of diethyl ether. Perform the test with 20 μL of this solution as directed in the Identification under Royal Jelly: no spot other than the principal spot at around Rf value of 0.5 appears.

**d-3-Hydroxy-cis-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride** C₂₀H₂₄N₂O₃S.HCl To 9 g of diltiazem hydrochloride add 50 mL of ethanol (99.5), and dissolve by heating at 80°C. To this solution add slowly 50 mL of a solution of potassium hydroxide in ethanol (99.5) (33 in 500) dropwise, and heat for 4 hours with stirring. Cool in an ice bath, filter, and evaporate the filtrate to dryness. Dissolve the residue in ethanol (99.5), add slowly a solution of hydrochloric acid in ethanol (99.5) (59 in 250) to make acidic, and filter. Add diethyl ether slowly to the filtrate, and filter the crystals produced. To the crystals add ethanol (99.5), heat to dissolve, add 0.5 g of activated charcoal, allow to stand, and filter. After cooling the filtrate in an ice-methanol bath, filter the crystals formed, and wash with diethyl ether. Further, add ethanol (99.5) to the crystals, and heat to dissolve. After cooling, filter the crystals produced, and dry under reduced pressure. White crystals or crystalline powder, having a slight, characteristic odor.

**Purity**—Dissolve 50 mg of d-3-hydroxy-cis-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(p-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride in chloroform to make exactly 10 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), chloroform, water and acetic acid (100) (12:10:3:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly iodine TS on the plate: any spot other than the principal spot does not appear.

**Water** <2.49>: not more than 1.0% (0.5 g).

**Content:** not less than 99.0%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.5 g of d-3-hydroxy-cis-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(p-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50 with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS = 40.89 mg of C₂₀H₂₄N₂O₃S.HCl

**d-3-Hydroxy-cis-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(p-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride** See d-3-hydroxy-cis-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride.

**N-(2-Hydroxyethyl)isonicotinamide nitric ester** C₈H₁₄N₂O₄ A white crystalline powder.

**Identification**—Determine the infrared absorption spectrum of N-(2-hydroxyethyl)isonicotinamide nitric ester as di-
Hydroxylamine perchlorate-ethanol (99.5) TS

Dissolve 10 g of hydroxylammonium chloride in 20 mL of water, and add ethanol (95) to make 200 mL. To this solution add, with stirring, 150 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and filter. Prepare before use.

Hydroxylamine TS, alkaline

Mix equal volumes of a solution of hydroxylammonium chloride in methanol (7 in 100) and a solution of sodium hydroxide in methanol (3 in 25), and filter. Prepare before use.

Hydroxylammonium chloride, pH 3.1

See hydroxylammonium chloride TS, pH 3.1.

Hydroxylammonium chloride hydrochloride TS

Acidify 100 mL of a solution of iron (III) chloride hexahydrate in ethanol (95) (1 in 200) with hydrochloric acid, and dissolve 1 g of hydroxylammonium chloride in the solution.

Hydroxylammonium chloride TS

Dissolve 20 g of hydroxylammonium chloride in water to make 65 mL, transfer it to a separator, add 2 to 3 drops of thymol blue TS, then add ammonia solution (28) until the solution exhibits a yellow color. Shake well after adding 10 mL of a solution of sodium N,N-diethyldithiocarbamate trihydrate (1 in 25), allow to stand for 5 minutes, and extract this solution with 10 to 15 mL portions of chloroform. Repeat the extraction until 5 mL of the extract does not exhibit a yellow color, upon adding 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) and shaking it. Add 1 to 2 drops of thymol blue TS; add dropwise dilute hydrochloric acid to this aqueous solution until it exhibits a red color, then add water to make 100 mL.

Hydroxylammonium chloride TS, pH 3.1

Dispose 6.9 g of hydroxylammonium chloride in 80 mL of water, adjust the pH to 3.1 by adding dilute sodium hydroxide TS, and add water to make 100 mL.

Hydroxylamine perchlorate-ethanol (99.5) TS

Dilute 2.99 mL of hydroxylamine perchlorate TS with ethanol (99.5) to make 100 mL.

Storage—Preserve in tight containers, in a cold place.

Hydroxylamine perchlorate TS

An ethanol (99.5) solution which contains 13.4% of hydroxylamine perchlorate.

Storage—Preserve in tight containers, in a cold place.

Hydroxylamine TS

Dissolve 10 g of hydroxylammonium chloride in 20 mL of water, and add ethanol (95) to make 200 mL. To this solution add, with stirring, 150 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and filter. Prepare before use.

Hydroxylamine TS, alkaline

Mix equal volumes of a solution of hydroxylammonium chloride in methanol (7 in 100) and a solution of sodium hydroxide in methanol (3 in 25), and filter. Prepare before use.

Hydroxylammonium chloride hydrochloride TS, pH 3.1

See hydroxylammonium chloride TS, pH 3.1.
retention time of capsaicin, the total area of the peaks other than 4-hydroxy-3-methoxybenzyl nonyl acid amide is not larger than the peak area of 4-hydroxy-3-methoxybenzyl nonyl acid amide from the standard solution.

3-(3-Hydroxy-4-methoxyphenyl)-2-(E)-propenyl acid

C₉H₁₀O₄ White to light yellow, crystals or crystalline powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 230°C (with decomposition).

**Identification**—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 215 nm and 219 nm, between 238 nm and 242 nm, between 290 nm and 294 nm, and between 319 nm and 323 nm.

**Purity** Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 2 μL of this solution as directed in the Identification (1) under Hochuekkito Extract: no spot other than the principle spot of around RI 0.6 appears.

3-(3-Hydroxy-4-methoxyphenyl)-2-(E)-propenyl acid (E)-ferulic acid TS for thin-layer chromatography

Dissolve 1 mg of 3-(3-hydroxy-4-methoxyphenyl)-2-(E)-propenyl acid and 1 mg of (E)-ferulic acid in 2 mL of methanol.

2-[4-(2-Hydroxymethyl)-1-piperazinyl] propanesulfonic acid

C₁₀H₁₉NO₄ White to light yellow crystals. Melting point: about 175°C (with decomposition).

**Purity** Related substances—Dissolve 0.5 mg of hypaconitine for purity in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Splay evenly dilute sulfuric acid on the plate, and heat at 105°C for 10 minutes: the spots other than the principal spot at the RI value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

**Hypaconitine for purity** C₁₁H₁₇NO₁₀ White, crystals or crystalline powder. Soluble in acetonitrile, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water. Melting point: about 175°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of hypaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3500 cm⁻¹, 1728 cm⁻¹, 1278 cm⁻¹, 1118 cm⁻¹, 1099 cm⁻¹ and 714 cm⁻¹.

**Absorbance** <2.24> E¹%cm (230 nm): 217 – 252 [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa), phosphorus (V) oxide, 40°C], ethanol (99.5), 200 mL.

**Purity** Related substances—(1) Dissolve 5.0 mg of hypaconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of hypaconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of hypaconitine and the solvent obtained with the sample solution is not larger than the peak area of hypaconitine with the standard solu-
**Hypoxanthine**

*C5H4N4O* White crystals or crystalline powder. Freely soluble in ammonia TS, sparingly soluble in dilute hydrochloric acid and in hot water, very slightly soluble in water, and practically insoluble in methanol.

**Purity** Related substances—Dissolve 5.0 mg of hypoxanthine in 100 mL of a solution of ammonia solution (28) in methanol (1 in 10) to make exactly 100 mL. Proceed with this solution as directed in the Purity (4) under Mercaptopurine Hydrate: any spot other than the principal spot at the Rf value of about 0.2 does not appear.

**Content:** not less than 97.0% and not more than 103.0%.

**Assay**—Weigh accurately about 0.15 g of hypoxanthine, previously dried at 105°C for 3 hours, and dissolve in phosphate buffer solution, pH 7.0, to make exactly 1000 mL. Pipet 10 mL of this solution, and dilute with phosphate buffer solution, pH 7.0, to make exactly 250 mL. Read the absorbance *A* of this solution at the wavelength of 250 nm as directed under Ultraviolet-visible Spectrophotometry *C2.24* using phosphate buffer solution, pH 7.0, as the blank solution.

\[
\text{Amount (mg) of } C_5H_4N_4O = \frac{A}{779} \times 250,000
\]

**Ibuprofen** *C13H18O2* [Same as the namesake monograph]

**Icariin for thin-layer chromatography** *C21H20O12* Light yellow crystals. Very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 234°C (with decomposition).

**Imidazole** *C3H4N2* White crystalline powder. Very soluble in water and in methanol.

**Melting point** *C2.60*: 89 – 92°C

**Absorbance** *C2.24*: *E*<sub>1% 1cm</sub> (313 nm): not more than 0.031 (8 g, water, 100 mL).

**Imidazole for Karl Fischer method** See the Water Determination *C2.48*.

**Imidazole for thin-layer chromatography** *C3H4N2* White, crystalline powder. Very soluble in water and in methanol, and freely soluble in ethyl acetate and in dichloromethane.

**Melting point** *C2.60*: 89 – 92°C

**Purity** Related substances—Dissolve 10 mg of imidazole for thin-layer chromatography in exactly 20 mL of dichloromethane, and proceed with this solution as directed in the Purity (6) under Clotrimazole: any spot other than the principal spot does not appear.

**Imidazole TS** Dissolve 8.25 g of imidazole in 65 mL of water, adjust the pH to 6.8 with 5 mol/L hydrochloric acid TS, and add water to make 100 mL.

**Imidobenzyl** *C8H13N* White to light brown crystals or crystalline powder, having a slight, characteristic odor.

**Melting point** *C2.60*: 104 – 110°C

**Purity** (1) Clarity of solution—Dissolve 1.0 g of imidobenzyl in 20 mL of methanol by heating on a water bath: the solution is clear.

(2) Related substances—Proceed as directed in the Purity (6) under Carbamazepine: any spot other than the principal spot at the Rf value of about 0.9 does not appear.

**Nitrogen** *C1.08*: 6.8 – 7.3%.
2,2'-Iminodiethanol hydrochloride C₆H₁₃NO₂.HCl
A pale yellow liquid.
*Refractive index* <2.45> n₀₂ = 1.515 - 1.519
*Specific gravity* <2.56> d₅₀ = 1.259 - 1.263
*Water* <2.48>: less than 0.1%.

Imipramine hydrochloride C₁₈H₂₁N₂.HCl [Same as the namesake monograph]

Indigo carmine C₁₈H₂₃Na₂O₄S₂ [K 8092, Special class]

Indigo carmine TS Dissolve 0.20 g of indigo carmine in water to make 100 mL. Use within 60 days.

2,3-Indolinedione C₈H₇NO₂ [K 8089, Special class]

Indometacin C₁₉H₁₆ClNO₄ [Same as the namesake monograph]

Insulin human [Same as the monograph Insulin Human (Genetical Recombination)]

Interleukin-2 dependent mouse natural killer cell (NKC3)
Fractionate using discontinuous concentration gradient method cells obtained by removing adhesive cells and phagocyteic cells from C3H/He mouse spleen cells. Then, cultivate in soft agar containing interleukin-2 the cell fraction with potent NK activity and obtain the colonies. From the cell lines obtained, one of the cell lines dependent on interleukin-2 that grows in liquid medium and serially subcultured in liquid medium containing interleukin-2 is identified as NKC3.

Iodine 1 [K 8920, Special class]

Iodine for assay I [Same as the monograph Iodine]

Iodine monobromide IBr Blackish brown crystals or masses. It dissolves in water, in ethanol (95), in diethyl ether, in carbon disulfide and in acetic acid (100).
*Melting point* <2.60>: 40°C
*Storage*—Preserve in light-resistant glass containers, in a cold place.

Iodine-starch TS To 100 mL of starch TS add 3 mL of dilute iodine TS.

Iodine trichloride ICl₃ [K 8403, Special class]

Iodine TS Dissolve 14 g of iodine in 100 mL of a solution of potassium iodide (2 in 5), add 1 mL of dilute hydrochloric acid, and dilute with water to make 1000 mL (0.05 mol/L).
*Storage*—Preserve in light-resistant containers.

Iodine TS, dilute To 1 volume of iodine TS add 4 volumes of water.

0.002 mol/L Iodine TS Measure exactly 1 mL of 0.5 mol/L iodine TS, add water to make exactly 250 mL, pipet 10 mL of the solution, and add water to make exactly 100 mL. Prepare before use.

0.5 mol/L Iodine TS To 12.7 g of iodine and 25 g of potassium iodide add 10 mL of water, triturate, and add water to make 100 mL.

Iodoethane C₂H₃I A colorless or a dark-brown, clear liquid, having diethyl ether-like odor.
*Distilling range* <2.57>: 71.0 - 72.5°C, not less than 94 vol%.

Iodomethane CH₃I [K 8919, Special class]

Iodomethane for assay CH₃I Clear, colorless liquid. On exposure to light, it liberates iodine and becomes brown. Miscible with ethanol (95) and with diethyl ether, and sparingly soluble in water. Use the distillate obtained between 42.2°C and 42.6°C.
*Specific gravity* <2.56> d₅₀: 2.27 - 2.28.

Purity—Perform the test with 1 µL of iodomethane for assay as directed under Gas Chromatography according to the operating conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of iodomethane by the area percentage method: it shows the purity of not less than 99.8%. Adjust the detection sensitivity so that the peak height of iodomethane from 1 µL of methyl iodide for assay is about 80% of the full scale.
*Content*: not less than 98.0%. Assay—Proceed as directed in the Assay under Isopropyl iodide for assay.

Each mL of 0.1 mol/L silver nitrate VS = 14.19 mg of CH₃I

5-Iodouracil for liquid chromatography C₁₉H₁₅N₃O₂
White, crystalline powder. Melting point: about 275°C (with decomposition).

Purity—Dissolve 3 mg of 5-iodouracil for liquid chromatography in a mixture of diluted methanol (1 in 25) to make 10 mL. Perform the test with 10 µL of this solution as directed under Liquid Chromatography according to the operating conditions in the Purity under Idoxuridine Eye Drops. Measure each peak area by the automatic integration method over a time span of twice as long as the retention time of the principal peak, and calculate the amount of 5-iodouracil by the area percentage method: It shows the purity of not less than 98.5%.
*Content*: not less than 98.5%. Assay—Weigh accurately about 5 mg of 5-iodouracil for liquid chromatography, previously dried at 60°C for 3 hours under reduced pressure, dissolve in water to make exactly 250 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry, and determine the absorbance A at the wavelength of maximum absorption at about 282 nm.

Amount (mg) of 5-iodouracil (C₁₉H₁₅N₃O₂)

\[ A \times 265 \times 2500 \]

Iotalamic acid for assay C₁₁H₁₃I₃N₂O₇ [Same as the monograph Totalamic Acid]

Iron Fe Iron in the forms of strips, sheets, granules or wires. Fe: not less than 97.7%. It is attracted by a magnet.

Iron (II) sulfate heptahydrate FeSO₄·7H₂O [K 8978, Special class]

Iron (II) sulfate TS Dissolve 8 g of iron (II) sulfate heptahydrate in 100 mL of freshly boiled and cooled water. Prepare before use.

Iron (II) sulfide FeS [K 8948, for hydrogen sulfide development]

Iron (II) tartrate TS Dissolve 1 g of iron (II) sulfate heptahydrate, 2 g of potassium sodium tartrate tetrahydrate and 0.1 g of sodium hydrogen sulfite in water to make 100 mL.
Iron (II) thiocyanate TS  Add 3 mL of dilute sulfuric acid to 35 mL of water, and remove the dissolved oxygen by boiling the solution. Dissolve 1 g of iron (II) sulfate heptahydrate in this hot solution, cool, and then dissolve 0.5 g of potassium thiocyanate. When the solution is pale red in color, decolorize by adding reduced iron, separate the excess of reduced iron by decanting, and preserve the solution with protection from oxygen. Do not use a solution showing a pale red color.

Iron (II) trisodium pentacyanoferrate (III) dihydrate TS  Add 0.012 g of sodium pentacyanonitrosylferrate (III) to 2.3 mL of ammonia water, shake, stopper closely, and allow to stand in a refrigerator overnight. Add this solution to 10 mL of ethanol (99.5), filter a yellow colored precipitate by suction, wash with ether (99.5), dry, and preserve in a desiccator. Before using, dissolve in water to make a solution of 1.0 mg/mL, and store in a refrigerator. Use within 7 days after preparation.

Iron (III) chloride-acetic acid TS  Dissolve 0.1 g of iron (III) chloride hexahydrate in diluted acetic acid (3 in 100) to make 100 mL.

Iron (III) chloride hexahydrate  
FeCl₃·6H₂O  [K 8142, Special class]

Iron (III) chloride-Iodine TS  Dissolve 5 g of iron (III) chloride hexahydrate and 2 g of iodine in a mixture of 50 mL of acetone and 50 mL of a solution of tartaric acid (1 in 5).

Iron (III) chloride-methanol TS  Dissolve 1 g of iron (III) chloride hexahydrate in methanol to make 100 mL.

Iron (III) chloride-potassium hexacyanoferrate (III) TS  Dissolve 0.1 g of potassium hexacyanoferrate (III) in 20 mL of iron (III) chloride TS. Prepare before use.

Iron (III) chloride-pyridine TS, anhydrous  Heat gradually 1.7 g of iron (III) chloride hexahydrate by direct application of flame, melt, and solidify. After cooling, dissolve the residue in 100 mL of chloroform, add 8 mL of pyridine, and filter.

Iron (III) chloride TS  Dissolve 9 g of iron (III) chloride hexahydrate in water to make 100 mL (1/3 mol/L).

Iron (III) chloride TS, acidic  To 60 mL of acetic acid (100) add 5 mL of sulfuric acid and 1 mL of iron (III) chloride hexahydrate.

Iron (III) chloride TS, dilute  Dilute 2 mL of iron (III) chloride hexahydrate TS with water to make 100 mL. Prepare before use.

Iron (III) nitrate enehydrate  
Fe(NO₃)₃·9H₂O  [K 8559, Special class]

Iron (III) nitrate TS  Dissolve 1 g of iron (III) nitrate enehydrate in hydrochloric acid-potassium chloride buffer solution (pH 2.0) to make 300 mL.

Iron (III) perchlorate-ethanol TS  Dissolve 0.8 g of iron (III) perchlorate hexahydrate in perchloric acid-ethanol TS to make 100 mL.

Storage—Preserve in tight containers, in a cold place.

Iron (III) perchlorate hexahydrate  
Fe(ClO₄)₃·6H₂O  Hygroscopic, light purple crystals, and a solution in ethanol (99.5) (1 in 125) is clear and orange in color.
Isoniazid (C\textsubscript{6}H\textsubscript{7}N\textsubscript{3}O). For assay, dissolve 0.1 g of isoniazid in a mixture of 50 mL of methanol and 0.12 mL of hydrochloric acid, and add methanol to make 200 mL.

**Isonicotinic acid** White, crystals or powder. Melting point: about 315°C (decomposition).

**Isonicotinic acid amide** C\textsubscript{6}H\textsubscript{5}N\textsubscript{2}O White, crystals or crystalline powder.

**Isobutyl parahydroxybenzoate** C\textsubscript{11}H\textsubscript{14}O\textsubscript{3} Colorless, clear liquid, odorless. Freely soluble in water, in ethanol (95) and in chloroform, and practically insoluble in water.

Melting point 2\textsubscript{2.60}°: 75 – 77°C
Residue on ignition 2\textsubscript{2.44}°: not more than 0.1%.
Content: not less than 98.0%. Assay—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Isobutyl salicylate C\textsubscript{17}H\textsubscript{20}N\textsubscript{2}S.HCl White, odorless, crystalline powder. Odorless. Freely soluble in water, in ethanol (95), in acetone and in diethyl ether, and practically insoluble in water.

Column: A column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography, about 2 m in length, packed with siliceous earth for gas chromatography, 180 to 250 \(\mu\)m in particle diameter, coated with polyethylene glycol 20 M for gas chromatography at the ratio 4:1.

Dissolve 0.02 to 0.05 mg of cytochrome C, trypsinogen, lentil-lectin basic band, lentil-lectin acidic band, horse myoglobin basic band, horse myoglobin acidic band, b-lactoglobulin A, in 0.1 mL of saccharose solution (3 in 10).

**Isopropyl 4-aminobenzoate** NH\textsubscript{2}C\textsubscript{6}H\textsubscript{4}COOCH(CH\textsubscript{3})\textsubscript{2}

Pale brown crystals.

Melting point 2\textsubscript{2.60}°: 83 – 86°C

**Isopropyl benzoate** C\textsubscript{6}H\textsubscript{5}COOCH(CH\textsubscript{3})\textsubscript{2} A clear, colorless liquid, having a characteristic odor.

**Isopropylamine-ethanol TS** To 20 mL of isopropylamine add ethanol (99.5) to make 100 mL. Prepare before use.

**Isopropylamine-ethanol TS** To 20 mL of isopropylamine add ethanol (99.5) to make 100 mL. Prepare before use.

**Isoniazid** C\textsubscript{6}H\textsubscript{7}N\textsubscript{3}O White, crystals or powder. Melting point: about 315°C (decomposition).

**Isonicotinic acid amide** C\textsubscript{6}H\textsubscript{5}N\textsubscript{2}O

White, crystals or powder.

**Isopropyl benzoate** C\textsubscript{6}H\textsubscript{5}COOCH(CH\textsubscript{3})\textsubscript{2} A clear, colorless liquid, having a characteristic odor.

**Isopropylamine-ethanol TS** To 20 mL of isopropylamine add ethanol (99.5) to make 100 mL. Prepare before use.

**Isoniazid** C\textsubscript{6}H\textsubscript{7}N\textsubscript{3}O

White, crystals or powder. Melting point: about 315°C (decomposition).

**Isopropyl benzoate** C\textsubscript{6}H\textsubscript{5}COOCH(CH\textsubscript{3})\textsubscript{2} A clear, colorless liquid, having a characteristic odor.

**Isopropylamine-ethanol TS** To 20 mL of isopropylamine add ethanol (99.5) to make 100 mL. Prepare before use.

**Isoniazid** C\textsubscript{6}H\textsubscript{7}N\textsubscript{3}O

White, crystals or powder. Melting point: about 315°C (decomposition).

**Isonicotinic acid amide** C\textsubscript{6}H\textsubscript{5}N\textsubscript{2}O

White, crystals or powder.

**Isopropyl benzoate** C\textsubscript{6}H\textsubscript{5}COOCH(CH\textsubscript{3})\textsubscript{2} A clear, colorless liquid, having a characteristic odor.

**Isopropylamine-ethanol TS** To 20 mL of isopropylamine add ethanol (99.5) to make 100 mL. Prepare before use.

**Isoniazid** C\textsubscript{6}H\textsubscript{7}N\textsubscript{3}O

White, crystals or powder. Melting point: about 315°C (decomposition).

**Isonicotinic acid amide** C\textsubscript{6}H\textsubscript{5}N\textsubscript{2}O

White, crystals or powder.

**Isopropyl benzoate** C\textsubscript{6}H\textsubscript{5}COOCH(CH\textsubscript{3})\textsubscript{2} A clear, colorless liquid, having a characteristic odor.

**Isopropylamine-ethanol TS** To 20 mL of isopropylamine add ethanol (99.5) to make 100 mL. Prepare before use.
Specific gravity <2.56> d 15°: 1.700 – 1.710

Purity—Perform the test with 1 μL of isopropyl iodide for assay as directed under Gas Chromatography <2.02> according to the operating conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of isopropyl iodide by the area percentage method: It shows the purity of not less than 99.8%. Adjust the detection sensitivity so that the peak height of isopropyl iodide from 1 μL of isopropyl iodide for assay is about 80% of the full scale.

Content: not less than 98.0%. Assay—Transfer 10 mL of ethanol (95) into a brown volumetric flask, weigh accurately, add 1 mL of isopropyl iodide for assay, and weigh accurately again. Add ethanol (95) to make exactly 100 mL, pipet 20 mL of this solution into the second brown volumetric flask, add exactly 50 mL of 0.1 mol/L silver nitrate VS and then 2 mL of nitric acid, stopper, shake occasionally for 2 hours in a dark place, and allow to stand overnight in a dark place. Shake occasionally for 2 hours, add water to make exactly 100 mL, and filter through dry filter paper. Discard the first 20 mL of the filtrate, pipet the next 50 mL, make exactly 100 mL, and filter through dry filter paper. Add 2 mL of nitric acid, stopper, shake occasionally for 2 hours, add water to make exactly 100 mL, and filter through dry filter paper. Discard the first 20 mL of the filtrate, pipet the next 50 mL, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L silver nitrate VS = 17.00 mg of C 3 H 1 I

Isopropyl myristate C 17 H 35 O 2 Colorless, clear, oily liquid, and odorless. Congeals at about 5°C. Soluble in 90% alcohol, miscible with many organic solvents and with solid oils, and insoluble in water, in glycerin and in propylene glycol.

Refractive index <2.45> n D 20: 1.432 – 1.436
Specific gravity <2.56> d 15°: 0.846 – 0.854
Saponification value <1.13>: 202 – 212
Acid value <1.13>: not more than 1.
Iodine value <1.13>: not more than 1.
Residue on ignition <2.44>: not more than 0.1% (1 g).

Isopropyl myristate for sterility test C 17 H 35 O 2 Transfer 100 mL of isopropyl myristate into a centrifuge tube, add 100 mL of twice-distilled water, and shake vigorously for 10 minutes. Then centrifuge at a rate of 1800 revolutions per minute for 20 minutes, separate the supernatant liquid (isopropyl myristate layer), and determine the pH of the residual water layer: not less than 5.5.

Treat isopropyl myristate which meets the requirements of pH determination as follows: 500 mL of isopropyl myristate, which has met the requirements of pH determination, is percolated through a 15-cm high layer of activated alumina filled in a glass column 20 mm in diameter and 20 cm in length with a slightly positive pressure in order to facilitate adequate flow, and then sterilized by filtration.

Isopropyl parahydroxybenzoate C 10 H 12 O 3 Odorless and colorless fine crystals, or white, crystalline powder. Freely soluble in ethanol (95), in acetone and in diethyl ether, and very slightly soluble in water.

Melting point <2.60>: 84 – 86°C
Residue on ignition <2.44>: not more than 0.1%

Content: not less than 99.0%. Assay—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS = 180.2 mg of C 6 H 12 O 6

4-Isopropylphenol C 8 H 14 O White to reddish yellow, crystals or crystalline powder.

Melting point <2.60>: 59 – 63°C

Isosorbide dinitrate for assay C 6 H 8 N 2 O 8 [Same as the monograph Isosorbide Dinitrate. It contains not less than 99.0% of isosorbide dinitrate (C 6 H 8 N 2 O 8) meeting the following additional specifications.]

Purity Related substances—Dissolve 50 mg of isosorbide dinitrate for assay in 50 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of isosorbide dinitrate obtained from the sample solution is not larger than the peak area of isosorbide dinitrate from the standard solution.

Operating conditions
Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating procedures in the Assay under Isosorbide Dinitrate Tablets.

Time span of measurement: About 2 times as long as the retention time of isosorbide dinitrate beginning after the solvent peak.

System suitability
Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 50 mL. Confirm that the peak area of isosorbide dinitrate obtained from 10 μL of this solution is equivalent to 7 to 13% of that of isosorbide dinitrate from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosorbide dinitrate are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isosorbide dinitrate is not more than 2.0%.

Isotonic sodium chloride solution [Same as the namesake monograph]

Isosuprine hydrochloride for assay C 18 H 23 NO 3 .HCl [Same as the monograph Isosuprine Hydrochloride]

Japanese acid clay Natural hydrous aluminum silicate, grayish white powder, having a particle size of about 74 μm.

Loss on drying <2.41>: not more than 10% (1 g, 105°C, 4 hours).

Water adsorbing capacity: not less than 2.5%. Weigh accurately about 10 g of Japanese acid clay in weighing bottle, allow to stand for 24 hours with cover in a chamber, in which humidity is maintained to 80% by means of sulfuric acid (specific gravity 1.19), reweigh, and determine the increase of mass of the sample.

Jesaconitine for purity C 42 H 49 NO 12 A white powder.
Freely soluble in acetonitrile, in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification—Determine the infrared absorption spectrum of jesaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25. It exhibits absorption at the wave numbers of about 3150 cm\(^{-1}\), 875 cm\(^{-1}\), 1715 cm\(^{-1}\), 1607 cm\(^{-1}\), 1281 cm\(^{-1}\), 1259 cm\(^{-1}\), 1099 cm\(^{-1}\) and 772 cm\(^{-1}\).

Absorbance is equivalent to 3.5 to 6.5 m\(^{-1}\) for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), coulometric titration].

Josamycin \(\text{C}_{26}\text{H}_{28}\text{Cl}_{2}\text{N}_{4}\text{O}_{4}\) [Same as the namesake monograph]

Josamycin propionate \(\text{C}_{26}\text{H}_{27}\text{O}_{16}\) [Same as the namesake monograph]

Kainic acid See kainic acid hydrate.

Kainic acid for assay See kainic acid hydrate.

Kainic acid hydrate \(\text{C}_{10}\text{H}_{15}\text{NO}_{4}\text{H}_{2}\text{O}\) [Same as the namesake monograph]

Kainic acid hydrate for assay See kainic acid hydrate.

Kanamycin sulfate \(\text{C}_{18}\text{H}_{33}\text{N}_{2}\text{O}_{11} \cdot \text{H}_{2}\text{SO}_{4}\) [Same as the namesake monograph]

Karl Fischer TS See Water Determination 2.48.

Kerosene It is mainly a mixture of hydrocarbons in the methane series, and a colorless, clear liquid, having not a disagreeable, characteristic odor.

Specific gravity is about 0.80.

Distilling range is 180 – 300°C.

Ketoconazole \(\text{C}_{26}\text{H}_{28}\text{Cl}_{2}\text{N}_{4}\text{O}_{3}\) [Same as the namesake monograph]

Ketoconazole for assay \(\text{C}_{26}\text{H}_{28}\text{Cl}_{2}\text{N}_{4}\text{O}_{3}\) [Same as the monograph Ketoconazole.

Kininogen Produced by purifying from bovine plasma. Dissolve an appropriate amount of kininogen in 0.02 mol/L phosphate buffer solution, pH 8.0 so that 10 mL of the solution contains 1 mg of kininogen, and use this solution as the sample solution. Perform the following tests with the sample solution: it meets the requirement of each test.

(i) Immediately after the sample solution is prepared, add 0.1 mL of a solution of trichloroacetic acid (1 in 5) to 0.5 mL of the sample solution, shake, and centrifuge. To 0.5 mL of the supernatant liquid add 0.5 mL of gelatin-tris buffer solution, pH 8.0, and shake. To 0.1 mL of this solution add 1.9 mL of gelatin-tris buffer solution. Proceed with 0.1 mL of this solution as directed in the Purity (2) under Kallidinogenase, and determine the amount of kinin: kinin is not detected.

(ii) Warm 0.5 mL of the sample solution at 30 ± 0.5°C for 20 minutes, and proceed as directed in (i): kinin is not detected.

(iii) Perform the test with 0.5 mL of the sample solution as directed in the Purity (2) under Kallidinogenase: the decomposition of bradykinin is not observed.

(iv) To 0.5 mL of the sample solution add 0.5 mL of 0.02 mol/L phosphate buffer solution, pH 8.0 containing 500 μg of crystalline trypsin, previously warmed at 30 ± 0.5°C for 5 minutes, warm this solution at 30 ± 0.5°C for 5 minutes, add 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Then boil for 3 minutes, cool in ice immediately, and centrifuge. To 0.5 mL of the supernatant liquid add 0.5 mL of gelatin-tris buffer solution, pH 8.0, and shake. To 0.1 mL of this solution add 0.9 mL of trichloroacetic acid-gelatin-tris buffer solution. To 0.1 mL of this solution add trichloroacetic acid-gelatin-tris buffer solution to
make 20 mL, then proceed as directed in (i), and determine the amount, \( B_k \), of kinin per well. Calculate the kinin-releasing activity per mg by the following equation: not less than 10 \( \mu g \) bradykinin equivalent per mg.

\[
\text{Kinin-releasing activity per mg (\( \mu g \) bradykinin equivalent/mg)} = \frac{B_k}{0.0096}
\]

**Kininogen TS** Dissolve a sufficient quantity of kininogen in 0.02 mol/L phosphate buffer solution, pH 8.0 to prepare a solution having an ability in each mL to release kinin corresponding to not less than 1 \( \mu g \) of bradykinin.

**Labetalol hydrochloride** \( C_{19}H_{24}N_2O_3\cdot HCl \) [Same as the namesake monograph]

**Labetalol hydrochloride for assay** \( C_{19}H_{24}N_2O_3\cdot HCl \) [Same as the monograph Labetalol Hydrochloride. However, when dried, it contains not less than 99.0% of labetalol hydrochloride (\( C_{19}H_{24}N_2O_3\cdot HCl \)).]

**Lactic acid** \( CH_3CH(OH)COOH \) [K 8726, Special class]

**Lactic acid TS** Dissolve 12.0 g of lactic acid in water to make 100 mL.

**Melting point** \( <2.60^\circ C \): not less than 118°C

**Purity**—Dissolve 0.10 g of lactic acid in 10 mL of a mixture of methanol and water (3:2), and perform the test with 10 \( \mu L \) of this solution as directed in the Identification (2) under Erythromycin Lactobionate: the spot other than the principal spot is not found.

**\( \beta \)-Lactoglobulin** Prepare from milk. White to light yellow powder.

\[ \text{Nitrogen content} <1.08\% : \text{not less than 14\% (calculated on the dried basis)} \]

**Lactose** See lactose monohydrate.

**\( \alpha \)-Lactalbumin** White powder. Derived from milk. Molecular weight of about 14,200.

**Lactobionic acid** \( C_{12}H_{22}O_12 \) Colorless crystals or white crystalline powder, having no odor.

\[ \text{Melting point} <2.25^\circ C \: \text{to} \: 118^\circ C \]

**Purity**—Dissolve 0.10 g of lactobionic acid in 10 mL of a mixture of methanol and water (3:2), and perform the test with 10 \( \mu L \) of this solution as directed in the Identification (2) under Erythromycin Lactobionate: the spot other than the principal spot is not found.

**Lactose monohydrate** \( C_{12}H_{22}O_{11} \cdot H_2O \) [Same as the monograph Lactose].

**Lactose substrate TS** Dissolve 6.0 g of lactose monohydrate in diluted disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 (1 in 10) to make 100 mL.

**Lactose substrate TS for \( \beta \)-galactosidase (penicillium)** Dissolve 6.0 g of lactose monohydrate in diluted disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 (1 in 10) to make 100 mL.

**Lanthanum-alizarin complexone TS** To 1 mL of ammonia water (28) add 10 mL of water. To 4 mL of this solution add 4 mL of a solution of ammonium acetate (1 in 5) and 192 mg of alizarin complexone, and label this solution as alizarin complexone stock solution. Dissolve 41 g of sodium acetate trihydrate in 400 mL of water, and add 24 mL of acetic acid (100). To this solution add the total volume of the alizarin complexone stock solution, add 400 mL of acetone, and label this solution as alizarin complexone solution. To 10 mL of diluted hydrochloric acid (1 in 6) add 163 mg of lanthanum (III) oxide, heat to dissolve, and label this solution as lanthanum solution. To the alizarin complexone solution add the lanthanum solution, and mix. After cooling, adjust to pH 4.7 with acetic acid (100) or ammonia water (28), and add water to make 1000 mL. Prepare before use.

**Lanthanum chloride TS** To 58.65 g of lanthanum (III) oxide add 100 mL of hydrochloric acid, and boil. After cooling, add water to make 1000 mL.

**Lanthanum (III) oxide** \( La_2O_3 \) White crystals.

\[ \text{Loss on ignition} <2.43\% : \text{not more than 0.5\% (1 g, 1000°C, 1 hour)} \]

**Lauromacrogol** [Same as the namesake monograph]

**Lead acetate** See lead (II) acetate trihydrate.

**Lead acetate TS** See lead (II) acetate TS.

**Lead monoxide** See lead (II) oxide.

**Lead nitrate** See lead (II) nitrate.

**Lead dioxide** See lead (IV) oxide.

**Lead subacetate TS** Place the yellowish mixture, obtained by triturating 3 g of lead (II) acetate trihydrate and 1 g of lead (II) oxide with 0.5 mL of water, in a beaker, and heat on a water bath, covering with a watch glass, until it shows a homogeneous, white to reddish white color. Then add 9.5 mL of hot water in small portions, cover it again with a watch glass, and set it aside. Decant the supernatant liquid, and adjust the specific gravity to 1.23 to 1.24 (15°C) by adding water. Preserve in tightly stoppered bottles.

**Lead subacetate TS, dilute** To 2 mL of lead subacetate TS add freshly boiled and cooled water to make 100 mL. Prepare before use.

**Lead (II) acetate trihydrate** \( Pb(CH_3COO)_2 \cdot 3H_2O \) [K 8374, Special class]

**Lead (II) acetate** To 9.5 g of lead (II) acetate trihydrate add freshly boiled and cooled water to make 100 mL. Preserve in tightly stoppered bottles (0.25 mol/L).

**Lead (II) nitrate** \( Pb(NO_3)_2 \) [K 8563, Special class]
Levothyroxine sodium hydrate for thin-layer chromatography

- A supernatant liquid of a solution in dilute acetic acid (1 in 100) responds to Quality Tests (1-09) (3) for lead salt.
- Leucine $\text{C}_9\text{H}_{19}\text{NO}_2$ [Same as the namesake monograph]
- Leucine for assay $\text{C}_9\text{H}_{19}\text{NO}_2$ [Same as the monograph 1-Leucine. When dried, it contains not less than 99.0% of 1-Leucine ($\text{C}_9\text{H}_{19}\text{NO}_2$).]
- Levallorphan tartrate for assay $\text{C}_{17}\text{H}_{25}\text{NO}_2\cdot\text{C}_4\text{H}_6\text{O}_6$ [Same as the monograph Levallorphan Tartrate. When dried, it contains not less than 99.0% of $\text{C}_{17}\text{H}_{25}\text{NO}_2\cdot\text{C}_4\text{H}_6\text{O}_6$.]
- Levothyroxine sodium See levothyroxine sodium hydrate.
- Levothyroxine sodium for thin-layer chromatography
  See levothyroxine sodium hydrate for thin-layer chromatography.
- Levothyroxine sodium hydrate $\text{C}_{19}\text{H}_{25}\text{NO}_2\cdot\text{C}_4\text{H}_6\text{O}_6$ [Same as the namesake monograph]
- Levothyroxine sodium hydrate for thin-layer chromatography $\text{C}_{19}\text{H}_{25}\text{NO}_2\cdot\text{C}_4\text{H}_6\text{O}_6$ [Same as the monograph Levothyroxine Sodium Hydrate. Proceed the test as directed in the Identification (3) under Levothyroxine Sodium Hydrate.]
- Limonene $\text{C}_{10}\text{H}_{16}$ Clear and colorless liquid, having a characteristic odor.
  - Refractive index $<2.45$ $n^p_{D} = 1.427 - 1.474$
  - Specific gravity $<2.56$ $d^p_{20} = 0.841 - 0.846$
  - Melting point $<2.60^\circ$: 176 - 177°C
- Purity Related substances—Dissolve 0.1 g of limonene in 25 mL of hexane and use this solution as the sample solution. Perform the test with 2 $\mu$L of the sample solution as directed under Gas Chromatography (2.02) according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of limonene: it is not less than 97.0%.
- Operating conditions
  Proceed the operating conditions in the Assay under Eucalyptus Oil except detection sensitivity and time span of measurement.
  - Detection sensitivity: Measure 1 $\mu$L of limonene, add hexane to make 100 $\mu$L, and adjust the detection sensitivity so that the peak height of limonene obtained from 2 $\mu$L of this solution is 40% to 60% of the full scale.
  - Time span of measurement: About 3 times as long as the retention time of limonene beginning after the solvent peak.
- Limonin for thin-layer chromatography $\text{C}_{26}\text{H}_{30}\text{O}_8$
  White crystals or crystalline powder. Slightly soluble in methanol and in ethyl acetate, and practically insoluble in water and in ethanol (99.5). Melting point: about 290°C.
- Identification Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared-visible Spectrophotometry (2.22): it exhibits absorption at the wave numbers of about 1759 cm$^{-1}$, 1709 cm$^{-1}$, 1166 cm$^{-1}$, 798 cm$^{-1}$ and 601 cm$^{-1}$.
- Purity Related substances—Dissolve 1 mg of limonin for thin-layer chromatography in 1 mL of ethyl acetate, and perform the test with 1 $\mu$L of this solution as directed in the Identification (2) under Orenge dokuto Extract: no spot other than the principal spot ($R_f$ value is about 0.4) appears.
- (Z)-Ligustilide for thin-layer chromatography $\text{C}_{12}\text{H}_{14}\text{O}_2$
  A clear, yellow-grown liquid, having a characteristic odor. Miscible with methanol and with ethanol (99.5), and practically insoluble in water.
- Identification—Determine the absorption spectrum of a solution in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits a maximum between 320 nm and 324 nm.
- Purity Related substances—Dissolve 1 mg in 10 mL of methanol. Proceed the test with 1 $\mu$L of this solution as directed in the Identification (5) under Hochuekikuto Exctract: no spot other than the principle spot of around $R_f$ 0.6 appears.
- Lithrothyroline sodium $\text{C}_{10}\text{H}_{13}\text{I}_3\text{NaO}_4$ [Same as the namesake monograph]
- Lithrothyroline sodium for thin-layer chromatography
  See Lithrothyroline Sodium. Proceed as directed for the Identification (1) under Lithrothyroline Sodium Tablets: any spot other than the principal spot at the $R_f$ value of 0.3 to 0.4 does not appear.
- Liquid paraffin See paraffin, liquid.
- Liquiritin for thin-layer chromatography $\text{C}_{17}\text{H}_{20}\text{O}_9$ A clear, yellow-grown liquid, having a characteristic odor. Miscible with methanol and with ethanol (99.5), and practically insoluble in water. Melting point: about 210°C (with decomposition).
- Identification—Determine the absorption spectrum of a solution in dilute acetic acid (1 in 2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 215 nm and 219 nm, and between 275 nm and 279 nm.
- Purity Related substances—Dissolve 1.0 mg in 1 mL of methanol, and perform the test with 1 $\mu$L of this solution as directed in the Identification (5) under Kakkonto Extract: no spot other than the principal spot ($R_f$ value is about 0.4) appears.
- Lisinopril See lisinopril hydrate.
- Lisinopril for assay See lisinopril hydrate for assay.
- Lisinopril hydrate $\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_5\cdot\text{H}_2\text{O}$ [Same as the namesake monograph]
- Lisinopril hydrate for assay $\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_5\cdot\text{H}_2\text{O}$ [Same as the monograph Lisinopril Hydrate. It contains not less than 99.5% of lisinopril ($\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_5$: 405.49), calculated on the anhydrous basis.]
- Lithium acetate dihydrate $\text{CH}_3\text{COOLi}\cdot\text{H}_2\text{O}$ Colorless crystals.
  Dilute acetic acid insoluble substances—To 40.0 g of lithium acetate dihydrate add 45 mL of water, heat in a water bath to dissolve, cool, then dissolve in dilute acetic acid, and filter by suction. Wash the filter with water, dry the filter at
105 ± 2°C for 1 hour, and weigh the mass of the residue after cooling: not more than 0.0025%.  

**Content:** not less than 97.0%. **Assay—**Weigh accurately 0.3 g of lithium acetate dihydrate, add exactly 50 mL of acetic acid (100) and exactly 5 mL of acetic anhydride, dissolve by heating in a water bath, and titrate with 0.1 mol/L perchloric acid VS after cooling (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 10.20 mg of CH₃COOLi.2H₂O

**Lithium bromide** LiBr White crystals or crystalline powder. It is hygroscopic.

**Purity** (1) Chloride <1.0%: not more than 0.1%.  
(2) Sulfate <1.14%: not more than 0.01%.

**Lithium chloride** LiCl White crystals or masses.  

**Identification—**Perform the test as directed under Flame Coloration Test (1) <1.0%: a persistent red color appears.

**Lithium perchlorate** LiClO₄ White, crystals or crystalline powder.

**Content**: not less than 98%. **Assay—**Accurately weigh about 0.2 g of lithium perchlorate, dissolve in 30 mL of water. Transfer the solution to a chromatographic column, prepared by pouring about 25 mL of strongly acidic ion-exchange resin (H type) for column chromatography into a chromatographic tube about 11 mm in inside diameter and about 300 mm in height (after adding 200 mL of 1 mol/L hydrochloric acid TS and flowing at a flow rate of 3 – 4 mL per minute, wash the chromatographic column with water until the color of the rinse water changes to yellowish red when adding methyl orange TS to the eluate), and flow at a flow rate of 3 – 4 mL per minute. Then, wash the column with about 30 mL of water at a flow rate of 3 – 4 mL per minute 5 times. Combine the rinse water and the eluate, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 10.64 mg of LiClO₄

**Lithium sulfate** See lithium sulfate monohydrate.

**Lithium sulfate monohydrate** Li₂SO₄ . H₂O [K 8994, Special class]

**Lithocholic acid for thin-layer chromatography** C₂₄H₄₀O₃ White crystals or crystalline powder. Soluble in acetic acid (100) and exactly 5 mL of acetic anhydride, dissolve by heating in a water bath, and titrate with 0.1 mol/L perchloric acid VS after cooling (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 10.20 mg of CH₃COOLi.2H₂O

Prepare before use. The constituents except dextrose and sodium hydrogen carbonate can be made up in concentrated stock solutions, stored in a dark place, and diluted before use.

**Loganin for assay** Loganin for thin-layer chromatography meeting the following additional specifications.

**Absorbance** <2.24> E<sub>1</sub>₉₅₀ (235 nm): 275 – 303 (dried in a desiccator (silica gel) for 24 hours, 5 mg, methanol, 500 mL).

**Purity** Related substances—Dissolve 2 mg of loganin for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of loganin is not larger than the peak area of loganin from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Goshajinkigan Extract.

Time span of measurement: About 3 times as long as the retention time of loganin.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of loganin obtained from 10 μL of this solution is equivalent to 3.5 to 6.5 μL of that of loganin from 10 μL of the standard solution.

**System performance and system repeatability:** Proceed as directed in the system suitability in the Assay (1) under Goshajinkigan Extract.

**Loganin for component determination** See loganin for assay.

**Loganin for thin-layer chromatography** C<sub>17</sub>H₂₆O₁₀ White, crystals or crystalline powder. Soluble in methanol and very slightly soluble in ethanol (99.5). Melting point: 221 – 227°C.

**Purity** Related substances—Dissolve 1.0 mg of loganin for thin-layer chromatography in 2 mL of methanol.
Perform the test with 10 μL of this solution as directed in the Identification under Cornus Fruit: any spot other than the principal spot at the RF value of about 0.4 does not appear.

**Lovastatin**

C_{27}H_{46}O_{4} White crystals or crystalline powder. Soluble in acetonitrile and in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

**Optical rotation** $\angle 2.493: \alpha [\beta ]_{D}^{20} +325 \pm 340^\circ$ (50 mg calculated on the dried basis, acetonitrile, 10 mL, 100 mm).

**Loss on drying** $\angle 2.475$: Not more than 1.0% (1 g, under reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

**Low-molecular weight heparin for calculation of molecular mass.**

It is a low-molecular weight heparin with a disaccharide unit prepared, and display the molecular mass distribution between 600 and more than 10,000. When the average of molecular mass of Low-molecular weight heparin international standard is determined with reference to this, the difference compared as a reference with the Low-molecular weight heparin international standard is not less than 5%.

**Luteolin for thin-layer chromatography** C_{16}H_{18}O_{6}

Light yellow to yellow-brown crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 310°C (with decomposition).

**Purity** Related substances—Dissolve 1.0 mg of luteolin for thin-layer chromatography in 1 mL of methanol. Proceed the test with 10 μL of this solution as directed in the Identification under Chrysanthemum Flower: any spot other than the principal spot of RF about 0.7 does not appear.

**Lysate reagent** A lyophilized product obtained from amebocyte lysate of horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). Amebocyte lysate preparations which do not react to β-glucans are available: they are prepared by removing the G factor reacting to β-glucans from amebocyte lysate or by inhibiting the G factor reacting system of amebocyte lysate.

**Lysate TS** Dissolve a lysate reagent in water for bacterial endotoxins test, or in a suitable buffer, by gentle stirring.

**Lysil endopeptidase** White powder or masses. An exotxin produced by *Achromobacter*. Molecular weight: 27,500.

**1-Lysine hydrochloride** C_{6}H_{12}N_{2}O_{2}.HCl [Same as the namesake monograph]

**Macrogol 600** HOCH_{2}(CH_{2}OCH_{2})_{n}CH_{2}OH, n = 11 – 13 Clear, colorless, viscous liquid or a white, petrolatum-like solid, having a faint, characteristic odor. Very soluble in water, in ethanol (95), in acetone and in macrogol 400, soluble in diethyl ether, and practically insoluble in petroleum benzine. Congealing point: 18 – 23°C

**Average molecular weight:** When perform the test as directed in the Average molecular weight test under Macrogol 400, it is between 570 and 630.

**4-(N-Maleimidylmethyl)-cyclohexane-1-carboxylate-N-hydroxysuccinimide ester** C_{19}H_{18}O_{5} Colorless crystals. Being decomposed by acid and alkaline treatment.

**Magnesia TS** Dissolve 5.5 g of magnesium chloride hexahydrate and 7 g of ammonium chloride in 65 mL of water, add 35 mL of ammonia TS, allow the mixture to stand for a few days in tightly stoppered bottles, and filter. If the solution is not clear, filter before use.

**Magnesium Mg** [K 8875, Special class]

**Magnesium chloride** See magnesium chloride hexahydrate.

**Magnesium chloride hexahydrate** MgCl_{2}.6H_{2}O [K 8159, Special class]

**Magnesium nitrate** See magnesium nitrate hexahydrate.

**Magnesium nitrate hexahydrate** Mg(NO_{3})_{2}.6H_{2}O [K 8567, Special class]

**Magnesium oxide** MgO [K 8432, Special class]

**Magnesium powder** Mg [K 8876, Special class]

**Magnesium sulfate** See magnesium sulfate heptahydrate.

**Magnesium sulfate heptahydrate** MgSO_{4}.7H_{2}O [K 8995, Special class]

**Magnesium sulfate TS** Dissolve 12 g of magnesium sulfate hexahydrate in water to make 100 mL (0.5 mol/L).

**Magnolol for assay** Use magnolol for thin-layer chromatography meeting the following additional specifications. Absorbance $\angle 2.47$ $E_{1}%^{10}$ (290 nm): 270 – 293 (10 mg, methanol, 500 mL). Use the sample dried in a desiccator (silica gel) for not less than 1 hour for the sample.

**Purity** Related substances—Dissolve 5.0 mg of magnolol for assay in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography $\angle 2.07$ according to the following conditions, and determine the area of each peak from these solutions by the automatic integration method: the total area of peaks other than the peak of magnolol from the sample solution is not larger than the peak area of magnolol from the standard solution. Operating conditions:

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Magnolia Bark.

**Time span of measurement:** About 3 times as long as the retention time of magnolol.

**System suitability**

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay under Magnolia Bark.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of magnolol obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

**Magnolol for component determination** See magnolol for assay.

**Magnolol for thin-layer chromatography** C_{18}H_{22}O_{2} Odorless, white crystals or crystalline powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble.
in water. Melting point: about 102°C.

**Identification**—Determine the absorption spectrum of a solution of mannitol for thin-layer chromatography in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits a maximum between 287 nm and 291 nm.

**Purity** Related substances—Dissolve 1.0 mg of mannitol for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.07>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane, acetone and acetic acid (100) (20:15:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot of around Rt value of 0.5 does not appear.

**Malachite green** See malachite green oxalate.

**Malachite green oxalate** C₂₂H₁₄N₂O₁₂ [K 8878, Malachite green (oxalate), Special class]

**Maleic acid** C₇H₆O₄ A white crystalline powder.

**Identification**—Determine the infrared absorption spectrum of maleic acid as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1706 cm⁻¹, 1876 cm⁻¹, 1587 cm⁻¹, 1567 cm⁻¹, 1436 cm⁻¹, 1263 cm⁻¹, 876 cm⁻¹ and 786 cm⁻¹.

**4-(Maleimidomethyl)cyclohexylcarbolic acid-N-hydroxy-succinimide ester** C₁₆H₁₉N₃O₃ White powder. Practically insoluble in water and in ethanol (95).

**Maltose** See maltose monohydrate.

**Maltose monohydrate** C₁₂H₂₂O₁₁ ⋅ H₂O [Same as the namesake monograph].

**Maltotriose** C₁₈H₃₂O₁₆ A white powder.

**Identification**—Determine the infrared absorption spectrum of maltotriose as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3420 cm⁻¹, 1,153 cm⁻¹ and 1024 cm⁻¹.

**Manganese dioxide** MnO₂ Black to black-brown, masses or powder.

**Identification**—To 0.5 g add 20 mL of water and 3 mL of hydrogen peroxide (30). Alkalinate the solution with ammonia solution (28) while cooling, and add 25 mL of hydrogen sulfide TS: pale red precipitates appear.

**Manganese (II) nitrate hexahydrate** Mn(NO₃)₂ ⋅ 6H₂O [K 8568, Special class]

**d-Mannitol** C₆H₁₂O₆ [Same as the monograph d-Mannitol]

**d-Mannosamine hydrochloride** C₆H₁₂N₂O₄ ⋅ HCl White powder. Melting point: about 168°C (with decomposition). Optical rotation <2.49> [α]D²⁴: +4.2 — 3.2° (0.4 g, water, 20 mL, 100 mm).

**d-Mannose** C₆H₁₀O₅ White crystal or crystalline powder. It is very soluble in water. Melting point: about 132°C (with decomposition).
graph]

**Mentha herb** [Same as the namesake monograph]

**Menthol** C_{10}H_{20}O [Same as the monograph dl-Menthol or l-Menthol]

**l-Menthol for assay** [Same as the monograph l-Menthol. It contains not less than 99.0% of C_{10}H_{20}O and meets the following additional specifications.]

- **Optical rotation** \( < 2.0^\circ \) \( [\alpha]_D^20 \): \(-48.0^\circ \) to \(-51.0^\circ \) (2.5 g, ethanol (95), 25 mL, 100 mm).

**Purity** Related substances—Dissolve 0.10 g of l-menthol for assay in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of this solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 5 \( \mu \)L each of the sample solution and standard solution (1) as directed under Gas Chromatography \( < 2.02 > \) according to the following conditions, measure each peak area of these solutions by the automatic integration method: the total peak area of l-menthol from the sample solution is not larger than the peak area of l-menthol from the standard solution (1).

**Operat conditions**

Proceed the operating conditions in the Assay under Mentha Oil except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add dichloromethane to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of l-menthol obtained from 5 \( \mu \)L of the standard solution (2) can be measured, and the peak height of l-menthol from 5 \( \mu \)L of the standard solution (1) is about 20% of the full scale.

**Time span of measurement:** About twice as long as the retention time of l-menthol beginning after the solvent peak.

**Mepivacaine hydrochloride for assay** C_{15}H_{22}N_{2}O.HCl [Same as the monograph Mepivacaine Hydrochloride. When dried, it contains not less than 99.0% of C_{15}H_{22}N_{2}O.HCl].

- **Detection sensitivity** so that the peak area of 2-mercaptoethanol is 3 – 4 times.

**Time span of measurement:** About 7 times as long as the retention time of 2-mercaptoethanol.

**Mercaptopurine** See mercaptopurine hydrate.

**Mercaptopurine hydrate** C_{10}H_{12}N_{4}S.H_{2}O [Same as the namesake monograph]

**Mercuric acetate** See mercury (II) acetate.

**Mercuric acetate TS for nonaqueous titration** See mercury (II) acetate TS for nonaqueous titration.

**Mercuric chloride** See mercury (II) chloride.

**Mercury Hg** [K 8572, Special class]

**Mercury (II) acetate** Hg(CH_{3}COO)_{2} White crystals or crystalline powder.

Identification—\( (1) \) Dissolve 1 g of mercury (II) chloride in 1 mL of dilute nitric acid (1 in 7), add 20 mL of water, and use this as the sample solution. To 10 mL of the sample solution add 0.8 mL of iron (III) chloride TS: a red-brown color is developed.

\( (2) \) To 10 mL of the sample solution obtained in \( (1) \) add 2 mL of potassium iodate TS: a red precipitate is produced. Preserve in a light-resistant tight container.

**Mercury (II) acetate TS for nonaqueous titration** Dissolve 5 g of mercury (II) acetate in acetic acid (100 mL) for nonaqueous titration to make 100 mL.

**Mercury (II) chloride HgCl_{2}** [K 8139, Special class]

**Mercury (II) chloride TS** Dissolve 5.4 g of mercury (II) chloride in water to make 100 mL.

**Mesaconitine for purity** C_{3}H_{7}NO_{11} White, crystals or crystalline powder. Slightly soluble in acetonitrile and in ethanol (99.5), very slightly soluble in diethyl ether, and practically insoluble in water. Melting point: about 190°C (with decomposition).

Identification—Determine the infrared absorption spectrum of mesaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry \( < 2.25 > \): it exhibits absorption at the wave numbers of about 3510 cm\(^{-1}\), 1713 cm\(^{-1}\), 1277 cm\(^{-1}\), 1116 cm\(^{-1}\), 1098 cm\(^{-1}\) and 717 cm\(^{-1}\).

Absorbance \( < 2.25 \times E_{1%}^{1 cm} (230 nm): 211 – 247 \) [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), ethanol (99.5), 200 mL].

Purity Related substances—\( (1) \) Dissolve 5.0 mg of mesaconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( < 2.03 > \). Spot 20 \( \mu \)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test...
as directed in the Identification under Processed Aconite Root: the spot other than the principal spot is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of mesaconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of mesaconitine and the solvent is not larger than the peak area of mesaconitine with the standard solution.

Operating conditions
Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity under Processed Aconite Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust the flow rate so that the retention time of mesaconitine is about 19 minutes.

Time span of measurement: About 3 times as long as the retention time of mesaconitine.

System suitability
Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of mesaconitine obtained from 10 μL of this solution is equivalent to 3.5 to 6.5

System performance: Dissolve 1 mg each of aconitine for purity, hypaconitine for purity and mesaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10 μL of this solution under the above operating conditions, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mesaconitine is not more than 1.5%

Water (2.48): not more than 1.0% [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide) for 4 hours. It is white, odorless crystals. Recrystallize in the same manner, and dry the crystals in a desiccator in vacuum, phosphorus (V) oxide) for 4 hours. It is white, odorless crystals. Absorbance <2.24> E<sub>1%1 cm</sub> (242 nm): 321 – 328 (1 mg, methanol, 100 mL).

Specific gravity <2.56> d<sub>4</sub>: 0.850 – 0.860

Metacresol purple CH<sub>3</sub>CH<sub>2</sub>H<sub>2</sub>OH [K 8889, Special class]

Metacresol purple TS Dissolve 0.10 g of metacresol purple in 13 mL of 0.01 mol/L sodium hydroxide TS, and add water to make 100 mL.

Metacycline hydrochloride C<sub>27</sub>H<sub>42</sub>O<sub>3</sub>N<sub>2</sub>HCl Yellow to dark yellow, crystals or crystalline powder.

Purity Related substances—Dissolve 20 mg of metacycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20 μL of the sample solution as directed in the Purity (2) under Doxycycline Hydrochloride Hydrate, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total area of peaks other than metacycline is not more than 10%.

Metallic sodium See sodium.

Metanil yellow C<sub>18</sub>H<sub>14</sub>N<sub>3</sub>NaO<sub>3</sub>S Yellow-brown powder. Sparingly soluble in water, and very slightly soluble in ethanol (95%) and in N,N-dimethylformamide.

Metanil yellow TS Dissolve 0.1 g of metanil yellow in 200 mL of N,N-dimethylformamide.

Metaphosphoric acid HPO<sub>3</sub> A colorless, deliquescent stick or masses.

Identification—(1) Dissolve 1 g of metaphosphoric acid in 50 mL of water, and use this as the sample solution. To 10 mL of the sample solution add 0.2 mL of ammonia TS and 1 mL of silver nitrate TS: a yellowish white precipitate is produced.

(2) To 10 mL of the sample solution obtained in (1) add 10 mL of albumin TS: a white precipitate is produced.

Metaphosphoric acid-acetic acid TS Dissolve 15 g of metaphosphoric acid and 40 mL of acetic acid (100) in water to make 500 mL. Preserve in a cold place, and use within 2 days.

Metenolone enanthate C<sub>18</sub>H<sub>14</sub>N<sub>3</sub>NaO<sub>3</sub>S [Same as the name-sake monograph]

Metenolone enanthate for assay C<sub>18</sub>H<sub>14</sub>N<sub>3</sub>NaO<sub>3</sub>S To 1 g of metenolone enanthate add 30 mL of water, and add slowly 70 mL of methanol with warming to dissolve. Filter while hot, and allow the filtrate to stand on a water bath for 30 minutes. Allow to stand overnight in a cold place, collect the crystals thus formed, and wash with a small amount of diluted methanol (1 in 3). Recrystallize in the same manner, and dry the crystals in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours. It is white, odorless crystals.

Absorbance <2.24> E<sub>1%1 cm</sub> (242 nm): 321 – 328 (1 mg, methanol, 100 mL).

Optical rotation <2.49> [α]<sub>D</sub>: +40 – +42° (0.2 g, chloroform, 10 mL, 100 mm).

Melting point <2.60>: 69 – 72°C

Purity Related substances—Dissolve 50 mg of metenolone enanthate for assay in chloroform to make exactly 10 mL, and use this solution as the sample solution. Proceed with 10 μL of this solution as directed in the Purity (3) under Metenolone Enanthate: any spot other than the principal spot does not appear.

Metformin hydrochloride for assay C<sub>6</sub>H<sub>11</sub>N<sub>2</sub>Cl [Same as the monograph Metformin Hydrochloride. When dried, it contains not less than 99.0% of metformin hydrochloride (C<sub>6</sub>H<sub>11</sub>N<sub>2</sub>Cl)].

Methanesulfonic acid CH<sub>3</sub>SO<sub>3</sub>H Clear, colorless liquid or colorless or white, crystalline mass, having a characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether.

Conspicuousness <2.42>: 15 – 20°C
Specific gravity <2.56> d<sub>4</sub>: 1.483 – 1.488
Content: not less than 99.0%. Assay—Weigh accurately about 2 g of methanesulfonic acid, dissolve in 40 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS
Methanesulfonic acid TS To 35 mL of methanesulfonic acid add 20 mL of acetic acid (100) and water to make 500 mL.

0.1 mol/L Methanesulfonic acid TS To 4.8 g of methanesulfonic acid add water to make 500 mL.

Methanol CH₃OH [K 8891, Special class]

Methanol, anhydrous CH₃O To 1000 mL of methanol add 5 g of magnesium powder. If necessary, add 0.1 mL of mercury (II) chloride TS to start the reaction. After the evolving of a gas is stopped, distillate the solution, and preserve the distillate protecting from moisture. Water content per mL is not more than 0.3 mg.

Methanol-free ethanol See ethanol (95), methanol-free.

Methanol-free ethanol (95) See ethanol (95), methanol-free.

Methanol, purified Distil methanol before use.

Methionin See L-methionine.

1-Methionine C₇H₁₅NO₂S [Same as the namesake monograph]

Methotrexate C₂₀H₂₂N₈O₅ [Same as the namesake monograph]

4-Methoxybenzaldehyde C₈H₉O₂ Clear, colorless to pal yellow liquid. Miscible with methanol and with ethanol (99.5), and slightly soluble in water. Congealing point: 3 – 8°C.

Identification—Determine the infrared absorption spectrum of 4-methoxybenzaldehyde as directed in the ATR method under Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 3421 cm⁻¹, 2923 cm⁻¹, 1610 cm⁻¹, 1460 cm⁻¹, 1220 cm⁻¹, and 1031 cm⁻¹.

Purity Related substances—Perform the test with 0.2 μL of 4-methoxybenzaldehyde as directed under Gas Chromatography: according to the following conditions. Determine each peak by the automatic integration method: the total area of the peaks other than the peak of 4-methoxy-4-methylphenol is not more than 3.0%.

Operating conditions
Detector: A hydrogen flame-ionization detector.
Column: A fused silica column 0.25 mm in inside diameter and 60 m in length, coated inside with polydimethylsiloxane for gas chromatography in 0.25 to 0.5 μm in thickness.

Column temperature: Inject at a constant temperature of about 100°C, raise the temperature to 130°C at a rate of 5°C per minute, raise to 140°C at a rate of 2°C per minute, raise to 200°C at a rate of 15°C per minute, and maintain at 200°C for 2 minutes.

Injection port temperature: 200°C.
Detector temperature: 250°C.
Carrier gas: Helium.
Flow rate: Adjust the flow rate so that the retention time of 2-methoxy-4-methylphenol is about 10 minutes.  
Split ratio: 1:50.

System suitability  
System performance: Dissolve 60 mg of 2-methoxy-4-methylphenol in methanol to make 100 mL, and use this solution as the solution for system suitability test. Proceed with 1 μL of the solution for system suitability test under the above operating conditions, the symmetry factor of the peak of 2-methoxy-4-methylphenol is not more than 1.5.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 2-methoxy-4-methylphenol is not more than 2.0%.

1-Methoxy-2-propanol C7H12O2  A colorless, clear liquid.

Clarity of solution—To 5 mL of 1-methoxy-2-propanol add 20 mL of water, and mix: the solution is clear.
Refractive index <2.45> nD20: 1.402 – 1.405  
Specific gravity <2.56> d20: 0.920 – 0.925  
Water <2.49> not more than 0.5% (5 g).

Content: not less than 98.0%. Assay—Proceed as directed under Gas Chromatography <2.02> using the area percentage method according to the following conditions:
Operating conditions  
Detector: Thermal conductivity detector.  
Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm) coated with polyethylene glycol 20 M for gas chromatography in 20%.

Column temperature: A constant temperature of about 90°C.
Carrier gas: Helium.

Flow rate: A constant flow rate of 20 mL per minute.

Methyl acetate CH3COOCH3  [K 8382, Special class]

p-Methyl aminophenol sulfate See 4-methyl aminophenol sulfate.

4-Methyl aminophenol sulfate (HOCH2NHCH2)2H2SO4  White to pale yellow or very pale grayish white, crystals or crystalline powder. Melting point: about 260°C (with decomposition).

p-Methyl aminophenol sulfate TS  See 4-methyl aminophenol sulfate TS.

4-Methyl aminophenol sulfate TS  Dissolve 0.35 g of 4-methyl aminophenol sulfate and 20 g of sodium hydrogen sulfite in water to make 100 mL. Prepare before use.

2-Methylnaphthopyridine C9H7N  A pale yellow liquid.

Specific gravity <2.56> d<sub>20</sub>: 1.050 – 1.065  
Boiling point <2.57>: 200 – 202°C  
Water <2.49>: less than 0.1%.

2-Methylnaphthopyridine for Karl Fischer method  See Water Determination <2.487>.

Methyl behenate C32H52O2  White, odorless and tasteless, scaly crystals or powder. Dissolves in acetone, in diethyl ether and in chloroform.
Melting point <2.60>: 54°C  
Saponification value <1.13>: 155.5 – 158.5  
Methyl benzoate C9H8O2  Clear, colorless liquid.

Refractive index <2.45> nD20: 1.515 – 1.520  
Specific gravity <2.56> d20: 1.087 – 1.095

Purity—Dissolve 0.1 mL of methyl benzoate in the mobile phase in Assay under Thiamine Hydrochloride to make 50 mL. Perform the test as directed under Liquid Chromatography <2.01> with 10 μL of this solution according to the Assay under Thiamine Hydrochloride. Measure each peak area by the automatic integration method in a range about twice the retention time of methyl benzoate, and calculate the amount of methyl benzoate by the area percentage method: it shows the purity of not less than 99.0%.

Methyl benzoate for estriol test C9H8O2  Clear, colorless liquid, having a characteristic odor.

Refractive index <2.45> nD20: 1.515 – 1.520  
Specific gravity <2.56> d20: 1.087 – 1.095  
Acid value <1.13>: not more than 0.5.

D(+)-α-Methylbenzylamine C7H9(CH2CH3)NH2  Colorless or pale yellow clear liquid, having an amine like odor. Miscible with ethanol (95) and with acetone, and slightly soluble in water.

Refractive index <2.45> nD20: 1.524–1.529  
Optical rotation <2.49> [α]D<sub>20</sub>: +37 – +41° (50 mm).

Content: not less than 98.0%. Assay—Perform the test with exact 0.6 μL of D(+)-α-methylbenzylamine as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of D(+)-α-methylbenzylamine.

Operating conditions  
Detector: Hydrogen flame-ionization detector.  
Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 250 μm in particle diameter) coated with polyethylene glycol 20 M for gas chromatography and potassium hydroxide at the ratio of 10% and 5%, respectively.

Column temperature: A constant temperature of about 140°C.
Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of D(+)-α-methylbenzylamine is about 5 minutes.

Selection of column: To 5 mL of D(+)-α-methylbenzylamine add 1 mL of pyridine. Proceed with 0.6 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pyridine and p(+)α-methylbenzylamine in this order with the resolution between these peaks being not less than 3.

Time span of measurement: About 3 times as long as the retention time of D(+)-α-methylbenzylamine beginning after the solvent peak.

3-Methyl-1-butanol C8H16O  [K 8051, Special class]

3-Methylbutyl acetate CH3COOCH2CH2CH(CH3)2  A clear and colorless liquid. Boiling point: about 140°C.

Specific gravity <2.56> d20: 0.868 – 0.879  
Preserve in a light-resistant tight container.

Methyl cellosolve  See 2-methoxyethanol.

Methyl docosanate C32H64O2  White, tabular crystals or crystalline powder.

Melting point <2.60>: 51.0 – 56.0°C
Methyldopa  See methyldopa hydrate.
Methyldopa for assay  See methyldopa hydrate for assay.
Methyldopa hydrate  \( C_{10}H_{13}NO_4 \cdot \frac{1}{2}H_2O \) [Same as the namesake monograph]
Methyldopa hydrate for assay  \( C_{10}H_{13}NO_4 \cdot \frac{1}{2}H_2O \) [Same as the monograph Methyldopa Hydrate. It contains not less than 99.0% of methyldopa \( (C_{10}H_{13}NO_4) \), calculated on the anhydrous basis.]
\( N,N' \)-Methylenebisacrylamide  \( CH_2(NHCOCHCH_2)_2 \) White crystalline powder. Content: not less than 97.0%.
Methylene blue  See methylene blue trihydrate.
Methylene blue-sulfuric acid-monobasic sodium phosphate TS  See methylene blue-sulfuric acid-sodium dihydrogenphosphate TS.
Methylene blue-sulfuric acid-sodium dihydrogenphosphate TS  To 30 mL of a solution of methylene blue (1 in 1000) add 500 mL of water, 6.8 mL of sulfuric acid and 50 g of sodium dihydrogenphosphate dihydrate, dissolve, and add water to make 1000 mL.
Methylene blue trihydrate  \( C_{16}H_{16}ClN_3S \cdot 3H_2O \) [K 8997, Special class]
Methylene blue TS  Dissolve 0.1 g of methylene blue trihydrate in water to make 100 mL. Filter if necessary.
\( dl \)-Methylenebisacrylamide hydrochloride  \( C_{11}H_{17}NO \cdot HCl \) [Same as the namesake monograph]
\( dl \)-Methylenebisacrylamide hydrochloride for assay  [Same as the monograph \( dl \)-Methylenebisacrylamide Hydrochloride]
Methylergometrine maleate for assay  \( C_{29}H_{32}N_6O_5 \cdot C_5H_8O_4 \) [Same as the monograph Methylergometrine Maleate. When dried, it contains not less than 99.0% of methylergometrine maleate \( (C_{29}H_{32}N_6O_5 \cdot C_5H_8O_4) \).]
Methyl ethyl ketone  See 2-butanone.
Methyl iodide  See iodomethane.
Methyl iodide for assay  See iodomethane for assay.
Methyl isobutyl ketone  See 4-methyl-2-pentanone.
3-O-Methylmethyldopa for thin-layer chromatography  \( C_{13}H_{15}NO_4 \)

Purity  Related substances—Dissolve 5 mg of 3-O-methylmethyldopa for thin-layer chromatography in methanol to make exactly 100 mL. Perform the test with 20 \( \mu L \) of this solution as directed in the Purity (5) under Methyldopa: any spot other than the principal spot at the \( R_f \) value of about 0.7 does not appear.

2-Methyl-5-nitroimidazole for thin-layer chromatography  \( C_{6}H_{12}N_2O_2 \) White crystalline powder. Slightly soluble in water and in acetone. Melting point: about 253°C (with decomposition).

Purity  Related substances—Dissolve 40 mg of 2-methyl-5-nitroimidazole for thin-layer chromatography in 8 mL of acetone, and use as the sample solution. Pipet 2.5 mL of the sample solution, add acetone to make exactly 100 mL, and use as the standard solution. Perform the test as directed in the Purity (3) under Metronidazole: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Methyl orange  \( C_{16}H_{18}ClN_3S \cdot 3H_2O \) [K 8993, Special class]
Methyl orange-boric acid TS  Add 0.5 g of methyl orange and 5.2 g of boric acid in 500 mL of water, and dissolve by warming on a water bath. After cooling, wash this solution with three 50-mL portions of chloroform.
Methyl orange TS  Dissolve 0.1 g of methyl orange in 100 mL of water, and filter if necessary.
Methyl orange-xylene cyanol FF TS  Dissolve 1 g of methyl orange and 1.4 g of xylene cyanol FF in 500 mL of dilute ethanol.
Methyl parahydroxybenzoate  \( \text{HOCH}_2\text{COOCH}_3 \) [Same as the namesake monograph]
4-Methylpentan-2-ol  \( \text{C}_6\text{H}_{14} \) A clear and colorless, volatile liquid.

Refractive index  \( <2.45> : \text{about} 1.411 \)
Specific gravity  \( <2.56> : \text{about} 0.802 \)
Boiling point  \( <2.57> : \text{about} 132°C \)

Methyl-2-pentanone  \( \text{CH}_3\text{COCH}_2\text{CH}(\text{CH}_3)_2 \) [K 8903, Special class]
3-Methyl-1-phenyl-5-pyrazolone  \( \text{C}_10\text{H}_{10}N_2 \) [K 9548, Special class]
Methyl prednisolone  \( \text{C}_{22}\text{H}_{36}O_3 \) [Same as the namesake monograph]
2-Methyl-1-propanol  \( \text{CH}_3\text{CH}_2\text{CH}_2\text{OH} \) [K 8811, Special class]

\( N \)-Methylpyrrolidine  \( \text{C}_4\text{H}_9\text{N} \) Colorless, clear liquid, having a characteristic order.

Identification—Determine the spectrum of \( N \)-methylpyrrolidine in a solution of deuterated chloroform for nuclear magnetic resonance spectroscopy (4 in 50) as directed under Nuclear Magnetic Resonance Spectroscopy \( <2.27> \) \( (\text{H}) \): it exhibits a big signal, at around \( \delta \) 2.3 ppm.

Content: not less than 95%.—Assay—Put 30 mL of water in a beaker, weigh accurately the beaker, add dropwise about 0.15 g of \( N \)-methylpyrrolidine, weigh accurately the beaker again, and titrate \( <2.50> \) with 0.05 mol/L sulfuric acid VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.05 mol/L sulfuric acid VS = 8.515 mg of \( \text{C}_4\text{H}_9\text{N} \)

Methyl red  \( \text{C}_{12}\text{H}_{15}\text{N}_2 \) [K 8896, Special class]
Methyl red-methylene blue TS  Dissolve 0.1 g of methyl red and 0.1 g of methylene blue in ethanol (95) to make 100 mL, and filter if necessary. Preserve in light-resistant containers.
Methyl red TS  Dissolve 0.1 g of methyl red in 100 mL of ethanol (95), and filter if necessary.
Methyl red, dilute  Dissolve 25 mg of methyl red in 100 mL of ethanol (99.5), and filter if necessary. Prepare before use.

Methyl red TS for acidity or alkalinity test  To 0.1 g of
methyl red add 7.4 mL of 0.05 mol/L sodium hydroxide VS or 3.7 mL of 0.1 mol/L sodium hydroxide VS, triturate to dissolve in a mortar, and add freshly boiled and cooled water to make 200 mL. Preserve in light-resistant, glass-stoppered bottles.

**Methyrosaniline chloride** See crystal violet.

**Methyrosaniline chloride TS** See crystal violet TS.

**Methyl salicylate** C₇H₆O₃ [Same as the namesake monograph]

**Methylsilicone polymer** for gas chromatography Prepared for gas chromatography.

**Methyltestosterone** C₁₉H₂₉NO₃ [Same as the namesake monograph]

1-Methyl-1H-tetrazole-5-thiol C₆H₈N₅S White, crystals or crystalline powder.

**Identification** (1) Determine the ultraviolet-visible absorption spectrum of a solution of 1-methyl-1H-tetrazole-5-thiol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry < 2.24: it exhibits a maximum between 222 nm and 226 nm.

(2) Determine the infrared absorption spectrum of 1-methyl-1H-tetrazole-5-thiol according to the potassium bromide disk method under Infrared Spectrophotometry < 2.25: it exhibits absorption at the wave numbers of about 3060 cm⁻¹, 2920 cm⁻¹, 2780 cm⁻¹, 1500 cm⁻¹, 1430 cm⁻¹ and 1410 cm⁻¹.

**Melting point** < 2.60: 125 – 129°C

**Purity** Related substances—Dissolve 0.1 g of 1-methyl-1H-tetrazole-5-thiol in exactly 100 mL of water. Perform the test with 1 μL of this solution as directed in the Purity (4) under Cefmetazole Sodium: any spot other than the principal spot at the Rf value of about 0.77 does not appear.

1-Methyl-1H-tetrazole-5-thiol for liquid chromatography C₆H₈N₅S White, crystals or crystalline powder. Very soluble in methanol, and freely soluble in water.

**Melting point** < 2.60: 123 – 127°C

**Loss on drying** < 2.47: not more than 1.0% (1 g, in vacuum, phosphorous(V) oxide, 2 hours).

**Content:** not less than 99.0%. Assay—Weigh accurately about 2 g of 1-methyl-1H-tetrazole-5-thiol, previously dried, dissolve in 80 mL of N,N-dimethylformamide, and titrate < 2.50 with 0.1 mol/L sodium methoxide VS (indicator: 3 drops of thymol blue-N,N-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS = 11.61 mg of C₆H₈N₅S

**Methylthymol blue** C₁₇H₁₉N₃O₁₁S [K 9552]

**Methylthymol blue-potassium nitrate indicator** Mix 0.1 g of methylthymol blue with 9.9 g of potassium nitrate, and triturate until the mixture becomes homogeneous.

**Sensitivity**—When 0.02 g of methylthymol blue-potassium nitrate indicator is dissolved in 100 mL of 0.02 mol/L sodium hydroxide VS, the solution is slightly blue in color. On adding 0.05 mL of 0.01 mol/L barium chloride VS to this solution, the solution shows a blue color, then on the subsequent addition of 0.1 mL of 0.01 mol/L disodium ethylenediaminetaacetate VS, it becomes colorless.

**Methyethylol blue-sodium chloride indicator** Mix 0.25 g of methylthymol blue and 10 g of sodium chloride, and grind to homogenize.

**Methyl yellow** C₁₄H₁₁N₃ [K 8494, Special class]

**Methyl yellow TS** Dissolve 0.1 g of methyl yellow in 200 mL of ethanol (95).

**Metoclopramide for assay** C₁₄H₂₁Cl₄N₂O₂ [Same as the monograph Metoclopramide. When dried, it contains not less than 99.0% of metoclopramide (C₁₄H₂₁Cl₄N₂O₂).]

**Metoprol tartrate for assay** (C₅H₇NO₄)₃C₂H₆O₆ [Same as the monograph Metoprol Tartrate. When dried, it contains not less than 99.5% of metoprol tartrate ((C₅H₇NO₄)₃C₂H₆O₆).]

**Metronidazole** C₁₅H₁₂N₃O [Same as the namesake monograph]

**Metronidazole for assay** C₁₅H₁₂N₃O [Same as the monograph Metronidazole. It meets the following additional requirement.]

**Related substances**—Weigh accurately about 25 mg of metronidazole for assay, dissolve in a mixture of water and methanol (4:1) to make exactly 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mixture of water and methanol (4:1) to make exactly 30 mL. Pipet 2.5 mL of this solution, add the mixture of water and methanol (4:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the standard solution and standard solution as directed under Liquid Chromatography < 2.01 according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than metronidazole is not more than the peak area of metronidazole with the standard solution.

**Operating conditions**
- **Detector**, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Metronidazole Tablets.
- **Time span of measurement:** About 4 times as long as the retention time of metronidazole.

**System suitability**
- **Test for required detectability:** Measure exactly 2 mL of the standard solution, add a mixture of water and methanol (4:1) to make exactly 20 mL. Confirm that the peak area of metronidazole obtained with 10 μL of this solution is equivalent to 7 to 13% of that with the standard solution.
- **System performance:** When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metronidazole are not less than 3000 and not more than 1.5, respectively.

**System repeatability**: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metronidazole is not more than 2.0%.

**Miconazole nitrate** C₁₈H₂₂Cl₂N₃O.HNO₃ [Same as the namesake monograph]

**Microplates** Polystyrene plates with an inside diameter of 7 (upper edge) to 6.4 (lower edge) mm, and 11.3 mm thickness. Have 96 flat-bottomed truncated cone-shaped wells.
Milk casein  See casein, milk.

Milk of lime  Place 10 g of calcium oxide in a mortar, and add gradually 40 mL of water under grinding.

Minocycline hydrochloride  C_{23}H_{27}N_{3}O_{7}.HCl  [Same as the namesake monograph]

Mixture of petroleum hexamethyl tetracosane branching hydrocarbons (L) for gas chromatography  Prepared for gas chromatography.

Molecular weight markers for teceleukin  Dissolve 0.4 mg of lysozyme, soy trypsin inhibitor, carbonic anhydrase, egg white albumin, bovine serum albumin, and phosphorylase b in 200 μL of diluted glycerin (1 in 2).

Molybdenum (III) oxide  MoO_{3}  A white to yellowish green powder.

Identification—Dissolve 0.5 g in 5 mL of ammonia solution (28), acidify 1 mL of this solution with a suitable amount of nitric acid, add 5 mL of sodium phosphate TS, and warm: yellow precipitates appear.

Molybdenum (III) oxide-citric acid TS  To 54 g of molybdenum (III) oxide and 11 g of sodium hydroxide add 200 mL of water, and dissolve by heating while stirring. Separately, dissolve 60 g of citric acid monohydrate in 250 mL of water, and add 140 g of hydrochloric acid. Mix these solutions, filter if necessary, add water to make 1000 mL, and add a solution of potassium bromate (1 in 100) until a yellow-green color appears.

Storage—Preserve in tightly stoppered containers, protected from light.

Molybdenum trioxide  See molybdenum (III) oxide.

Molybdenum trioxide-citric acid TS  See molybdenum (III) oxide-citric acid TS.

Monobasic ammonium phosphate  See ammonium dihydrogenphosphate.

0.02 mol/L  Monobasic ammonium phosphate TS  See 0.02 mol/L ammonium dihydrogenphosphate TS.

Monobasic potassium phosphate  See potassium dihydrogenphosphate.

Monobasic potassium phosphate for pH determination  See potassium dihydrogenphosphate for pH determination.

0.05 mol/L  Monobasic potassium phosphate, pH 3.0  See 0.05 mol/L potassium dihydrogenphosphate, pH 3.0.

0.05 mol/L  Monobasic potassium phosphate TS  See 0.05 mol/L potassium dihydrogenphosphate TS.

0.02 mol/L  Monobasic potassium phosphate TS  See 0.02 mol/L potassium dihydrogenphosphate TS.

0.05 mol/L  Monobasic potassium phosphate TS  See 0.05 mol/L potassium dihydrogenphosphate TS.

0.2 mol/L  Monobasic potassium phosphate TS  See 0.2 mol/L potassium dihydrogenphosphate TS.

0.2 mol/L  Monobasic potassium phosphate TS for buffer solution  See 0.2 mol/L potassium dihydrogenphosphate TS for buffer solution.

Monobasic sodium phosphate  See sodium dihydrogenphosphate dihydrate.

0.05 mol/L  Monobasic sodium phosphate TS, pH 2.6  See 0.05 mol/L sodium dihydrogenphosphate TS, pH 2.6.

0.05 mol/L  Monobasic sodium phosphate TS, pH 3.0  See 0.05 mol/L sodium dihydrogenphosphate TS, pH 3.0.

0.1 mol/L  Monobasic sodium phosphate TS, pH 3.0  See 0.1 mol/L sodium dihydrogenphosphate TS, pH 3.0.

0.05 mol/L  Monobasic sodium phosphate TS  See 0.05 mol/L sodium dihydrogenphosphate TS.

0.1 mol/L  Monobasic sodium phosphate TS  See 0.1 mol/L sodium dihydrogenphosphate TS.

2 mol/L  Monobasic sodium phosphate TS  See 2 mol/L sodium dihydrogenphosphate TS.

Monoethanolamine  See 2-Aminoethanol.

Morphine hydrochloride  See morphine hydrochloride hydrate.

Morphine hydrochloride for assay  See morphine hydrochloride hydrate for assay.

Morphine hydrochloride hydrate  C_{17}H_{19}NO_{3}.HCl.3H_{2}O  [Same as the namesake monograph]

Morphine hydrochloride hydrate for assay  C_{17}H_{19}NO_{3}.HCl.3H_{2}O  [Same as the monograph Morphine Hydrochloride Hydrate. It contains not less than 99.0% of morphine hydrochloride (C_{17}H_{19}NO_{3}.HCl), calculated on the anhydrous basis.]

3-(N-Morpholino)propanesulfonic acid  C_{7}H_{12}NO_{3}S

White crystalline powder, freely soluble in water, and practically insoluble in ethanol (99.5).

Melting point <2.60>: 275 – 280°C

0.02 mol/L  3-(N-Morpholino)propanesulfonic acid buffer solution, pH 7.0  Dissolve 4.2 g of 3-(N-morpholino)propanesulfonic acid in 900 mL of water, adjust the pH to 7.0 with dilute sodium hydroxide TS, and add water to make 1000 mL.

0.1 mol/L  3-(N-Morpholino)propanesulfonic acid buffer solution, pH 7.0  Dissolve 20.92 g of 3-(N-morpholino)propanesulfonic acid in 900 mL of water, adjust the pH to 7.0 with sodium hydroxide TS, and add water to make 1000 mL.

0.02 mol/L  3-(N-Morpholino)propanesulfonic acid buffer solution, pH 8.0  Dissolve 4.2 g of 3-(N-morpholino)propanesulfonic acid in 700 mL of water, adjust the pH to 8.0 with dilute sodium hydroxide TS, and add water to make 1000 mL.

Mosapride citrate for assay  See mosapride citrate hydrate for assay.

Mosapride citrate hydrate for assay  C_{21}H_{25}ClFN_{3}O_{3}.C_{6}H_{8}O_{7}.2H_{2}O  [Same as the monograph Mosapride Citrate Hydrate. It contains not less than 99.0% of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{3}.C_{6}H_{8}O_{7}) calculated on the anhydrous basis.]
an autoclave for 15 minutes at 121°C to make the PBS(–) solution. Dissolve 0.3 g of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide in this PBS(–) solution to make 100 mL. Sterilize by membrane filtration (pore size, 0.45 μm), and store in a cool place shielded from light.

Murexide C₉H₇N₂O₂ Red-purple powder. Practically insoluble in water, in ethanol (95) and in diethyl ether. 

Purity Clarity of solution—Dissolve 10 mg of murexide in 100 mL of water: the solution is clear. 

Residue on ignition <2.44%: not more than 0.1% (1 g).

Sensitivity—Dissolve 10 mg of murexide in 2 mL of ammonium-ammonium chloride buffer solution, pH 10.0, and add water to make 100 mL, and use this solution as the sample solution. Separately, add 2 mL of ammonium ammonium chloride buffer solution, pH 10.0, to 5 mL of diluted Standard Calcium Solution (1 in 10), add water to make 25 mL, and render the solution to pH 11.3 with sodium hydroxide VS: a green color develops. Add subsequently 0.2 mL of 0.1 mol/L sodium hydroxide VS: a bright yellow color develops.

Water <2.46%: 7.0–11.5% (0.5 g, volumetric titration, direct titration).

Content: not less than 95.0%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.5 g of 2-naphthalenesulfonic acid hydrate, dissolve in 30 mL of water, and titrate <2.59% with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 20.82 mg of C₉H₇O₃S

1-Naphthol C₁₀H₇OH [K 8698, Special class] Preserve in light-resistant containers.

2-Naphthol C₁₀H₇OH [K 8699, Special class] Preserve in light-resistant containers.

α-Naphthol See 1-naphthol.

β-Naphthol See 2-naphthol.

p-Naphtholbenzein C₂₇H₂₀O₃ [K 8693, Special class] See p-naphtholbenzein.

p-Naphtholbenzein TS Dissolve 0.2 g of p-naphtholbenzein in acetic acid (100) to make 100 mL.

Purity Clarity and color of solution—Dissolve 0.1 g of p-naphtholbenzein in 100 mL of ethanol (95): the solution is red in color and clear. 

Sensitivity—Add 100 mL of freshly boiled and cooled water to 0.2 mL of a solution of p-naphtholbenzein in ethanol (95) (1 in 1000), and add 0.1 mL of 0.1 mol/L sodium hydroxide VS: a green color develops. Add subsequently 0.2 mL of 0.1 mol/L hydrochloric acid VS: the color of the solution changes to yellow-red.

p-Naphtholbenzein TS See p-naphtholbenzein TS.

1-Naphthol-sulfuric acid TS Dissolve 1.5 g of 1-naphthol in 50 mL of ethanol (95), add 3 mL of water and 7 mL of sulfuric acid, and mix well. Prepare before use.

1-Naphthol TS Dissolve 6 g of sodium hydroxide and 16 g of anhydrous sodium carbonate in water to make 100 mL. In this solution dissolve 1 g of 1-naphthol. Prepare before use.

2-Naphthol TS Dissolve 1 g of 2-naphthol in sodium carbonate TS to make 100 mL. Prepare before use.

α-Naphthol TS See 1-naphthol TS.

β-Naphthol TS See 2-naphthol TS.

1-Naphthylamine C₁₀H₁₂N₂ [K 8692, Special class] Preserve in light-resistant containers.
α-Naphthylamine See 1-naphthylamine.

\(\text{N}-(1\text{-Naphthyl})-\text{N}^\prime\text{-diethylethylenediamine oxalate}\) See \(\text{N},\text{N}-\text{diethyl}-\text{N}^\prime\text{-1-naphthylethylenediamine oxalate}\).

\(\text{N}-(1\text{-Naphthyl})-\text{N}^\prime\text{-diethylethylenediamine oxalate-acetone TS}\) See \(\text{N},\text{N}-\text{diethyl}-\text{N}^\prime\text{-1-naphthylethylenediamine oxalate-acetone TS}\).

\(\text{N}-1\text{-Naphthylethylenediamine dihydrochloride}\)
\[\text{C}_{10}\text{H}_{7}\text{N}\text{HCH}_{2}\text{CH}_{2}\text{NH}_{2}.2\text{HCl} \quad [\text{K} 8197, \text{Special class}]\]

Naringin dihydrate for thin-layer chromatography

Naringin for thin-layer chromatography

Neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)

An amido binding condensate of neocarzinostatin and styrene-maleic acid alternating copolymer partial butyl ester in a rate of 2:3. Average molecular mass: about 28,400. A pale yellow powder.

Identification—Dissolve 4 mg of the substance to be examined in 0.05 mol/L phosphate buffer solution, pH 7.0 to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry. <2.24>: it exhibits a maximum between 266 nm and 270 nm, and shoulders between 257 nm and 262 nm, between 286 nm and 291 nm and between 318 nm and 348 nm.

Absorbance <2.24> \(E_{10}^{100}(268 \text{ nm})\): 13.0 – 17.5 [4 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 10 mL]

Purity—Proceed as directed in the Purity (3) under Zinostatin Stimalamer, with the exception of without using of (iii) Standard solution, and changing (iv) Sample solution, (v) Procedure and (vii) Determination as follows:

(iv) Sample solution Dissolve 3.0 mg of the substance to be examined in the buffer solution for sample to make 10 mL.

(v) Procedure Mount the gel in the electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the top reservoir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100 \(\mu\text{L}\) of the sample solution onto the surface of the gel, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as a bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached at 5 cm from the upper end of the gel.

(vii) Determination Determine the peak area, \(A_T\), of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) and the total area, \(A\), of the peaks other than neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3), based on the absorbance at 600 nm of the gel determined by using a densitometer. Calculate the amount of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) by the following formula: not less than 90.0%.

Amount (%) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) = \(\frac{[A_T/(A_T + A)] \times 100}{\ aspiration time of neocarzinostatin is about 21 minutes.

Time span of measurement: About 2 times as long as the retention time of neocarzinostatin.

System suitability

System performance: When the procedure is run with 0.25 mL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of neocarzinostatin are not less than 2000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 0.25 mL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of neocarzinostatin is not more than 2.0%.

Water <2.40> Not more than 10.0% (10 mg, coulometric titration).
**JP XVI**

**General Tests / 9.41 Reagents, Test Solutions**

**Water** [2.48] Not more than 12.0% (10 mg, coulometric titration).

**Neutral alumina containing 4% of water** Take 50 g of neutral alumina for column chromatography, previously dried at 105°C for 2 hours, in a tight container, add 2.0 mL of water, shake well to make homogeneous, and allow to stand for more than 2 hours.

**Neutral detergent** Synthetic detergent containing anionic or non-ionic surfactant, and pH of its 0.25% solution is between 6.0 and 8.0. Dilute to a suitable concentration before use.

**Neutralized ethanol** See ethanol, neutralized.

**Neutral red** C_{18}H_{17}N_{4}Cl Slightly metallic, dark green powder or masses.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3310 cm⁻¹, 3160 cm⁻¹, 1621 cm⁻¹, 1503 cm⁻¹, 1323 cm⁻¹, 1199 cm⁻¹ and 732 cm⁻¹.

**Neutral red TS** Dissolve 0.1 g of neutral red in acetic acid (100) to make 100 mL.

**Nicardipine hydrochloride for assay** C_{26}H_{29}N_{3}O_{6}.HCl [Same as the monograph Nicardipine Hydrochloride. When dried, it contains not less than 99.0% of nicardipine hydrochloride (C_{26}H_{29}N_{3}O_{6}.HCl).]

**Nicergoline for assay** C_{24}H_{26}BrN_{3}O_{3} [Same as the monograph Nicergoline, or Nicergoline purified according to the method below. When dried, it contains not less than 99.0% of nicergoline (C_{24}H_{26}BrN_{3}O_{3}), and when perform the test of the Purity (2) under Nicergoline, the total area of the peaks other than nicergoline from the sample solution is not more than 2.5 times the peak area of nicergoline from the standard solution.

Method of purification: Dissolve 1 g of Nicergoline in 20 mL of acetonitrile, allow to stand in a dark place for about 36 hours, filter, and dry the crystals so obtained at 60°C for 2 hours in vacuum.]

**Nicomol for assay** C_{16}H_{12}N_{4}O [Same as the monograph Nicomol. When dried, it contains not less than 99.0% of C_{16}H_{12}N_{4}O_{3}.]

**Nicotinamide** C_{6}H_{6}N_{2}O [Same as the namesake monograph]

**β-Nicotinamide adenine dinucleotide (β-NAD)** C_{21}H_{27}N_{7}O_{14}P_{2} [K 9802]

**Content:** not less than 94.5%. **Assay:**—Weigh accurately about 25 mg of β-nicotinamide-adenine dinucleotide, oxidized form, and dissolve in water to make exactly 25 mL. Pipet 0.2 mL of this solution, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 10 mL, and use this solution as the sample solution. Determine the absorbances, A_1 and A_0, of the sample solution and 0.1 mol/L phosphate buffer solution, pH 7.0, at 260 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

\[
\text{Amount (mg) of } C_{21}H_{27}N_{7}O_{14}P_{2} = \frac{0.6634 \times 10}{17.6 \times 0.20} \times (A_1 - A_0) \times 25
\]

**β-Nicotinamide adenine dinucleotide TS** Dissolve 40 mg of β-nicotinamide adenine dinucleotide, oxidized form in 10 mL of water. Prepare before use.

**Nifedipine** C_{17}H_{14}N_{2}O_{6} [Same as the namesake monograph]

**Nile blue** C_{29}H_{32}ClIN_{2}O Blue-green powder.

**Ninhydrin** C_{6}H_{6}N_{2}O [K 8870, Special class]

**Ninhydrin TS** Dissolve 0.2 g of ninhydrin in water to make 10 mL. Prepare before use.

**Ninhydrin-acetic acid TS** Dissolve 1.0 g of ninhydrin in 50 mL of ethanol (95), and add 10 mL of acetic acid (100).

**Ninhydrin-tartric acid-acetic acid TS** Dissolve 70 g of citric acid, monohydrate in 500 mL of water, add 58 mL of acetic acid (100), 70 mL of a solution of sodium hydroxide (21 in 50) and water to make 1000 mL. In 100 mL of this solution dissolve 0.2 g of ninhydrin.

**Ninhydrin-stannous chloride TS** See ninhydrin-tin (II) chloride TS.

**Ninhydrin-sulfuric acid TS** Dissolve 0.1 g of ninhydrin in 100 mL of sulpheric acid. Prepare before use.

**Ninhydrin-tartric acid-chloride TS** Dissolve 21.0 g of citric acid in water to make 200 mL, adjust the pH to 5.6 ± 0.2 by adding sodium hydroxide TS, add water to make 500 mL, and dissolve 1.3 g of tin (II) chloride. To 50 mL of the solution, add 50 mL of a 2-methoxethanol solution of ninhydrin (2 in 50). Prepare before use.

**0.2% Ninhydrin-water saturated 1-butanol TS** Dissolve 2 g of ninhydrin in 1-butanol saturated with water to make 1000 mL.

**Nitrendipine for assay** [Same as the monograph Nitrendipine. It, when dried, contains not less than 99.0% of nitrendipine (C_{20}H_{20}ClN_{3}O_{6}) and meets the following requirement. When perform the test as directed in the Purity (2) under Nitrendipine, the area of the peak of nitrendipine from the standard solution, and the area of the peak other than nitrendipine and the dimethyl ester is not larger than 1/2 times the peak area of nitrendipine from the standard solution, and the total area of the peak other than nitrendipine is not larger than 1/2 times the peak area of nitrendipine from the standard solution.]

**Nitric acid** HNO_{3} [K 8541, Special class, Concentration: 69 – 70%, Density: about 1.42 g/mL]

**Nitric acid, dilute** Dilute 10.5 mL of nitric acid with water to make 100 mL (10%).

**Nitric acid, fuming** [K 8739, Special class, Concentration: not less than 97%, Density: 1.52 g/mL]

**Nitric acid TS, 2 mol/L** Dilute 12.9 mL of nitric acid...
with water to make 100 mL.

2',2'-2'-Nitrolitriethanol  \((\text{CH}_2\text{CH}_2\text{OH})_3\text{N}\)  [K 8663, Special class]

2',2'-2'-Nitrolitriethanol buffer solution, pH 7.8
Dissolve 149.2 g of 2',2',2'-nitrolitriethanol in about 4500 mL of water, adjust to pH 7.8 with diluted 6 mol/L hydrochloric acid TS (2 in 3), and add water to make 5000 mL.

3-Nitroaniline  \(\text{C}_6\text{H}_5\text{NO}_2\)  Yellow crystals or crystalline powder.
**Melting point** <2.60>: 112 – 116°C

4-Nitroaniline  \(\text{C}_6\text{H}_4\text{NO}_2\)  Yellow to yellowish-red crystals or crystalline powder.
**Melting point** <2.60>: 147 – 150°C. Preserve in a light-resistant tight container.

p-Nitroaniline  See 4-nitroaniline.

p-Nitroaniline-sodium nitrite TS  See 4-nitroaniline-sodium nitrite TS.

3-Nitroaniline-sodium nitrite TS  To 90 mL of a solution of 0.3 g of 4-nitroaniline in 100 mL of 10 mol/L hydrochloric acid TS add 10 mL of a solution of sodium nitrite (1 in 20), and mix well. Prepare before use.

O-Nitrobenzaldehyde  See 2-nitrobenzaldehyde.

2-Nitrobenzaldehyde  \(\text{O}_2\text{NC}_6\text{H}_4\text{CHO}\)  Pale yellow crystals or crystalline powder.
**Melting point** <2.60>: 42 – 44°C

Nitrobenzene  \(\text{C}_6\text{H}_5\text{NO}_2\)  [K 8723, Special class]

p-Nitrobenzenediazonium chloride TS  See 4-nitrobenzenediazonium chloride TS.

4-Nitrobenzenediazonium chloride TS  To 90 mL of a solution of 0.3 g of 4-nitroaniline in 100 mL of 10 mol/L hydrochloric acid TS add 10 mL of a solution of sodium nitrite (1 in 20), and mix well. Prepare before use.

p-Nitrobenzenediazonium chloride TS for spraying  See 4-nitrobenzenediazonium chloride TS for spraying.

4-Nitrobenzenediazonium chloride TS for spraying  Dissolve 1.1 g of 4-nitroaniline in 1.5 mL of hydrochloric acid, add 1.5 mL of water, and then add a solution prepared by dissolving 0.5 g of sodium nitrite in 5 mL of water, while cooling in an ice bath. Prepare before use.

p-Nitrobenzenediazonium fluoride TS  See 4-nitrobenzenediazonium fluoride TS.

4-Nitrobenzenediazonium fluoride TS  Dissolve 1.1 g of 4-nitroaniline in 1.5 mL of hydrochloric acid, add 1.5 mL of water, and then add a solution prepared by dissolving 0.5 g of sodium nitrite in 5 mL of water, while cooling in an ice bath. Prepare before use.

p-Nitrobenzenediazonium fluoride TS for spraying  See 4-nitrobenzenediazonium fluoride TS for spraying.

4-Nitrobenzenediazonium fluoride TS for spraying  Dissolve 0.4 g of 4-nitroaniline in 60 mL of 1 mol/L hydrochloric acid TS, and add, while cooling in an ice bath, sodium nitrite TS until the mixture turns potassium iodide-starch paper to blue in color. Prepare before use.

p-Nitrobenzenediazonium hydrochloride  See 4-nitrobenzenediazonium hydrochloride.

4-Nitrobenzenediazonium hydrochloride  \(\text{O}_2\text{NC}_6\text{H}_4\text{NBF}_4\)  Pale yellowish white, almost odorless powder. Freely soluble in dilute hydrochloric acid, slightly soluble in water, and very slightly soluble in ethanol (95) and in chloroform. Melting point: about 148°C (with decomposition).

**Identification**—Add 1 mL each of a solution of phenol (1 in 1000) and sodium hydroxide TS to 10 mL of a solution of 4-nitrobenzenediazonium hydrochloride (1 in 1000): a red color develops.

**Loss on drying** <2.41>: not more than 1.0% (1 g, silica gel, 2 hours).

p-Nitrobenzoyl chloride  See 4-nitrobenzoyl chloride.

4-Nitrobenzoyl chloride  \(\text{O}_2\text{NC}_6\text{H}_4\text{COCl}\)  Light yellow crystals.
**Melting point** <2.60>: 70 – 74°C

**Content**: not less than 98.0%. Assay—Weigh accurately about 0.5 g of 4-nitrobenzoyl chloride, add an excess of silver nitrate-ethanol TS, and boil under a reflux condenser for 1 hour. After cooling, filter the precipitate, wash with water, dry at 105°C to constant mass, and weigh. The mass of 4-nitrobenzoyl chloride, multiplied by 1.107, represents the mass of 4-nitrobenzoyl chloride (\(\text{C}_6\text{H}_4\text{CINO}_3\)).

p-Nitrobenzyl chloride  See 4-nitrobenzyl chloride.

4-Nitrobenzyl chloride  \(\text{O}_2\text{NC}_6\text{H}_4\text{CHCl}\)  Light yellow crystals or crystalline powder. Soluble in ethanol (95).
**Melting point** <2.60>: 71 – 73°C

**Content**: not less than 98.0%. Assay—Weigh accurately about 0.5 g of 4-nitrobenzyl chloride, add 15 mL of a solution prepared by dissolving 4 g of silver nitrate in 10 mL of water and adding ethanol (95) to make 100 mL, and heat on a water bath under a reflux condenser for 1 hour. After cooling, filter the precipitate with a glass filter, wash with water, dry at 105°C to constant mass, and weigh. The mass of the precipitate represents the amount of silver chloride (AgCl: 143.32).

Amount (mg) of 4-nitrobenzyl chloride
= amount (mg) of silver chloride (AgCl: 143.32) \(\times\) 1.197

4-(4-Nitrobenzy1)pyridine  \(\text{C}_6\text{H}_4\text{NO}_2\)  Pale yellow, crystalline powder. Freely soluble in acetone, and soluble in ethanol (95).
**Melting point** <2.60>: 69 – 71°C

**Nitroethane**  \(\text{C}_2\text{H}_5\text{NO}_2\)  
**Density** <2.50>: 1.048 – 1.053 g/cm\(^3\) (20°C) 
**Water** <2.48>: not more than 0.1%.

**Nitrogen**  [Same as the namesake monograph]

Nitrogen monoxide  NO  A colorless gas. Prepare by adding sodium nitrite TS to a solution of iron (II) sulfate heptahydrate in dilute sulfuric acid. Nitrogen monoxide from a metal cylinder may be used.

Nitromethane  \(\text{CH}_3\text{NO}_2\)  [K 9523, Special class]

3-Nitrophenol  \(\text{C}_6\text{H}_4\text{NO}_3\)  A light yellow crystalline powder.
**Melting point** <2.60>: 96 – 99°C

4-Nitrophenol  \(\text{C}_6\text{H}_4\text{NO}_3\)  [K 8721, Special class]

o-Nitrophenyl-β-D-galactopyranoside  See 2-nitrophenyl-β-D-galactopyranoside.

2-Nitrophenyl-β-D-galactopyranoside  \(\text{C}_6\text{H}_4\text{NO}_3\)  White crystalline powder. Odorless. It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.
**Melting point** <2.60>: 193 – 194°C

**Purity** Clarity and color of solution—A solution of 2-nitrophenyl-β-D-galactopyranoside (1 in 100) is clear and colorless.

**Loss on drying** <2.41>: not more than 0.1% (0.5 g, 105°C, 2 hours).

**Content**: not less than 98.0%. Assay—Weigh accurately
about 0.05 g of 2-nitrophenyl-β-D-galactopyranoside, previously dried, dissolve in water to make exactly 100 mL. Pipet 20 mL of this solution, and add water to make exactly 50 mL. Determine the absorbance, A, of this solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry 2.24:

\[
A = \frac{\text{Amount (mg) of 2-nitrophenyl-β-D-galactopyranoside}}{133} \times 25,000
\]

1-Nitroso-2-naphthol C_{10}H_{7}NO_{2} 
A yellow-brown to red crystalline powder.

Melting point < 260°: 106 – 110°C.
Preserve in a light-resistant tight container.

1-Nitroso-2-naphthol TS Dissolve 0.06 g of 1-nitroso-2-naphthol in 80 mL of acetic acid (100), and add water to make 100 mL.

α-Nitroso-β-naphthol See 1-nitroso-2-naphthol.

α-Nitroso-β-naphthol TS See 1-nitroso-2-naphthol TS.

Nitrous oxide N_{2}O Colorless and odorless gas. Use nitrous oxide from a metal cylinder.

Cells derived from mouse NK cells.

NN Indicator Mix 0.5 g of 2-hydroxy-1-(2′-hydroxy-4′-sulfo-1′-naphthylazo)-3-naphthoic acid with 50 g of anhydrous sodium sulfate, and triturate until the mixture becomes homogeneous.

Nodakenin for thin-layer chromatography C_{20}H_{24}O_{9}
White powder. Slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5). Melting point: about 220°C (with decomposition).

Identification—Determine the absorption spectrum of a solution of nodakenin for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24; it exhibits a maximum between 333 nm and 337 nm.

Optical rotation < 2.49° [α]_{D}^{20}: +50 – +68° (5 mg, methanol, 10 mL, 100 mm).

Purity Related substances—Dissolve 1 mg of nodakenin for thin-layer chromatography in 3 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed with 5 μL each of these solutions as directed in the Identification (2) under Peucedanum Root: the spot other than the principal spot of around Rf value of 0.3 from the sample solution is not more intense than the spot from the standard solution.

Nonylphenoxypoly(ethylenoxy)ethanol for gas chromatography Prepared for gas chromatography.

Normal agar media for teceleukin Dissolve 5.0 g of meat extract, 10.0 g of peptone, 5.0 g of sodium chloride, and 15.0 to 20.0 g of agar in water to make 1000 mL, and sterilize. Adjust the pH to 6.9 to 7.1.

n-Octadecane C_{18}H_{38}
Colorless or white solid at ordinary temperature.

Purity Clarity of solution—A solution of n-octadecane in chloroform (1 in 25) is clear.

Octadecylsilanized silica gel for pretreatment Prepared for pretreatment.

n-Octane C_{8}H_{18}

Specific gravity < 2.56* d_{20}^{20}: 0.700 – 0.705

Purity—Perform the test with 2 μL of n-octane as directed under Gas Chromatography 2.02 according to the conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of n-octane by the area percentage method: not less than 99.0%.

Octane, iso A colorless liquid. Practically insoluble in water. Miscible with diethyl ether and with chloroform.

Purity—Determine the absorbances of isooctane at 230 nm, 250 nm and 280 nm as directed under Ultraviolet-visible Spectrophotometry 2.24, using water as the blank solution: these values are not more than 0.050, 0.010 and 0.005, respectively.

1-Octanol CH_{3}(CH_{2})_{2}CH_{2}OH [K 8213, Special class]
Octyl alcohol See 1-octanol.

n-Octylbenzene C_{10}H_{12} Clear and colorless liquid, having a characteristic odor.
Specific gravity < 2.56* d_{20}^{20}: 0.854 – 0.863
Distillation test < 2.57°: 263 – 265°C, not less than 95 vol%.

Ofl oxacin C_{18}H_{36}FN_{3}O_{4} [Same as the namesake monograph]

Ofo l oxacin demethyl substance (±)-9-Fluoro-2,3-dihydro-3-methyl-7-oxo-7H-10-(1-piperaziny1)-pirido[1, 2, 3-de] [1, 4]benzoxazine-6-carboxylic acid C_{17}H_{16}FN_{3}O_{4} White to light green-yellowish white, crystals or crystalline powder.

Identification—Determine the infrared absorption spectrum of ofloxacin demethyl substance as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25; it exhibits absorption at the wave numbers of about 3050 cm\(^{-1}\), 2840 cm\(^{-1}\), 1619 cm\(^{-1}\), 1581 cm\(^{-1}\), 1466 cm\(^{-1}\), 1267 cm\(^{-1}\), 1090 cm\(^{-1}\), 1051 cm\(^{-1}\) and 816 cm\(^{-1}\).

Oleic acid C_{18}H_{32}O
Occurs as a colorless or pale yellow transparent liquid and has a slightly distinct odor. It is miscible with ethanol (95) and with diethyl ether, and practically insoluble in water.

Specific gravity < 2.56* d_{20}^{20}: about 0.9

Content: not less than 99.0%. Assay—To 40 μL of oleic acid to be examined add 1 mL of a solution of boron trifluoride in methanol (3 in 20), mix, and heat on a water bath for 3 minutes. After cooling, add 10 mL of petroleum ether and 10 mL of water, shake, collect the ether layer after allowing to stand, and use as the sample solution. Perform the test with 0.2 μL of the sample solution as directed under Gas Chromatography 2.02 according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of methyl oleate by the area percentage method.

Operating conditions
Detector: A hydrogen flame-ionization detector
Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (149 – 177 μm) coated with methyl polyacrylate in a rate of 5 – 10%.

Column temperature: A constant temperature of about 220°C.
Carrier gas: Helium.
Flow rate: Adjust the flow rate so that the retention time of methyl oleate is about 10 minutes.
Time span of measurement: About 2 times as long as the retention time of methyl oleate, beginning after the solvent peak.

**Olive oil** [Same as the namesake monograph]

**Ophiopogon tuber** [Same as the namesake monograph]

**Orcine** C₉H₉O₃ White to light red-brown crystals or crystalline powder, having an unpleasant, sweet taste. It turns to red in color when oxidized in air. Soluble in water, in ethanol (95), and in diethyl ether.

*Melting point* 93 – 94°C

**Orcine-ferric chloride TS** See orcine-iron (III) chloride TS.

**Orcine-iron (III) chloride TS** Dissolve 10 mg of orcine in 1 mL of a solution of iron (III) chloride hexahydrate in hydrochloric acid (1 in 1000). Prepare before use.

**Ordinary agar medium** Dissolve 25 to 30 g of agar in 1000 mL of ordinary broth with the aid of heat, add water to make up for the loss, adjust the pH to between 6.4 and 7.0, and filter. Dispense the filtrate, and sterilize by autoclaving. When powdered agar is used, 15 to 20 g of it is dissolved.

**Ordinary broth** Dissolve 5 g of beef extract and 10 g of peptone in 1000 mL of water by gentle heating. Adjust the pH of the mixture between 6.4 and 7.0 after sterilization, cool, add water to make up for the loss, and filter. Sterilize the filtrate by autoclaving for 30 minutes at 121°C.

**Osthole for thin-layer chromatography** C₁₅H₁₄O₂ A white crystalline powder, having no odor. Freely soluble in methanol and in ethyl acetate, soluble in ethanol (99.5), and practically insoluble in water. Melting point: 83 – 84°C.

**Purity** Related substances—Dissolve 1.0 mg of osthole for thin-layer chromatography in 1 mL of methanol. Perform the test with 10 μL of this solution as directed in the Identification under Cnidium Monnieri Fruit: on spot appears other than the principal spot at around Rf 0.3.

**Oxalate pH standard solution** See pH Determination <2.54>.

Oxalic acid See oxalic acid dihydrate.

**Oxalic acid dihydrate** H₂C₂O₄·2H₂O [K 8519, Special class]

**Oxalic acid TS** Dissolve 6.3 g of oxalic acid dihydrate in water to make 100 mL (0.5 mol/L).

**Oxycodone hydrochloride for assay** See oxycodone hydrochloride hydrate for assay.

**Oxycodone hydrochloride hydrate for assay** C₁₈H₂₁NO₄·HCl·3H₂O [Same as the monograph Oxycodone Hydrochloride Hydrate. It contains not less than 99.0% of oxycodone hydrochloride (C₁₈H₂₁NO₄·HCl), calculated on the anhydrous basis.]

**Oxygen** O₂ [K 1101]

**2-Oxy-1-(2′-oxy-4′-sulfo-1′-naphthylazo)-3-naphthoic acid** See 2-hydroxy-1-(2′-hydroxy-4′-sulfo-1′-naphthylazo)-3-naphthoic acid.

**8-Oxyquinoline** See 8-quinolinol.

**Oxytocin** C₄₃H₆₆N₁₂O₁₂S₂ [Same as the namesake monograph]

**Paeonol for assay** C₉H₁₀O₃ Use paeonol for thin-layer chromatography meeting the following additional specifications.

**Purity** Related substances—Dissolve 5.0 mg of paeonol for assay in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μL each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area of these solutions by the automatic integration method: the total area of the peaks other than paeonol from the sample solution is not larger than the peak area of paeonol from the standard solution (1).

**Operating conditions**
Proceed the operating conditions in the Assay under Moutan Bark except detection sensitivity and time span of measurement.

**Detection sensitivity** Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of paeonol obtained from 10 μL of the standard solution (2) can be measured, and the peak height of paeonol from 10 μL of the standard solution (1) is about 20% of the full scale.

**Time span of measurement** About 3 times as long as the retention time of paeonol beginning after the solvent peak.

**Paeonol for component determination** See paeonol for assay.

**Paeonol for thin-layer chromatography** C₉H₁₀O₃ White, crystals or crystalline powder, having a specific odor. Freely soluble in methanol and in diethyl ether, and slightly soluble in water. Melting point: about 50°C

**Purity** Related substances—Dissolve 1.0 mg of paeonol for thin-layer chromatography in exactly 1 mL of methanol, and perform with 20 μL of this solution as directed in the Identification (2) under Peony Root: any spot other than the principal spot at the Rf value of about 0.3 does not appear.

**Palladium chloride** See palladium (II) chloride.

**Palladium chloride TS** See palladium (II) chloride TS.

**Palladium (II) chloride** PdCl₂ [K 8154, Special class]
Palladium (II) chloride TS  Dissolve 0.2 g of palladium (II) chloride in 500 mL of 0.25 mol/L sulfuric acid TS, by heating if necessary, cool, and add 0.25 mol/L sulfuric acid TS to make 1000 mL.

Palmitin chloride C_{20}H_{21}NO_4 \cdot HCl A yellow-brown crystalline powder.

Purity—Dissolve 1 mg of palmitin chloride in 10 mL of methanol, and use this solution as the sample solution. Proceed with 20 μL of the sample solution as directed in the Assay under Phellodendron Bark: when measure the peak areas 2 times as long as the retention time of palmitin, the total area of the peaks other than palmitin is not larger than 1/10 of the total area except the area of solvent peak.

Palmitic acid for gas chromatography C_{16}H_{32}O_2

<table>
<thead>
<tr>
<th>[K 8756, Special class]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic digest of casein</td>
</tr>
<tr>
<td>Pancreatic digest of gelatin</td>
</tr>
<tr>
<td>Papaic digest of soya bean</td>
</tr>
<tr>
<td>Papaverine hydrochloride C_{20}H_{21}NO_4 \cdot HCl [Same as the namesake monograph]</td>
</tr>
<tr>
<td>Papaverine hydrochloride for assay C_{20}H_{21}NO_4 \cdot HCl [Same as the monograph Papaverine Hydrochloride. When dried, it contains not less than 99.0% of papaverine hydrochloride (C_{20}H_{21}NO_4 \cdot HCl).]</td>
</tr>
<tr>
<td>Paraffin [Same as the namesake monograph]</td>
</tr>
<tr>
<td>Paraffin, light liquid [Same as the namesake monograph]</td>
</tr>
<tr>
<td>Parahydroxybenzoic acid C_{7}H_{6}O_3 White crystals.</td>
</tr>
<tr>
<td>Melting point &lt;2.60°: 212 – 216°C</td>
</tr>
<tr>
<td>Content: not less than 98.0%. Assay—Weigh accurately about 0.7 g of parahydroxybenzoic acid, dissolve in 50 mL of aceton, add 100 mL of water, and titrate &lt;2.50° with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.</td>
</tr>
<tr>
<td>Each mL of 0.5 mol/L sodium hydroxide VS = 69.06 mg of C_{7}H_{6}O_3</td>
</tr>
<tr>
<td>PBS containing bovine serum</td>
</tr>
<tr>
<td>Storage—Store in a cool place shielded from light.</td>
</tr>
<tr>
<td>PBS containing bovine serum albumin</td>
</tr>
<tr>
<td>Storage—Store in a cool, dark place.</td>
</tr>
<tr>
<td>Peanut oil [Same as the namesake monograph]</td>
</tr>
<tr>
<td>Pentane CH_{3}(CH_{2})<em>{3}CH</em>{3} Clear and colorless liquid.</td>
</tr>
<tr>
<td>Specific gravity &lt;2.56° d_{40}^{20}: 0.620 – 0.650</td>
</tr>
<tr>
<td>Distilling range &lt;2.57°: 35.5 – 37°C, not less than 98 vol%.</td>
</tr>
<tr>
<td>Peptic digest of animal tissue See Peptone, animal tissue.</td>
</tr>
<tr>
<td>Peptone</td>
</tr>
<tr>
<td>Peptone, animal tissue</td>
</tr>
<tr>
<td>Peptone, casein</td>
</tr>
<tr>
<td>Loss on drying &lt;2.41%: not more than 7% (0.5 g, 105°C, constant mass).</td>
</tr>
<tr>
<td>Residue on ignition &lt;2.44%: not more than 15% (0.5 g).</td>
</tr>
<tr>
<td>Degree of digestion—Dissolve 1 g of casein peptone in 10 mL of water, and perform the following test using this solution as the sample solution:</td>
</tr>
<tr>
<td>(1) Overlay 1 mL of the sample solution with 0.5 mL of a mixture of 1 mL of acetic acid (100) and 10 mL of dilute ethanol: no ring or precipitate forms at the junction of the two liquids, and on shaking, no turbidity results.</td>
</tr>
<tr>
<td>(2) Mix 1 mL of the sample solution with 4 mL of a saturated solution of zinc sulfate heptahydrate: a small quantity of precipitate is produced (proteoses).</td>
</tr>
<tr>
<td>(3) Filter the mixture of (2), and to 1 mL of the filtrate add 3 mL of water and 4 drops of bromine TS: a red-purple color is produced.</td>
</tr>
<tr>
<td>Nitrogen content &lt;1.08%: not less than 10% (105°C, constant mass, after drying).</td>
</tr>
<tr>
<td>Peptone, gelatin</td>
</tr>
<tr>
<td>Peptone, soybean</td>
</tr>
<tr>
<td>Perchloric acid HClO_4 [K 8223, Special class, Density: about 1.67 g/mL. Concentration: 70.0 – 72.0%]</td>
</tr>
<tr>
<td>Perchloric acid-dehydrated ethanol TS</td>
</tr>
<tr>
<td>Perchloric acid-ethanol TS</td>
</tr>
<tr>
<td>Performic acid</td>
</tr>
<tr>
<td>Storage—Store in a cool place.</td>
</tr>
<tr>
<td>Perillaldehyde for assay Perillaldehyde for thin-layer chromatography meeting the following specifications.</td>
</tr>
<tr>
<td>Absorbance &lt;2.24° E_{1%}^{230} (230 nm): 850 – 950 (10 mg, methanol, 2000 mL).</td>
</tr>
<tr>
<td>Purity Related substances—Dissolve 10 mg of perillaldehyde in 250 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography &lt;2.01&gt; according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of perillaldehyde from the sample solution is not larger than the peak of perillaldehyde from the standard solution.</td>
</tr>
<tr>
<td>Operating conditions</td>
</tr>
<tr>
<td>Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Perilla Herb.</td>
</tr>
<tr>
<td>Time span of measurement: About 3 times as long as the retention time of perillaldehyde beginning after the solvent peak.</td>
</tr>
<tr>
<td>System suitability</td>
</tr>
</tbody>
</table>
| Test for required detectability: Pipet 1 mL of the standard
solution, and add methanol to make exactly 20 mL. Confirm that the peak area of perillaldehyde obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of perillaldehyde from 10 μL of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Perilla Herb.

**Perillaldehyde for component determination**  See perillaldehyde for assay.

**Perillaldehyde for thin-layer chromatography** C₁₀H₁₄O₃ Colorless to light brown transparent liquid, having a characteristic odor. Miscible with methanol and with ethanol (99.5), and very slightly soluble in water.

**Identification**—Determine the infrared absorption spectrum of perillaldehyde for thin-layer chromatography as directed in the liquid film method under Infrared Spectrophotometry 2.25: it exhibits absorption at the wave numbers of about 3080 cm⁻¹, 2930 cm⁻¹, 1685 cm⁻¹, 1644 cm⁻¹, 1435 cm⁻¹ and 890 cm⁻¹.

**Purity** Related substances—Dissolve 1.0 mg of perillaldehyde for thin-layer chromatography in 10 mL of methanol, and perform the test with 10 μL of this solution as directed in the Identification under Perilla Herb: no spot other than the principal spot at around RT value of 0.5 appears.

**Peroxidase** Obtained from horse-radish. A red-brown powder. It is freely soluble in water. It contains about 250 units per mg. One unit indicates an amount of the enzyme powder. It is freely soluble in water. It contains about 250

**Peroxidase-labeled antibody stock solution** 1 w/v% bovine serum albumin-phosphate buffer-sodium chloride TS containing antibody fragment (Fab') bound to peroxidase.

**Peroxidase-labeled bradykinin** A solution of horseradish origin peroxidase-binding bradykinin in gelatin-phosphate buffer solution, pH 7.0. A colorless to light brown clear solution.

**Peroxidase-labeled bradykinin TS** To 0.08 mL of peroxidase-labeled bradykinin, 8 mg of sodium tetraborate decahydrate, 8 mg of bovine serum albumin and 0.8 mL of gelatin-phosphate buffer solution, pH 7.0 add water to make 8 mL, and lyophilize. Dissolve this in 8 mL of water. Prepare before use.

**Peroxidase-labeled rabbit anti-ECP antibody Fab' TS** Mix 1 volume of ECP standard substance (equivalent to about 1 mg of protein) and 1 volume of Freund's complete adjuvant, and then immunize rabbits subcutaneously in the back region and intramuscularly in the hind leg muscle with this solution 5 times at 2 week intervals. Harvest blood on the 10th day after completing the immunization to obtain rabbit antisemur. Rabbit anti-ECP antibody Fab' is obtained by preparing an immobilzed ECP column in which ECP standard substance is bound to agarose gel and then purifying by affinity column chromatography to obtain rabbit anti-ECP antibody which undergoes pepsin digestion to yield Fab'; which is reacted with 2-aminoethanethiol hydrochloride.

Horseradish peroxidase is reacted with maleimido reagent [4-(maleimidomethyl) cyclohexyl carbonic acid-N-hydroxy-succinimide imidoester] to yield maleimido peroxidase. Perform a coupling reaction by mixing rabbit anti-ECP antibody Fab' and maleimido peroxidase at 4°C to prepare peroxidase-labeled rabbit anti-ECP antibody Fab'. Take a specific amount of peroxidase-labeled rabbit anti-ECP antibody Fab' and dilute using PBS containing bovine serum albumin. The peroxidase-labeled rabbit anti-ECP antibody Fab' TS is a diluted solution with a concentration that gives a good calibration curve with assay characteristics.

**Description** Clear and colorless solution

**Identification** Pipet 100 μL of the TS to be examined into flat-bottomed microtest plates. When substrate buffer solution for celmoleukin is added to this, it immediately exhibits a dark violet color, which changes to yellowish-red with time.

**Perphenazine maleate for assay** C₂₁H₂₆ClN₃OS.2C₄H₄O₄ [Same as the monograph Perphenazine Maleate. When dried, it contains not less than 99.0% of perphenazine maleate (C₂₁H₂₆ClN₃OS.2C₄H₄O₄).]

**Pethidine hydrochloride for assay** C₁₉H₂₃NO₂.HCl [Same as the monograph Pethidine Hydrochloride. When dried, it contains not less than 99.0% of pethidine hydrochloride C₁₉H₂₃NO₂.HCl.]

**Petrolatum** [Same as the monograph Yellow Petroleum or White Petrolatum]

**Petroleum benzine** [K 8594, Special class]

**Petroleum ether** [K 8593, Special class]

**Phenacetin** C₁₀H₁₃NO₂ White crystals or crystalline powder. Soluble in ethanol (95), and very slightly soluble in water.

**Melting point** 2.60°: 134 – 137°C

**Loss on drying** 2.41°: not more than 0.5% (1 g, 105°C, 2 hours).

**o-Phenanthroline** See 1,10-phenanthroline monohydrate.

**o-Phenanthroline hydrochloride** See 1,10-phenanthroline chloride monohydrate.

**1,10-Phenanthroline monohydrate** C₁₂H₉N₂.H₂O [K 8789, Special class]

**1,10-Phenanthroline TS** See 1,10-phenanthroline TS.

**1,10-Phenanthroline monohydrate** Dissolve 0.15 g of 1,10-phenanthroline monohydrate in 10 mL of a freshly prepared ferrous sulfate heptahydrate solution (37 in 2500) and 1 mL of dilute sulfuric acid. Preserve in tightly stoppered containers.

**1,10-Phenanthroline chloride monohydrate** C₁₂H₉N₂Cl.H₂O [K 8202, Special class]

**Phenethyline hydrochloride** C₁₀H₁₅CH₂NH₂.HCl White crystals or crystalline powder.

**Melting point** 2.60°: 220 – 225°C

**Phenobarbital for assay** C₁₂H₁₂N₂O₃ [Same as the monograph Phenobarbital]

**Phenol** C₆H₅OH [K 8798, Special class]

**Phenol for assay** C₆H₅OH [K 8798, Phenol, Special class]

**Phenol-hydrochloric acid TS** Dissolve 0.2 g of phenol in
10 mL of 6 mol/L hydrochloric acid TS.

**Phenolphthalein** $C_{19}H_{14}O_5S$ [K 8799, Special class]

**Phenolphthalein-thymol blue TS** Solution A: Dissolve 0.1 g of phenolphthalein in diluted ethanol (99.5%) (4 in 5). Solution B: Dissolve 0.1 g of thymol blue in 50 mL of a mixture of ethanol and dilute sodium hydroxide TS, add water to make 100 mL. Mix 2 volumes of solution A and 3 volumes of solution B before use.

**Phenolphthalein TS** Dissolve 1 g of phenolphthalein in 100 mL of ethanol (95).

**Phenolphthalein TS, alkaline** See Alcohol Number Determination <1.01>.

**Phenol red** $C_{19}H_{14}O_5S$ [K 8800, Special class]

**Phenol red TS** Dissolve 0.1 g of phenol red in 100 mL of ethanol (95), and filter if necessary.

**Phenol red TS, dilute** To 253 mL of a solution of ammonium nitrate (1 in 9400) add 105 mL of 2 mol/L sodium hydroxide TS and 135 mL of a solution prepared by dissolving 24 g of acetic acid (100) in water to make 200 mL. To this solution add 25 mL of a solution prepared by dissolving 33 mg of phenol red in 1.5 mL of 2 mol/L sodium hydroxide TS and adding water to make 100 mL. If necessary, adjust the pH to 4.7.

**Phenol-sodium nitroprusside TS** See phenol-sodium pentacyanonitrosylferrate (III) TS.

**Phenol-sodium pentacyanonitrosylferrate (III) TS** Dissolve 5 g of phenol and 25 mg of sodium pentacyanorosylferrate (III) dihydrate in sufficient water to make 500 mL. Preserve in a dark, cold place.

**Phenolsulfonphthalein for assay** $C_{19}H_{14}O_5S$ [Same as the monograph Phenolsulfonphthalein. When dried, it contains not less than 99.0% of phenolsulfonphthalein ($C_{19}H_{14}O_5S$).]

**Phenylalanine** See L-phenylalanine.

**L-Phenylalanine** $C_9H_{11}NO_2$ [Same as the namesake monograph]

**Phenyl benzoate** $C_8H_6COOC_6H_5$ White crystals or crystalline powder, having a slight, characteristic odor. *Melting point* $<2.60^°$: 68 – 70°C

**Purity** Clarity of solution—Dissolve 1.0 g of phenyl benzoate in 20 mL of methanol: the solution is clear.

**25% Phenyl-25% cyanopropyl methyl polysiloxane polymer for gas chromatography** Prepared for gas chromatography.

**o-Phenylenediamine dihydrochloride** $H_2NC_6H_4NH_2\cdot2HCl$ White to pale yellow or pale red crystals or crystalline powder.

**Clarity**—a solution (1 in 20) is clear.

**Content**—not less than 98.0%. Assay—Weigh accurately about 0.15 g of o-phenylenediamine dihydrochloride, dissolve in 50 mL of water, and titrate $<2.50^°$ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 9.053 mg of $H_2NC_6H_4NH_2\cdot2HCl$

**Phenylfluorone** $C_{19}H_{12}O_5$ [K 9547]

**Phenylfluorone-ethanol TS** Dissolve 50 mg of phenylfluorone in 10 mL of a mixture of ethanol (95) and diluted hydrochloric acid (1 in 3), and add thanol (95) to make exactly 500 mL.

**n-Phenylglycine** $C_6H_7NO_2$ White, crystals or crystalline powder. Slightly soluble in water. *Loss on drying* $<2.40^°$: not more than 0.5% (1 g, 105°C, 3 hours).

**Content**—not less than 98.5%. Assay—Weigh accurately about 0.3 g of n-phenylglycine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate $<2.50^°$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 15.12 mg of $C_6H_7NO_2$

**Phenyldrazine** $C_6H_7NHNH_2$ Colorless or light yellow, clear liquid, having a faint aromatic odor.

**Phenyldrazine hydrochloride** See phenyldrazinium chloride.

**Phenyldrazine hydrochloride TS** See phenyldrazinium chloride TS.

**Phenyldrazinium chloride** $C_6H_7NHNH_2\cdotHCl$ [K 8203, Special class]

**Phenyldrazinium chloride TS** Dissolve 65 mg of phenyldrazinium chloride recrystallized from dilute ethanol, in 100 mL of a solution previously prepared by adding cautiously 170 mL of sulfuric acid to 80 mL of water.

**Phenyl isothiocyanate** $C_8H_9NO_2$ Prepared for amino acid analysis or biochemistry.

**1-phenyl-3-methyl-5-pyrazolone** See 3-methyl-1-phenyl-5-pyrazolone.

50% Phenyl-50% methyl polysiloxane for gas chromatography Prepared for gas chromatography.
35% Phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography.

50% Phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography.

65% Phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography.

Phenylpiperazine hydrochloride See phenylpiperazine monohydrochloride.

Phenylpiperazine monohydrochloride C₁₅H₁₄N₂·HCl A white powder. Melting point: about 247°C (with decomposition).

Phenytoin for assay C₁₅H₁₂N₂O₂ [Same as the monograph Phenytoin. It meets the following requirements.]

Purity Related substances—Dissolve 25 mg of phenytoin for assay in 50 mL of the mobile phase, and use this solution as the sample solution. To exactly 1 mL of the sample solution add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total peak area of phenytoin obtained from the standard solution is not less than 1.5 times that with phenytoin obtained from the sample solution.

Retention time of phenytoin, beginning after the solvent peak.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column, column temperature, and flow rate: Proceed as directed in the operating conditions in the Assay under Phenytoin.
Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution, pH 3.5 and acetonitrile for liquid chromatography (11:9).

Time span of measurement: About 5 times as long as the retention time of phenytoin, beginning after the solvent peak.

System suitability
Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of phenytoin obtained from 10 µL of this solution is equivalent to 8 to 12% of that with 10 µL of the standard solution.

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of phenytoin are not less than 6000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phenytoin is not more than 2.0%.

Phloroglucin See phloroglucinol dihydrate.

Phloroglucin dihydrate See phloroglucinol dihydrate.

Phloroglucinol dihydrate C₆H₃(OH)₃·2H₂O White to pale yellow, crystals or crystalline powder. Melting point <2.60>: 215 – 219°C (after drying). Loss on drying <2.41>: 18.0 – 24.0% (1 g, 105°C, 1 hour).

Phosphatase, alkaline Obtained from bovine small intestine. A white to grayish white or yellowish brown, lyophilized powder. It contains about 1 Units per mg, not containing any saline. One unit indicates an amount of the enzyme which produces 1 µmol of 4-nitrophenol at 37°C and pH 9.8 in 1 minute from 4-nitrophenyl phosphate used as the substrate.

Phosphatase TS, alkaline Dissolve 0.1 g of alkaline phosphatase in 10 mL of boric acid-magnesium chloride buffer solution, pH 9.0. Prepare before use.

Phosphate-buffered sodium chloride TS Dissolve 8.0 g of sodium chloride, 0.2 g of potassium chloride, 2.9 g of disodium hydrogen phosphate dodecahydrate, and 0.2 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.01 mol/L Phosphate buffer-sodium chloride TS, pH 7.4 Dissolve 2.93 g of disodium hydrogen phosphate dodecahydrate (NaH₂PO₄·12H₂O), 0.25 g of potassium dihydrogen phosphate (KH₂PO₄), and 9.0 g of sodium chloride in water to make 1000 mL.

Phosphate buffer solution for assay of bupleurum root To 100 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 59 mL of sodium hydrogen phosphate TS.

Phosphate buffer solution for component determination of bupleurum root See phosphate buffer solution for assay of bupleurum root.

Phosphate buffer solution for microplate washing Dissolve 0.62 g of sodium dihydrogen phosphate dihydrate, 9.48 g of disodium hydrogen phosphate dodecahydrate (NaH₂PO₄·12H₂O), 0.25 g of potassium dihydrogen phosphate (KH₂PO₄), and 9.0 g of sodium chloride in 1000 mL. Dilute this solution 10 times with water before use.

Phosphate buffer solution for pancreatin Dissolve 3.3 g of anhydrous disodium hydrogen phosphate, 1.4 g of potassium dihydrogen phosphate, and 0.33 g of sodium chloride in water to make 100 mL.

Phosphate buffer solution for processed aconite root Dissolve 19.3 g of disodium hydrogen phosphate dodecahydrate in 3660 mL of water, and add 12.7 g of phosphoric acid.

Phosphate buffer solution, pH 3.0 Dissolve 136 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.0 with phosphoric acid.

0.02 mol/L Phosphate buffer solution, pH 3.0 Dissolve 3.1 g of sodium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with dilute phosphoric acid (1 in 10).

Phosphate buffer solution, pH 3.1 Dissolve 136.1 g of potassium dihydrogen phosphate in 500 mL of water, and add 6.3 mL of phosphoric acid and water to make 1000 mL.

0.02 mol/L Phosphate buffer solution, pH 3.5 Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10).

0.05 mol/L Phosphate buffer solution, pH 3.5 To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add a suitable amount of a solution of phosphoric acid (49 in
0.1 mol/L Phosphate buffer solution, pH 4.5  Dissolve 13.61 g of potassium dihydrogen phosphate in 750 mL of water, adjust to pH 4.5 with potassium hydroxide TS, and add water to make 1000 mL.

0.1 mol/L Phosphate buffer solution, pH 5.3  Dissolve 0.44 g of disodium hydrogen phosphate dodecahydrate and 13.32 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 5.3 with sodium hydroxide TS or phosphoric acid, and add water to make 1000 mL.

1/15 mol/L Phosphate buffer solution, pH 5.6  Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate in 750 mL of water, adjust to pH 5.6 with potassium hydroxide TS, and add water to make 1000 mL.

Phosphate buffer solution, pH 5.9  Dissolve 6.8 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 5.9 with diluted potassium hydroxide TS (1 in 10), and add water to make 1000 mL.

Phosphate buffer solution, pH 6.0  Dissolve 8.63 g of potassium dihydrogen phosphate and 1.37 g of anhydrous disodium hydrogen phosphate in 750 mL of water, adjust the pH to 6.0 with sodium hydroxide TS or diluted phosphoric acid (1 in 15), and add water to make 1000 mL.

0.05 mol/L Phosphate buffer solution, pH 6.0  To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution add 5.70 mL of 0.2 mol/L sodium hydroxide TS and water to make 200 mL.

Phosphate buffer solution, pH 6.2  Dissolve 9.08 g of potassium dihydrogen phosphate in 1000 mL of water (solution A). Dissolve 9.46 g of disodium hydrogen phosphate in 1000 mL of water (solution B). Mix 800 mL of the solution A and 200 mL of the solution B, and adjust the pH to 6.2 with the solution A or the solution B if necessary.

Phosphate buffer solution, pH 6.5  Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 15.20 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

Phosphate buffer solution for antibiotics, pH 6.5  Dissolve 10.5 g of disodium hydrogen phosphate dodecahydrate and 5.8 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.5 with sodium hydroxide TS, and add water to make 1000 mL.

Phosphate buffer solution, pH 6.8  Dissolve 3.40 g of potassium dihydrogen phosphate and 3.55 g of disodium hydrogen phosphate in water to make 1000 mL.

0.01 mol/L Phosphate buffer solution, pH 6.8  Dissolve 1.36 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 6.8 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

0.1 mol/L Phosphate buffer solution, pH 6.8  Dissolve 6.4 g of potassium dihydrogen phosphate and 18.9 g of disodium hydrogen phosphate dodecahydrate in about 750 mL of water, adjust the pH to 6.8 with sodium hydroxide TS if necessary, and add water to make 1000 mL.

Phosphate buffer solution, pH 7.0  Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 29.54 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

0.05 mol/L Phosphate buffer solution, pH 7.0  Dissolve 4.83 g of dipotassium hydrogen phosphate and 3.02 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 7.0 with phosphoric acid or potassium hydroxide TS.

0.1 mol/L Phosphate buffer solution, pH 7.0  Dissolve 17.9 g of disodium hydrogen phosphate dodecahydrate in about 750 mL of water, adjust the pH to 7.0 with sodium hydroxide TS, and add water to make 1000 mL.

0.02 mol/L Phosphate buffer solution, pH 7.2  Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution, and 46.1 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

Phosphate buffer solution, pH 7.4  Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 39.50 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

0.03 mol/L Phosphate buffer solution, pH 7.5  Dissolve 4.083 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 7.5 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Phosphate buffer solution, pH 8.0  Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution, and 46.1 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 1000 mL.

0.02 mol/L Phosphate buffer solution, pH 8.0  To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution add 300 mL of water, adjust the pH to 8.0 with sodium hydroxide TS, and add water to make 1000 mL.

0.1 mol/L Phosphate buffer solution, pH 8.0  Dissolve 13.2 g of anhydrous disodium hydrogen phosphate and 0.91 g of potassium dihydrogen phosphate in about 750 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL.

0.1 mol/L Phosphate buffer solution for antibiotics, pH 8.0  Dissolve 16.73 g of dipotassium hydrogen phosphate and 0.523 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 8.0 with phosphoric acid, and add water to make 1000 mL.

0.2 mol/L Phosphate buffer solution, pH 10.5  Dissolve 34.8 g of dipotassium hydrogen phosphate in 750 mL of water, adjust to pH 10.5 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Phosphate buffer solution, pH 12  To 5.44 g of disodium hydrogen phosphate add 36.5 mL of sodium hydroxide TS and 40 mL of water, dissolve by shaking well, and add water to make 100 mL.

Phosphinic acid H₃PO₂  Colorless or pale yellow viscous liquid.

Identification—(1) To 0.5 mL of phosphinic acid add 0.5 mL of hydrogen peroxide (30) and 0.5 mL of dilute sulfuric acid (1 in 6), and evaporate to nearly dryness on a
Reagents, Test Solutions / General Tests

Phosphoric acid See phosphoric acid n-hydrate.

Phosphotungstic acid See phosphotungstic acid n-hydrate.

Phosphotungstic acid n-hydrate \( \text{P}_2\text{O}_5.\text{WO}_3.n\text{H}_2\text{O} \)

White to yellowish green, crystals or crystalline powder.

Identification—To 5 mL of a solution in 10 mL of acidic tin (II) chloride TS, and heat: blue precipitates appear.

Phosphotungstic acid TS Dissolve 1 g of phosphotungstic acid n-hydrate in water to make 100 mL.

\( \text{C}_8\text{H}_4\text{O}_3 \) Light yellow to yellow crystals.

Content—Not less than 99%. Assay—Dissolve 1 g of \( \text{C}_8\text{H}_4\text{O}_3 \) in 10 mL of ethanol (95); Proceed with 2 \( \mu \)L of this solution as directed in Gas Chromatography \( <2.02 \) according to the following conditions, and determine each peak area by the automatic integration method.

Content % = peak area of \( \text{C}_8\text{H}_4\text{O}_3 \)/total area of all peaks \( \times 100 \)

Operating conditions

Detector: A thermal conductivity detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliconeous earth for gas chromatography treated with acid and silane (177 – 250 \( \mu \)m), coated with methyl silicon polymer for gas chromatography in 10%.

Column temperature: A constant temperature of about 180°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of \( \text{C}_8\text{H}_4\text{O}_3 \) is 3 – 4 minutes.

Time span of measurement: About 7 times as long as the retention time of \( \text{C}_8\text{H}_4\text{O}_3 \), beginning after the solvent peak.

Phthalic anhydride \( \text{C}_8\text{H}_4\text{O}_3 \) White crystals or crystalline powder. Soluble in ethanol (95), sparingly soluble in water.

Sensitivity test—Dissolve 10 mg of phthalic anhydride in 1 mL of ammonia solution (28), and add water to make 100 mL. To 2 mL of this solution add 95 mL of water, 4 mL of ammonia solution (28), 50 mL of ethanol (95), and 0.1 mL of diluted barium chloride TS (1 in 5); the solution shows a blue-purple color which disappears on the addition of 0.15 mL of 0.1 mol/L sulfuric acid VS.

Phthalic acid \( \text{C}_8\text{H}_4\text{O}_3 \) Colorless or white crystalline powder. Soluble in methanol and in ethanol (95), sparingly soluble in water, and practically insoluble in chloroform.

Melting point: about 200°C (with decomposition).

Content—Not less than 98%. Assay—Weigh accurately about 2.8 g of phthalic acid, add exactly 50 mL of 1 mol/L sodium hydroxide VS and 25 mL of water, and dissolve by heating on a hot plate. After cooling, add 5 drops of phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS. Perform a blank determination, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS = 83.07 mg of \( \text{C}_8\text{H}_4\text{O}_3 \)

Phthalic anhydride \( \text{C}_8\text{H}_4\text{O}_3 \) White crystals or crystalline powder.

Melting point \( <2.60; \) 131 – 134°C.
Phthalimide $C_6H_5NO_2$ White to pale brown crystals or powder.

Melting point $<2.60^\circ$: 232 – 237°C

Clarity—1.0 g of phthalimide dissolves in 20 mL of sodium hydroxide TS as a slight turbid solution.

Content: not less than 98.0%. Assay—Weigh accurately about 0.3 g of the substance to be tested, dissolve in 40 mL of $N,N$-dimethylformamide, and titrate $<2.50$ with 0.1 mol/L sodium methoxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS $= 14.71$ mg of $C_6H_5NO_2$

Phytonadione $C_{31}H_{46}O_2$ [Same as the namesake monograph]

Picric acid See 2,4,6-trinitrophenol.

Picric acid-ethanol TS See 2,4,6-trinitrophenol-ethanol TS.

Picric acid TS See 2,4,6-trinitrophenol TS.

Picric acid TS, alkaline See 2,4,6-trinitrophenol TS, alkaline.

Pig bile powder for thin-layer chromatography A yellow-gray to yellow-brown powder, having a characteristic odor and a bitter taste. It is practically insoluble in water, in methanol and in ethanol (99.5).

Identification—To 0.1 g of pig bile powder for thin-layer chromatography in a screw-capped test tube, add 1 mL of sodium hydroxide solution (3 in 25), and shake. Heat the tube in an oil bath at 120°C for 4 hours, allow to cool to a lukewarm temperature, add 2 mL of 3 mol/L hydrochloric acid TS and 2 mL of ethyl acetate, shake at 50°C for 30 minutes, and separate ethyl acetate layer as the sample solution. Separately, dissolve 10 mg of hyodeoxycholic acid for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.07>$. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 10 cm, and air-dry the plate. Splay evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 10 minutes: spots equivalent to those described below appear.

<table>
<thead>
<tr>
<th>$R_f$ value</th>
<th>Color and shape of the spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Around 0</td>
<td>A strong spot, very dark blue</td>
</tr>
<tr>
<td>Around 0.08</td>
<td>A very dark blue spot</td>
</tr>
<tr>
<td>Around 0.1 – 0.2</td>
<td>A leading spot, very dark blue</td>
</tr>
<tr>
<td>Around 0.25</td>
<td>A strong spot, deep blue (corresponding to plantagouguanidinic acid)</td>
</tr>
<tr>
<td>Around 0.35</td>
<td>A strong spot, dark grayish blue (corresponding to geniposidic acid)</td>
</tr>
<tr>
<td>Around 0.45</td>
<td>A weak spot, grayish yellowish green</td>
</tr>
<tr>
<td>Around 0.50</td>
<td>A strong spot, deep yellow-green (corresponding to acteoside)</td>
</tr>
<tr>
<td>Around 0.6</td>
<td>A weak spot, light blue</td>
</tr>
<tr>
<td>Around 0.85</td>
<td>A deep blue spot</td>
</tr>
<tr>
<td>Around 0.9 – 0.95</td>
<td>A tailing spot, grayish blue</td>
</tr>
</tbody>
</table>

(2) Proceed as directed in the operating conditions under (1), except using a mixture of ethyl acetate, water and formic acid (6:1:1) as developing solvent: spots equivalent to those described below appear.

<table>
<thead>
<tr>
<th>$R_f$ value</th>
<th>Color and shape of the spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Around 0</td>
<td>A yellow-greenish dark gray spot</td>
</tr>
<tr>
<td>Around 0.05</td>
<td>A weak spot, dark grayish yellow-green</td>
</tr>
<tr>
<td>Around 0.2</td>
<td>A weak spot, dark green</td>
</tr>
<tr>
<td>Around 0.25</td>
<td>A strong spot, dark reddish purple (corresponding to geniposidic acid)</td>
</tr>
<tr>
<td>Around 0.35</td>
<td>A weak spot, bright blue</td>
</tr>
<tr>
<td>Around 0.4 – 0.45</td>
<td>A weak tailing spot, dull greenish blue</td>
</tr>
<tr>
<td>Around 0.45</td>
<td>A strong spot, deep yellow-green (corresponding to acteoside)</td>
</tr>
<tr>
<td>Around 0.5</td>
<td>A strong spot, deep blue (corresponding to plantagouguanidinic acid)</td>
</tr>
<tr>
<td>Around 0.95</td>
<td>A strong spot, dark grayish blue-green</td>
</tr>
<tr>
<td>Around 0.97</td>
<td>A dark grayish blue-green spot</td>
</tr>
</tbody>
</table>

Polyalkylene glycol for gas chromatography Prepared for gas chromatography.
Polyalkylene glycol monoether for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 15000-diepoxide for gas chromatography Prepared for gas chromatography.

Polyethylene glycol ester for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 20 M for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 400 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 600 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 1500 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 6000 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 2-nitroterephthalate for gas chromatography Prepared for gas chromatography.

Polymethyl acrylate for gas chromatography Prepared for gas chromatography.

Polymethylsiloxane for gas chromatography Prepared for gas chromatography.

Polyethylene glycol hydrogenated castor oil 60 A nonionic surfactant prepared by addition polymerization of ethylene oxide with hydrogenated castor oil. Average molar number of ethylene oxide added is about 60. A white or pale yellow petrolatum-like or waxy substance, having a faint, characteristic odor and a slight bitter taste. Very soluble in ethyl acetate and in chloroform, freely soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) To 0.5 g of polyoxyethylene hydrogenated castor oil 60 add 10 mL of water and 5 mL of ammonium thiocyanate-cobalt (II) nitrate TS, and shake thoroughly. Add 5 mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer.

(2) To 0.2 g of polyoxyethylene hydrogenated castor oil 60 add 0.5 g of potassium bisulfate, and heat: an acrolein-stain: a blue color develops in the chloroform layer.

(3) Add 5 mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer.

Identification (1) To 0.5 g of polyoxyethylene hydrogenated castor oil 60 add 10 mL of water and 5 mL of ammonium thiocyanate-cobalt (II) nitrate TS, and shake thoroughly. Add 5 mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer.

Polyethylene glycol 600 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 1500 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 6000 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 2-nitroterephthalate for gas chromatography Prepared for gas chromatography.

Polymethyl acrylate for gas chromatography Prepared for gas chromatography.

Polymethylsiloxane for gas chromatography Prepared for gas chromatography.
Viscosity <2.53>: 25.0 – 31.0 mm²/s. Weigh 4.000 g of polyvinyl alcohol I, previously dried, add 95 mL of water, allow to stand for 30 minutes, and heat to dissolve on a water bath under a reflux condenser for 2 hours while stirring. After cooling, add water to make 100.0 g, and mix. Allow to stand still to remove bubbles, and perform the test at 20 ± 0.1°C as directed in Method 1.

pH <2.54>—The pH of a solution of polyvinyl alcohol I (1 in 25) is between 5.0 and 8.0.

Clarity and color of solution—To 1.0 g of polyvinyl alcohol I add 20 mL of water, disperse by well stirring, heat on water bath under a reflux condenser for 2 hours while stirring. After cooling, add water to make 100.0 g, and mix. Allow to stand still to remove bubbles, and perform the test at 20°C for 2 hours, and cool: the solution is colorless and clear.

Saponification value: 98.0 – 99.0 mol%. Weigh accurately about 3.0 g of polyvinyl alcohol I, previously dried, transfer to a glass-stoppered conical flask, add 100 mL of water, and dissolve by heating on a water bath. After cooling, add exactly 25 mL of 0.1 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then add exactly 30 mL of 0.05 mol/L sulfuric acid VS, shake well, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction. However, when the volume of 0.1 mol/L sodium hydroxide VS consumed in the test is 25 mL or more, use about 2.0 g of the sample.

\[ S\text{aponification value (mol\%)} = 100 - \frac{60.05 - 0.42 A}{a} \]

where:
- \( A = \frac{0.6005 \times (a - b) f}{\text{amount (g) of the sample}} \)
- \( a \): Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test
- \( b \): Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank test
- \( f \): Molarity factor of 0.5 mol/L sodium hydroxide VS

Polyvinyl alcohol II—Colorless to white or pale yellow granules or powder. It is odorless, or has a faint odor of acetic acid. It is tasteless. Practically insoluble in ethanol (95) or in diethyl ether. To polyvinyl alcohol II add water, and heat: A clear, viscous solution is obtained. It is hygroscopic.

Viscosity <2.53>: 4.6 – 5.4 mm²/s. Weigh 4.000 g of polyvinyl alcohol II, previously dried, add 95 mL of water, allow to stand for 30 minutes, and dissolve by stirring on a water bath between 60°C and 80°C for 2 hours. After cooling, add water to make 100.0 g, and mix. Allow to stand still to remove bubbles, and perform the test at 20 ± 0.1°C as directed in Method 1.

pH <2.54>—The pH of a solution of polyvinyl alcohol II (1 in 25) is between 5.0 and 8.0.

Clarity and color of solution—To 1.0 g of polyvinyl alcohol II add 20 mL of water, disperse by well stirring, heat on a water bath for 2 hours, and cool: the solution is clear and colorless.

\[ S\text{aponification value (mol\%)} = 100 - \frac{60.05 - 0.42 A}{a} \]

where:
- \( A = \frac{0.6005 \times (a - b) f}{\text{amount (g) of the sample}} \)
- \( a \): Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test
- \( b \): Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank test
- \( f \): Molarity factor of 0.5 mol/L sodium hydroxide VS

Potassium bicarbonate—See potassium hydrogen carbonate.

Potassium biphthalate—See potassium hydrogen phthalate.

Potassium biphthalate buffer solution, pH 3.5—See potassium hydrogen phthalate buffer solution, pH 3.5.

Potassium biphthalate buffer solution, pH 4.6—See potassium hydrogen phthalate buffer solution, pH 4.6.

Potassium biphthalate buffer solution, pH 5.6—See potassium hydrogen phthalate buffer solution, pH 5.6.

Potassium biphthalate for pH determination—See potassium hydrogen phthalate for pH determination.

Potassium biphthalate (standard reagent)—See potassium hydrogen phthalate (standard reagent).

0.2 mol/L Potassium biphthalate TS for buffer solution—See 0.2 mol/L potassium hydrogen phthalate TS for buffer solution.

Potassium bisulfate—See potassium hydrogen sulfate.

Potassium bromate—KBrO₃ [K 8530, Special class]

Potassium bromide—KBr [K 8506, Special class]

Potassium bromide for infrared spectrophotometry—Crush homocrystals of potassium bromide or potassium bromide, collect a powder passed through a No. 200 (75 μm) sieve, and dry at 120°C for 10 hours or at 500°C for 5 hours. Prepare tablets with this powder, and determine the infrared absorption spectrum <2.25>: any abnormal absorption does not appear.

Potassium carbonate—K₂CO₃ [K 8615, Special class]

Potassium carbonate, anhydrous—See potassium carbonate.
Potassium carbonate-sodium carbonate TS Dissolve 1.7 g of potassium carbonate and 1.3 g of anhydrous sodium carbonate in water to make 100 mL.

Potassium chlorate KClO₃ [K 8207, Special class]

Potassium chloride KCl [K 8121, Special class]

Potassium chloride for conductivity measurement [K 8121, Potassium chloride for conductivity measurement]

Potassium chloride for infrared spectrophotometry Crush homocrystals of potassium chloride or potassium chloride (Special class), collect the powder passed through a No. 200 (75 μm) sieve, and dry at 120°C for 10 hours or at 500°C for 5 hours. Prepare tablets with this powder, and determine the infrared absorption spectrum: any abnormal absorption does not appear.

Potassium chloride-hydrochloric acid buffer solution To 250 mL of a solution of potassium chloride (3 in 20) add 55 mL of 2 mol/L hydrochloric acid TS and water to make 1000 mL.

Potassium chloride TS, acidic Dissolve 250 g of potassium chloride in water to make 1000 mL, and add 8.5 mL of hydrochloric acid.

0.2 mol/L Potassium chloride TS Dissolve 14.9 g of potassium chloride in water to make 1000 mL. Prepare before use.

Potassium chromate K₂CrO₄ [K 8312, Special class]

Potassium chromate TS Dissolve 10 g of potassium chromate in water to make 100 mL.

Potassium cyanide KCN [K 8443, Special class]

Potassium cyanide TS Dissolve 1 g of potassium cyanide in water to make 10 mL. Prepare before use.

Potassium dichromate K₂Cr₂O₇ [K 8517, Special class]

Potassium dichromate (standard reagent) K₂Cr₂O₇ [K 8005, Standard reagent for volumetric analysis]

Potassium dichromate-sulfuric acid TS Dissolve 0.5 g of potassium dichromate in diluted sulfuric acid (1 in 5) to make 100 mL.

Potassium dichromate TS Dissolve 7.5 g of potassium dichromate in water to make 100 mL.

Potassium dihydrogen phosphate KH₂PO₄ [K 9007, Special class]

Potassium dihydrogen phosphate for pH determination KH₂PO₄ [K 9007, for pH determination]

0.1 mol/L Potassium dihydrogen phosphate TS, pH 2.0 Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 1000 mL. Adjust the pH to 2.0 with phosphoric acid.

0.25 mol/L Potassium dihydrogen phosphate TS, pH 3.5 Dissolve 34 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 3.5 with phosphoric acid, and add water to make 1000 mL.

0.05 mol/L Potassium dihydrogen phosphate, pH 3.0 Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 3.0 with phosphoric acid.

0.05 mol/L Potassium dihydrogen phosphate TS, pH 4.7 Dissolve 6.80 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to exactly 4.7 with dilute sodium hydrochloride TS, and add water to make 1000 mL.

0.01 mol/L Potassium dihydrogen phosphate TS, pH 4.0 Dissolve 1.4 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 4.0 with phosphoric acid.

0.02 mol/L Potassium dihydrogen phosphate TS Dissolve 2.72 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.05 mol/L Potassium dihydrogen phosphate TS Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.33 mol/L Potassium dihydrogen phosphate TS Dissolve 4.491 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.1 mol/L Potassium dihydrogen phosphate TS Dissolve 13.61 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.2 mol/L Potassium dihydrogen phosphate TS Dissolve 27.22 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.2 mol/L Potassium dihydrogen phosphate TS for buffer solution Dissolve 27.218 g of potassium dihydrogen phosphate for pH determination in water to make 1000 mL.

Potassium disulfate K₂S₂O₇ [K 8783, Potassium Dioxide]

Potassium ferricyanide TS See potassium hexacyanoferrate (II) TS.

Potassium ferricyanide See potassium hexacyanoferrate (III) TS.

Potassium ferricyanide TS, alkaline See potassium hexacyanoferrate (III) TS, alkaline.

Potassium ferrocyanide TS See potassium hexacyanoferrate (I) trihydrate.

Potassium ferrocyanide TS See potassium hexacyanoferrate (II) TS.

Potassium guaiacolsulfonate C₇H₇KO₅S [Same as the namesake monograph]

Potassium hexacyanoferrate (II) trihydrate K₃Fe(CN)₆·3H₂O [K 8802, Special class]

Potassium hexacyanoferrate (II) TS Dissolve 1 g of potassium hexacyanoferrate (II) trihydrate in water to make 10 mL (½ mol/L). Prepare before use.

Potassium hexacyanoferrate (III) K₃Fe(CN)₆ [K 8801, Special class]

Potassium hexacyanoferrate (III) TS Dissolve 1 g of potassium hexacyanoferrate (III) in water to make 10 mL (½ mol/L). Prepare before use.

Potassium hexacyanoferrate (III) TS, alkaline Dissolve 1.65 g of potassium hexacyanoferrate (III) and 10.6 g of anhydrous sodium carbonate in water to make 100 mL. Preserve in light-resistant containers.
Potassium hexahydroxoantimonate (V)  \( \text{K}_2\text{H}_2\text{Sb}_2\text{O}_7 \)
White granules or crystalline powder.

*Identification*—To 1 g add 100 mL of water, and dissolve by warming. To 20 mL of this solution add 0.2 mL of sodium chloride TS: white precipitates appear. Rubbing the inside wall of the vessel with a glass rod accelerates the forming of the precipitates.

Potassium hexahydroxoantimonate (V) TS  To 2 g of potassium hexahydroxoantimonate (V) add 100 mL of water. Boil the solution for about 5 minutes, cool quickly, add 10 mL of a solution of potassium hydroxide (3 in 20), allow to stand for 1 day, and filter.

Potassium hydrogen carbonate  \( \text{KHCO}_3 \)  [K 8621, Special class]

Potassium hydrogen phthalate  \( \text{C}_6\text{H}_4\text{(COOK)}\text{(COOH)} \)  [K 8809, Special class]

Potassium hydrogen phthalate buffer solution, \( \text{pH} \) 3.5  Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 7.97 mL of 0.2 mol/L hydrochloric acid VS with water to make 200 mL.

Potassium hydrogen phthalate buffer solution, \( \text{pH} \) 4.6  Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 12.0 mL of 0.2 mol/L sodium hydroxide VS with water to make 200 mL.

0.3 mol/L Potassium hydrogen phthalate buffer solution, \( \text{pH} \) 4.6  Dissolve 61.26 g of potassium hydrogen phthalate in about 800 mL of water, adjust the \( \text{pH} \) to 4.6 with sodium hydroxide TS, and add water to make 1000 mL.

Potassium hydrogen phthalate buffer solution, \( \text{pH} \) 5.6  Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 39.7 mL of 0.2 mol/L sodium hydroxide VS with water to make 200 mL.

Potassium hydrogen phthalate for \( \text{pH} \) determination  \( \text{C}_6\text{H}_4\text{(COOK)}\text{(COOH)} \)  [K 8809, For \( \text{pH} \) determination]

Potassium hydrogen phthalate (standard reagent)  \( \text{C}_6\text{H}_4\text{(COOK)}\text{(COOH)} \)  [K 8805, Standard reagent for volumetric analysis]

0.2 mol/L Potassium hydrogen phthalate TS for buffer solution  Dissolve 40.843 g of potassium hydrogen phthalate for \( \text{pH} \) determination in water to make 1000 mL.

Potassium hydrogen sulfate  \( \text{KHSO}_4 \)  [K 8972, Special class]

Potassium hydroxide  \( \text{KOH} \)  [K 8574, Special class]

Potassium hydroxide-ethanol TS  Dissolve 10 g of potassium hydroxide in ethanol (95) to make 100 mL. Prepare before use.

0.1 mol/L Potassium hydroxide-ethanol TS  To 1 mL of dilute potassium hydroxide-ethanol TS add ethanol (95) to make 5 mL. Prepare before use.

Potassium hydroxide-ethanol TS, dilute  Dissolve 35 g of potassium hydroxide in 20 mL of water, and add ethanol (95) to make 1000 mL (0.5 mol/L). Preserve in tightly stoppered bottles.

Potassium hydroxide TS  Dissolve 6.5 g of potassium hydroxide in water to make 100 mL (1 mol/L). Preserve in polyethylene bottles.

0.02 mol/L Potassium hydroxide TS  Dilute 2 mL of potassium hydroxide TS with water to make 100 mL. Prepare before use.

0.05 mol/L Potassium hydroxide TS  Dilute 5 mL of potassium hydroxide TS with water to make 100 mL. Prepare before use.

8 mol/L Potassium hydroxide TS  Dissolve 52 g of potassium hydroxide in water to make 100 mL. Preserve in polyethylene bottles.

Potassium iodate  \( \text{KIO}_3 \)  [K 8922, Special class]

Potassium iodate (standard reagent)  \( \text{KIO}_3 \)  [K 8005, Standard reagent for volumetric analysis]

Potassium iodide  \( \text{KI} \)  [K 8913, Special class]

Potassium iodide for assay  [Same as the monograph Potassium Iodide]

Potassium iodide-starch TS  Dissolve 0.5 g of potassium iodide in 100 mL of freshly prepared starch TS. Prepare before use.

Potassium iodide TS  Dissolve 16.5 g of potassium iodide in water to make 100 mL. Preserve in light-resistant containers. Prepare before use (1 mol/L).

Potassium iodide TS, concentrated  Dissolve 30 g of potassium iodide in 70 mL of water. Prepare before use.

Storage—Preserve in light-resistant containers.

Potassium iodide TS, saturated  Saturate 20 g of potassium iodide in 10 mL of freshly boiled and cooled water. Prepare before use.

Potassium iodide-zinc sulfate TS  Dissolve 5 g of potassium iodide, 10 g of zinc sulfate, and 50 g of sodium chloride in water to make 200 mL.

Potassium methanesulfonate  \( \text{CH}_3\text{SO}_3\text{K} \)  White crystals or crystalline powder.

Purity  Clarity and color of solution—Dissolve 1.0 g of potassium methanesulfonate in 20 mL of water: the solution is transparent and colorless.

Content: not less than 98.0%. Assay—Dissolve about 0.1 g of potassium methanesulfonate, accurately weighed, in 10 mL of acetic acid (100), add 20 mL of acetic anhydride, and titrate \( <2.50\text{mL} \) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 13.42 mg of \( \text{CH}_3\text{SO}_3\text{K} \)

Potassium naphthoquinone sulfonate  See potassium 1,2-naphthoquinone-4-sulfonate.

Potassium 1,2-naphthoquinone-4-sulfonate  \( \text{C}_9\text{H}_6\text{O}_4\text{SO}_3\text{K} \)  [K 8696, \( \beta \)-Naphthoquinone-4-sulfonic acid potassium salt, Special class]

Potassium 1,2-naphthoquinone-4-sulfonate TS  Dissolve 0.5 g of potassium 1,2-naphthoquinone-4-sulfonate in water to make 100 mL. Prepare before use.

Potassium nitrate  \( \text{KNO}_3 \)  [K 8548, Special class]
Potassium nitrite  KNO₂ - A white to pale yellow crystalline powder. It is deliquescent.

Identification—(1) Dissolve 1 g of potassium nitrite in 20 mL of water, and use this as the sample solution. To 5 mL of the sample solution add 1 mL of sulfuric acid: a yellowish brown gas is evolved.

(2) The sample solution obtained in (1) responds to the Qualitative Tests <1.00> (1) for potassium salt. Preserve in a light-resistant tight container.

Potassium periodate  KIO₄ - [K 8249, Special class]

Potassium periodate TS - To 2.8 g of potassium periodate add 200 mL of water, dissolve by adding dropwise 20 mL of sulfuric acid under shaking, cool, and add water to make 1000 mL.

Potassium permanganate  KMnO₄ - [K 8247, Special class]

Potassium permanganate TS - Dissolve 3.3 g of potassium permanganate in water to make 1000 mL (0.02 mol/L).

Potassium permanganate TS, acidic - To 100 mL of potassium permanganate TS add 0.3 mL of sulfuric acid.

Potassium persulfate  K₂S₂O₈ - [K 8253, Special class]

Potassium pyroantimonate - See potassium hexahydroxoantimonate (V).

Potassium pyroantimonate TS - See potassium hexahydroxoantimonate (V) TS.

Potassium pyrophosphate  K₂P₂O₇ - White, crystalline powder, very soluble in water.

Melting point <2.60>: 1109°C

Potassium pyrosulfate - See potassium disulfate.

Potassium sodium tartrate - See potassium sodium tartarate tetrahydrate.

Potassium sodium tartrate tetrahydrate  KNaC₄H₄O₆·4H₂O - [K 8536, (±)-Potassium sodium tartrate tetrahydrate, Special class]

Potassium sulfate  K₂SO₄ - [K 8962, Special class]

Potassium sulfate TS - Dissolve 1 g of potassium sulfate in water to make 100 mL.

Potassium tartrate  2C₄H₆K₂O₆·H₂O - [K 8535, (±)-Potassium Tartrate-Water (2/1), Special class]

Potassium tellurite  K₂TeO₃ - White powder or small masses obtained by melting an equimolar mixture of tellurium dioxide and potassium carbonate in a stream of carbon dioxide. Soluble in water.

Content: not less than 90.0%. Assay—Dissolve about 1.0 g of potassium tellurite, accurately weighed, in 100 mL of water, add 5 mL of diluted acetic acid (31) (1 in 3), and boil. After cooling, filter by suction through a crucible glass filter (14G), previously dried at 105 ± 2°C for 1 hour to constant mass (b g). Wash the filtrate with water, dry the glass filter at 110°C for 3 hours, and measure the mass a (g).

\[
\text{Content (\%) of potassium tellurite (K}_2\text{TeO}_3) = \frac{(a - b) \times 1.5902}{S} \times 100
\]

S: Mass (g) of potassium tellurite taken.

Potassium tetroxalate for pH determination - See potassium trihydrogen dioxalate dihydrate for pH determination.

Potassium thiocyanate  KSCN - [K 9001, Special class]

Potassium thiocyanate TS - Dissolve 1 g of potassium thiocyanate in water to make 10 mL.

Potassium trihydrogen dioxalate dihydrate for pH determination  KH₃(C₂O₄)₂·2H₂O - [K 8474]

Potato extract - Prepared for microbial test.

Potato starch - Same as the namesake monograph.

Potato starch TS - Prepare as directed under starch TS with 1 g of potato starch.

Potato starch TS for amylolytic activity test - Dry about 1 g of potato starch, accurately weighed, at 105°C for 2 hours, and measure the loss. Weigh accurately an amount of potato starch, equivalent to 1.000 g on the dried basis, place into a conical flask, add 20 mL of water, and make it pasty by gradually adding 5 mL of a solution of sodium hydroxide (2 in 25) while shaking well. Heat in a water bath for 3 minutes while shaking, add 25 mL of water, and cool. Neutralize exactly with 2 mol/L hydrochloric acid TS, add 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0, and add water to make exactly 100 mL. Prepare before use.

Powdered tragacanth - Same as the namesake monograph.

(±)-Praeruptorin A for thin-layer chromatography  C₁₂H₁₇O₁₇ - White crystals or crystalline powder. Soluble in methanol, sparingly slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification—Determine the absorption spectrum of a solution of (±)-praeruptorin A for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 320 nm and 324 nm.

Melting point <2.60>: 152 – 156°C

Pravastatin sodium  C₂₅H₃₅NaO₇ - [Same as the namesake monograph]

Prazepam for assay  C₁₉H₁₇ClN₂O - [Same as the monograph Prazepam. When dried, it contains not less than 99.0% of C₁₉H₁₇ClN₂O.]

Prednisolone  C₂₁H₂₈O₅ - [Same as the namesake mono-
graph]

Prednisolone acetate $C_{23}H_{30}O_6$ [Same as the namesake monograph]

Prednisone $C_{21}H_{29}O_7$. White, crystalline powder. Slightly soluble in methanol, in ethanol (95) and in chloroform, and very slightly soluble in water.

Optical rotation $\angle 2.49^\circ$ [g] $\oplus$: +167 – +175° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm). Loss on drying $\angle 2.41^\circ$: not more than 1.0% (1 g, 105°C, 3 hours).

Content: 96.0 – 104.0%. Assay—Weigh accurately about 20 mg of prednisone, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, dilute with methanol to make exactly 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry $\angle 2.24^\circ$, and read the absorbance $A$ at the wavelength of maximum absorption at about 238 nm.

Amount (mg) of $C_{21}H_{29}O_7$: $A = \frac{440}{280} \times 20,000$

Probenecid $C_{17}H_{18}O_5\text{N}_2$ [Same as the namesake monograph]

Procainamide hydrochloride $C_{13}H_{21}N_3O\cdot HCl$ [Same as the namesake monograph]

Procainamide hydrochloride for assay $C_{13}H_{21}N_3O\cdot HCl$ [Same as the namesake monograph Procainamide Hydrochloride. When dried, it contains not less than 99.0% of procainamide hydrochloride ($C_{13}H_{21}N_3O\cdot HCl$).]

Propafenone hydrochloride for assay $C_{16}H_{21}N_2O_3\cdot HCl$ [Same as the namesake monograph Procaterol Hydrochloride. When dried, it contains not less than 99.5% of propafenone hydrochloride ($C_{16}H_{21}N_2O_3\cdot HCl$).] [Same as the namesake monograph Propranolol Hydrochloride. When dried, it contains not less than 99.0% of propafenone hydrochloride ($C_{16}H_{21}N_2O_3\cdot HCl$).]

Propylbenzoate $C_6H_5COOC_3H_7$. Clear, colorless liquid, having a characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether. Boiling point: about 82°C.

Refractive index $\angle 2.50^\circ$ $n_2^D$: 1.376 – 1.378

Specific gravity $\angle 2.50^\circ$ : $d_2^D$: 0.785 – 0.788

Purity (1) Ultraviolet absorbing substances—Perform the test with 2-propanol for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry $\angle 2.24^\circ$, using water as the blank: the absorbance at 230 nm is not more than 0.2; at 250 nm, not more than 0.03; and between 280 nm and 400 nm, not more than 0.01.

(2) Peroxide—Mix 100 mL of water and 25 mL of dilute sulfuric acid, and add 25 mL of a solution of potassium iodide (1 in 10). Add this solution to 20 g of 2-propanol for liquid chromatography. Stopper tightly, shake, allow to stand for 15 minute in a dark place, and titrate $\angle 2.50^\circ$ with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction (not more than 0.0005%).

Propranolol, iso See 2-propanol.

Propranolol hydrochloride for assay $C_{16}H_{22}N_2O_3\cdot HCl$ [Same as the namesake monograph Propranolol Hydrochloride. When dried, it contains not less than 99.5% of propranolol hydrochloride ($C_{16}H_{22}N_2O_3\cdot HCl$).]

Propanol, iso See Water determination.

Propanol, $CH_3CH_2CH_2OH$ [K 8838, Special class]

Propylene carbonate $C_4H_6O_3$. Colorless liquid. Refractive index $\angle 2.57^\circ$: 1.397 – 1.400

Distilling range $\angle 2.57^\circ$: 54 – 54°C, not less than 95 vol%.

Propylamine, iso $C_9H_{19}\text{CNH}_2$. Colorless liquid, having a characteristic, amine-like odor. Miscible with water, with ethanol (95) and with diethyl ether.

Refractive index $\angle 2.50^\circ$ $n_2^D$: 1.374 – 1.376

Specific gravity $\angle 2.50^\circ$ : $d_2^D$: 0.685 – 0.690

Distilling range $\angle 2.57^\circ$: 31 – 33°C, not less than 95 vol%.

Propylbenzoate $C_6H_5COOC_3H_7$. Clear, colorless liquid, having a characteristic odor.

Refractive index $\angle 2.50^\circ$ $n_2^D$: 1.498 – 1.503

Specific gravity $\angle 2.50^\circ$ : $d_2^D$: 1.022 – 1.027

Propylene carbonate $C_4H_6O_3$. Colorless liquid.

Boiling point $\angle 2.57^\circ$: 240 – 242°C

Water $\angle 2.48^\circ$: less than 0.1%.

Propylene carbonate for water determination See Water Determination $\angle 2.48^\circ$.

Propylene glycol $CH_2CH(OH)CH_2OH$ [K 8837, Special class]

Propylene glycol cefazolin $C_{11}H_{17}O_5\cdot S_2\cdot C_2H_3O_2$ [Same as the namesake monograph]

Propyler, iso $(CH_3)_2CHOCH(CH_3)_2$. Clear, colorless
liquid, having a characteristic odor. Not miscible with water. 

Refractive index \(< 2.45 > n_{20}^0: 1.368 – 1.369

Specific gravity \(< 2.56 > d_{20}^0: 0.723 – 0.725

Propyl parahydroxybenzoate

\( \text{HOCH}_2\text{H}_2\text{COOCCH}_2\text{CH}_3 \) [Same as the namesake monograph]

Propylthiouracil for assay \( \text{C}_9\text{H}_13\text{N}_2\text{O}_5 \) [Same as the monograph Propylthiouracil. When dried, it contains not less than 99.0% of propylthiouracil (\( \text{C}_9\text{H}_13\text{N}_2\text{O}_5 \)).]

Prostaglandin A\(_1\) \( \text{C}_{20}\text{H}_{19}\text{O}_4 \) White crystals or crystalline powder. Very soluble in ethanol (95) and in ethyl acetate, and very slightly soluble in water.

Purity Related substances—Dissolve 5 mg of prostaglandin A\(_1\) in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu \)L of each of the sample solution and standard solution as directed in the Assay (1) under Kakkonto Extract: the total area of the peaks other than pseudoephedrine and the solvent is not larger than the peak area of prostaglandin A\(_1\) from the standard solution. Perform the test with this solution as directed under Ultraviolet-spectrophotometry.

Operating conditions

Detector, column, column temperature, mobile phase, flow rate, and selection of columns: Proceed the operating conditions in the Assay of Alprostadil Alfadex.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of prostaglandin A\(_1\) obtained from 10 \( \mu \)L of the standard solution is 5 to 10\% of the full scale.

Time span of measurement: About twice as long as the retention time of prostaglandin A\(_1\) beginning after the solvent peak.

Protein digestive enzyme TS A solution of lysyl endopeptidase in 0.05 mol/L tris buffer solution, pH 8.6 (1 in 5000).

Pseudoephedrine hydrochloride \( \text{C}_{10}\text{H}_{12}\text{NO}.\text{HCl} \) White, crystals or crystalline powder. Freely soluble in water, in methanol and in acetic acid (100), soluble in ethanol (99.5), and practically insoluble in acetic anhydride. Melting point: 182 – 186\°C

Purity Related substances—Dissolve 1 mg in 10 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 10 \( \mu \)L of the sample solution for twice as long as the retention time of ephedrine as directed in the Assay (1) under Kakkonto Extract: the total area of the peaks other than pseudoephedrine and the solvent is not larger than 1/10 times the total area of the peaks other than the solvent.

Puerarin for thin-layer chromatography \( \text{C}_{21}\text{H}_{20}\text{O}_8 \) White crystalline powder. Freely soluble in methanol, and practically insoluble in diethyl ether. Melting point: about 188\°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of puerarin for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 2 \( \mu \)L of this solution as directed in the Identification under Pueraria Root: any spot other than the principal spot at the \( R_f \) value of about 0.4 does not appear.

Pullulanase An enzyme obtained from Klebsiella pneumoniae. White crystals. It contains not less than 30 units per mg. One unit is an enzymatic activity to produce 1 \( \mu \)mol of maltotriose from pullulan per minute at pH 5.0 and 30\°C.

Pullulanase TS A solution of pullulanase containing 10 units per mL.

Purified hydrochloric acid See hydrochloric acid, purified.

Purified methanol See methanol, purified.

Purified sulfuric acid See sulfuric acid, purified.

Purified water [Use the water prescribed by the monographs of Purified Water or Purified Water in Containers. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of the relevant test.]

Pyrazole \( \text{C}_7\text{H}_8\text{N}_2\) White to pale yellow crystals or crystalline powder.

Melting point \(< 2.60\°: 67 – 71\°C

Pyridine \( \text{C}_3\text{H}_7\text{N} \) [K 8777, Special class]

Pyridine-acetic acid TS Dilute 20 mL of pyridine with sufficient diluted acetic acid (100) (1 in 25) to make 100 mL. Prepare before use.

Pyridine, dehydrated \( \text{C}_3\text{H}_7\text{N} \) To 100 mL of pyridine add 10 g of sodium hydroxide, and allow to stand for 24 hours. Decant the supernatant liquid, and distill.

Pyridine for Karl Fischer method See Water Determination \(< 2.40\°.

0.2 mol/L Pyridine-formic acid buffer solution, pH 3.0

To 15.82 g of pyridine add 900 mL of water, shake well, adjust the pH to 3.0 with diluted formic acid (1 in 2), and add water to make 1000 mL.

Pyridine-pyrazoline TS Dissolve, with thorough shaking, 0.1 g of 3-methyl-1-phenyl-5-pyrazoline in 100 mL of water by heating between 65\°C and 70\°C, and cool below 30\°C. Mix this solution with a solution prepared by dissolving 0.02 g of bis-(1-phenyl-3-methyl-5-pyrazoline) in 20 mL of pyridine. Prepare before use.

Pyridoxine hydrochloride \( \text{C}_{13}\text{H}_{15}\text{NO}_3.\text{HCl} \) [Same as the namesake monograph]

\( \text{1-(2-Pyridylazo)-2-naphthol} \) Orange-yellow or orange-red powder.

Absorbance—Dissolve 25 mg of 1-(2-pyridylazo)-2-naphthol in methanol to make exactly 100 mL. Pipet 2.0 mL of this solution, and add methanol to make exactly 50 mL. Perform the test with this sample solution as directed under Ultraviolet-visible Spectrophotometry \(< 2.24\°, using methanol as the blank: absorbance at the wavelength of 470 nm is not less than 0.55.

Melting point \(< 2.60\°: 137 – 140\°C

Purity Clarity and color of solution—Dissolve 25 mg of 1-(2-pyridylazo)-2-naphthol in 100 mL of methanol: the solution is clear and orange-yellow.

Residue on ignition \(< 2.40\°: not more than 1.0%.

Sensitivity—On adding 50 mL of water, 30 mL of methanol and 10 mL of acetic acid-sodium acetate buffer solution, pH 5.5, to 0.2 mL of a solution of 1-(2-pyridylazo)-2-
naphthol in methanol (1 in 4000), the solution is yellow in color. Add 1 drop of a solution of copper (II) chloride dihydrate (1 in 600) to this solution: the solution is red-purple in color. Add a subsequent 1 drop of diluted 0.1 mol/L disodium dihydrogen ethylenediamine tetracacetate TS (1 in 10): the color of the solution changes to yellow again.

1-(4-Pyridyl)pyridinium chloride hydrochloride

C_{19}H_{26}N_8O_6.HCl White to yellowish white, crystalline powder. Very soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point <2.60>: 154 – 156°C

Pyrogallol C_{6}H_{4}(OH)_3 [K 8780, Special class]

1-Pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride

C_{19}H_{26}N_8O_6.HCl White to light powder. Freely soluble in water, in methanol and in acetic acid (100).

Absorbance <2.24> E_{1cm}^1% (316 nm): 242 – 268 (2 mg, water, 100 mL).

Optical rotation <2.49> [α]_D: –51 – –56° [0.1 g, diluted acetic acid (100) (1 in 2), 10 mL, 100 mm].

Purity Related substances—Dissolve 0.05 g of 1-pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.06>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

1-Pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride TS

Dissolve 25 mg of 1-pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride and 0.04 g of D-Mannitol in 2 to 3 mL of water, lyophilize, and add 16.7 mL of water to dissolve. To 1 volume of this solution add 9 volumes of water before use.

Pyrole C_{6}H_{5}N Clear, colorless liquid, having a characteristic odor. Soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Specific gravity <2.56> d_{20}^3: 0.965 – 0.975

Pyrophosphate buffer solution, pH 9.0

Dissolve 3.3 g of potassium pyrophosphate, 15 mg of dithiothreitol and 40 mg of disodium dihydrogen ethylenediamine tetracacetate dihydrate in 70 mL of water, adjust the pH with a solution of citric acid monohydrate (21 in 100) to exactly 9.0, and add water to make 100 mL.

0.05 mol/L Pyrophosphate buffer solution, pH 9.0

Dissolve 0.83 g of potassium pyrophosphate in 40 mL of water, adjust the pH with 1 mol/L hydrochloric acid VS to 9.0, and add water to make 50 mL. Adjust the temperature to 22 ± 2°C before use.

Quinapril hydrochloride for assay

C_{25}H_{30}N_2O_5.HCl [Same as the monograph Quinapril Hydrochloride. When performing the purity test (2) of Quinapril Hydrochloride, the area of the peaks, having the relative retention time of about

0.5 and about 2.0 to quinapril obtained from the sample solution, is not larger than the peak area of quinapril from the standard solution, the area of peak other than quinapril and above mentioned peak from the sample solution is not larger than 2/5 times the peak area of quinapril from the standard solution, and the total area of the peaks other than quinapril from the sample solution is not larger than 2 times the peak area of quinapril from the standard solution.]

Quinhydrone C_{6}H_{4}(OH)_2.C_{6}H_10O_2 Green crystals or crystalline powder.

Melting point <2.60>: 169 – 172°C

Quinidine sulfate See quinidine sulfate hydrate.

Quinidine sulfate hydrate (C_{20}H_{24}N_2O_2)_{2}.H_2SO_4.2H_2O [Same as the monograph monograph]

Quinine sulfate See quinine sulfate hydrate.

Quinine sulfate hydrate (C_{20}H_{24}N_2O_2)_{2}.H_2SO_4.2H_2O [Same as the monograph monograph]

Quinoline C_{6}H_{5}N [K 8279, Special class]

Quinoline TS Mix 50 mL of quinoline with 300 mL of diluted hydrochloric acid (1 in 6), previously heated, cool, and filter if necessary.

8-Quinolinol C_{6}H_{5}NO [K 8775, Special class]

Raney nickel catalyst Grayish black powder. An alloy containing 40 to 50% of nickel and 50 to 60% of aluminum.

Ranitidinediamine (C_{10}H_{18}N_2OS)_{2}.C_{4}H_{4}O_4 White to pale yellow crystalline powder.

Identification—Determine the infrared absorption spectrum of ranitidinediamine as directed in the paste method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 2780 cm⁻¹, 1637 cm⁻¹, 1015 cm⁻¹ and 788 cm⁻¹.

Content: not less than 95%. Assay—Weigh accurately about 0.1 g of ranitidinediamine, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple to green through blue (indicator: crystal violet TS). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 13.62 mg of (C_{9}H_{17}N_2O_4).[C_{9}H_{15}ClN_2O_4].

Rebamipide for assay C_{19}H_{15}ClN_2O_4 [Same as the monograph Rebamipide. When dried, it contains not less than 99.5% of rebamipide (C_{19}H_{15}ClN_2O_4).]

Reduced iron See iron powder.

Reference anti-interleukin-2 antibody for teceleukin Monoclonal antibody obtained from a fusion cell strain from mouse spleen cells sensitized to teceleukin and mouse melanoma cells, or alternately, rabbit antiserum towards human interleukin-2, that is purified using affinity chromatography. When determining the neutralizing activity, taking 1 neutralizing unit as the titer that neutralizes one unit of activity of teceleukin, contains at least 2000 neutralizing units per 1 mL.

Reinecke salt See reinecke salt monohydrate.

Reinecke salt monohydrate NH_{4}[Cr(NH_3)_2(SCN)_4].H_2O
Dark red crystals or crystalline powder.

Identification—Determine the infrared absorption spectrum of Reineke salt monohydrate as directed in the potassium bromide disk method as directed under Spectrophotometry \( \leq 2.25\% \): it exhibits absorption at the wave numbers of about 3310 cm\(^{-1} \), 2130 cm\(^{-1} \), 1633 cm\(^{-1} \), 1400 cm\(^{-1} \), 1261 cm\(^{-1} \) and 711 cm\(^{-1} \).

Reineke salt TS

To 20 mL of water add 0.5 g of Reineke salt monohydrate, shake frequently for 1 hour, then filter. Use within 48 hours.

Resazurin C\(_6\)H\(_5\)NNaO\(_4\) Brownish purple powder. It dissolves in water and the solution is purple in color.

Residue on ignition \( < 2.44\% \): not less than 28.5\% (1 g).

Resibufogenin for assay C\(_{25}\)H\(_{32}\)O\(_4\)xH\(_2\)O Odorless white crystalline powder.

Absorbance \( < 2.24\% \): \( E_{1\%}^{1\text{cm}} \) (300 nm): 131 – 145 (0.01 g, methanol, 250 mL), dried in a desiccator (silica gel) for 24 hours.

Purity Related substances—Weigh accurately 40 mg of resibufogenin for assay and proceed as directed in the Purity under bufalin for assay.

Content: not less than 98.0\%. Assay—Weigh accurately about 10 mg of resibufogenin for assay, previously dried in a desiccator (silica gel) for 24 hours, add methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20 \( \mu \)L of this solution as directed under Liquid Chromatography \( < 2.01\% \) according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of resibufogenin by the area percentage method.

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 300 nm).

Column: A stainless steel column about 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecysilanolized silica gel for liquid chromatography (5 to 10 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (1:1). Selection of column: Dissolve 0.01 g each of bufalin for assay, cinobufagin for assay and resibufogenin for assay in methanol to make 200 mL. Perform the test with 20 \( \mu \)L of this solution according to the above operating conditions, and calculate the resolution. Use a column giving elution of this solution according to the above operating conditions, and calculate the amount of resibufogenin from 20 \( \mu \)L of the standard solution (1) is about 20\% of the full scale.

Time span of measurement: About twice as long as the retention time of resibufogenin beginning after the peak of solvent.

Resibufogenin for component determination See resibufogenin for assay.

Resibufogenin for thin-layer chromatography C\(_{24}\)H\(_{32}\)O\(_4\)xH\(_2\)O White crystalline powder having no odor. It is freely soluble in acetone and in methanol.

Purity Related substances—Dissolve 5.0 mg of the substance to be tested in exactly 5 mL of acetone. Perform the test with 5 \( \mu \)L of this solution as directed in the Identification under Toad Venom: no other spots than the principal spot of around \( Rf \) 0.4 appear.

Resolving gel for celmoleukin Prepare the resolving gel in tris buffer solution, \( p\)H 8.8 using ammonium persulfate and \( N,N,N',N'\)-tetramethylethylenediamine so the concentrations of acrylamide and sodium lauryl sulfate are 13.5\% and 0.1\%, respectively.

Resorcin See resorcinol.

Resorcinol C\(_6\)H\(_4\)(OH)\(_2\) [K 9032, Special class]

Resorcinol sulfuric acid TS Dissolve 0.1 g of resorcinol in 10 mL of diluted sulfuric acid (1 in 10).

Resorcin TS Dissolve 0.1 g of resorcinol in 10 mL of hydrochloric acid. Prepare before use.

Resorcin sulfuric acid TS See resorcinol sulfuric acid TS.

Resorcin TS See resorcinol TS.

\( \Lambda \)-Rhamnose monohydrate C\(_{12}\)H\(_{22}\)O\(_4\) White crystalline powder having sweet taste. Freely soluble in water, and sparingly soluble in ethanol (95).

Optical rotation \( < 2.49\% \): \( [\alpha]_{D}^{24} \): + 7.8 – + 8.3° (1 g, 20 mL of water, 2 drops of ammonium TS, 100 mm).

Melting point \( < 2.60\% \): 87 – 91°C

Purity Related substances—Dissolve 1.0 mg of \( \Lambda \)-rhamnose monohydrate in 1 mL of water, and add methanol to make exactly 10 mL. Proceed with 20 \( \mu \)L of this solution as directed in the Identification (2) under Acacia: any spot other than the principal spot at the \( Rf \) value of about 0.5 does not appear.

Rhine for thin-layer chromatography C\(_9\)H\(_8\)O\(_2\) A yellow powder. Very slightly soluble in acetone, and practically insoluble in water, in methanol, and in ethanol (99.5). Melting point: about 320°C (with decomposition).

Identification—Determine the absorption spectrum of a solution in methanol (3 in 500,000) as directed under Ultraviolet-visible Spectrophotometry \( < 2.24\% \): it exhibits maxima between 228 nm and 232 nm, between 255 nm and 259 nm, and between 429 nm and 433 nm.

Purity Related substances—Dissolve 1.0 mg in 10 mL of acetone, and perform the test with 2 \( \mu \)L of this solution as directed in the Identification (1) under Daikokanzoto Extract: no spot other than the principal spot (\( RF \) value is about 0.3) appears.

Rhnycophylline for assay C\(_{25}\)H\(_{32}\)N\(_2\)O\(_4\) Rhnycophylline for thin-layer chromatography. It meets the following requirements.

Absorbance \( < 2.24\% \): \( E_{1\%}^{1\text{cm}} \) (245 nm): 473 – 502 (5 mg of the dried substance in a desiccator (silica gel) for 24 hours, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

Purity Related substances—Dissolve 5 mg of rhnycophylline for assay in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the sample
solution. Pipet 1 mL of the sample solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid-chromatography 2.07 according to the following conditions. Determine the peak area of each solution by the automatic integration method: the total area of the peaks other than rhyncophylline obtained from the sample solution is not larger than the peak area of rhyncophylline from the standard solution.  

Operating conditions  
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Uncaria Hook.  
Time span of measurement: About 4 times as long as the retention time of rhyncophylline, beginning after the solvent peak.  
System suitability  
Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 20 mL. Confirm that the peak area of rhyncophylline obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that with 20 μL of the standard solution.  
System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Uncaria Hook.  

Rhyncophylline for component determination  
See rhyncophylline for assay.  

Rhyncophylline for thin-layer chromatography  
C_{22}H_{28}N_{2}O_{4} White crystals or crystalline powder. Slightly soluble in ethanol (95.5) and in acetone, and practically insoluble in water. Melting point: 205 – 209°C.  
Identification—Determine the absorption spectrum of a solution of rhyncophylline for thin-layer chromatography in a mixture of methanol and dilute acetic acid (7:3) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits a maximum between 242 nm and 246 nm.  
Purity Related substances—Dissolve 1.0 mg of rhyncophylline for thin-layer chromatography in 1 mL of acetone, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography 2.03. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography and develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): no spot other than the principal spot at around Rf 0.5 appears.  

Riboflavin C_{17}H_{20}N_{4}O_{6} [Same as the namesake monograph]  
Riboflavin sodium phosphate C_{17}H_{28}N_{4}NaO_{6}P [Same as the namesake monograph]  
Risperidone for assay C_{23}H_{27}FN_{4}O_{2} [Same as the monograph Risperidone. It contains not less than 99.5% of risperidone (C_{18}H_{16}O_{8}N), calculated on the dried basis.]  
Ritodrine hydrochloride C_{17}H_{20}NO_{3}.HCl [Same as the namesake monograph]  

Rose Bengal  
See Microbial Limit Test for Crude Drugs 5.02.  
Rose Bengal TS  
See Microbial Limit Test for Crude Drugs 5.02.  

Rosmarinic acid for assay  
Rosmarinic acid for thin-layer chromatography. However, it meets the following requirements:  
Absorbance 2.24 E_{1\%}^{254 nm} (325 nm): 502 – 534 (5 mg, water, 500 mL).  
Purity Related substances—Conduct this procedure using light-resistant vessels. Dissolve 5 mg of rosmarinic acid for assay in 20 mL of the mobile phase, and use this as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.07 according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than rosmarinic acid from the sample solution is not larger than the peak area of rosmarinic acid from the standard solution.  
Operating conditions  
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).  
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).  
Column temperature: A constant temperature of about 40°C.  
Mobile phase: A mixture of water, acetonitrile and phosphoric acid (800:200:1).  
Flow rate: Adjust the flow rate so that the retention time of rosmarinic acid is about 14 minutes.  
Time span of measurement: About 4 times as long as the retention time of rosmarinic acid.  
System suitability  
Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of rosmarinic acid obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.  
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rosmarinic acid are not less than 5000 and not more than 1.5, respectively.  
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rosmarinic acid is not more than 1.5%.  

Rosmarinic acid for component determination  
See rosmarinic acid for assay.  

Rosmarinic acid for thin-layer chromatography  
C_{18}H_{16}O_{8} White to pale yellow crystals or crystalline powder. Freely soluble in ethanol (99.5), and slightly soluble in water. Melting point: about 170°C (with decomposition).  
Identification—Determine the absorption spectrum of a solution of rosmarinic acid for thin-layer chromatography (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits maxima between 215 nm and 219 nm and between 322 nm and 326 nm.
### Reagents, Test Solutions / General Tests

**Purity**  
Related substances—Conduct this procedure using light-resistance vessels. Dissolve 10 mg of rosmarinic acid for thin-layer chromatography in 2 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Proceed with 10 \( \mu L \) each of the sample solution and standard solution as directed in the Identification (2) under Hangekobukuto Extract: the spot other than the principal spot of around RF 0.5 from the sample solution is not more intense than the spot from the standard solution.

**Rosaxidine acetate hydrochloride**  
C\(_{19}\)H\(_{28}\)N\(_2\)O\(_4\).HCl  
[Same as the namesake monograph]

**RPMI-1640 powdered medium**  
Powder medium for cell culture containing 6 g of sodium chloride, 400 mg of potassium chloride, 800 mg of sodium dihydrogen phosphate, 100 mg of anhydrous calcium nitrate, 49 mg of anhydrous magnesium sulfate, 2 g of dextrose, 200 mg of L-arginine, 1 mg of glutathione, 50 mg of L-isoleucine, 15 mg of L-phenylalanine, 5 mg of L-tryptophan, 0.2 mg of biotin, 1 mg of nicotinamide, 1 mg thiamine hydrochloride, 300 mg of L-glutamine, 56.8 mg of L-asparagine, 10 mg of glycine, 50 mg of L-leucine, 20 mg of L-proline, 20 mg of L-lysine, 0.25 mg of \( \text{Ca}^{2+} \) calcium pantothenate, 5 \( \mu g \) of cyano-cobalamin, 1 mg of aminobenzoic acid, 20 mg of L-aspartic acid, 15 mg of L-histidine, 40 mg of L-lysine hydrochloride, 30 mg of L-serine, 20 mg of L-valine, 1 mg of folic acid, 1 mg of pyridoxine hydrochloride, 20 mg of L-glutamic acid, 20 mg of L-hydroxyproline, 15 mg of L-methionine, 20 mg of L-threonine, 3 mg of choline chloride, 35 mg of \( \text{I}^{-} \) I-insoluble in water.

**Saccharated pepsin**  
[Same as the namesake monograph]

**Saikosaponin a for assay**  
Use saikosaponin a for thin-layer chromatography meeting the following additional specifications.

**Purity**  
Related substances—

1. Dissolve 2.0 mg of saikosaponin a for assay in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solution. Proceed the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed in the Purity (2) under Bupleurum Root: the spot other than the principal spot around RF 0.4 is not larger and not more intense than the spot obtained with the standard solution.

2. Dissolve 10 mg of saikosaponin a for assay in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 20 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than saikosaponin a is not more than the peak area of saikosaponin a obtained with the standard solution.

**Operating conditions**

Detector, column: Proceed as directed in the operating conditions in the Assay under Bupleurum Root.

Column temperature: A constant temperature of about 40°C.

**Mobile phase** A mixture of water and acetonitrile (13:7).

**Flow rate** Adjust the flow rate so that the retention time of saikosaponin a is about 16 minutes.

**Time span of measurement** About 6 times as long as the retention time of saikosaponin a beginning after the solvent peak.

**System suitability**

Test for required detectability: Measure exactly 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of saikosaponin a obtained with 20 \( \mu L \) of this solution is equivalent to 3.5 to 6.5% of that with 20 \( \mu L \) of the standard solution.

**System performance** Dissolve 6 mg each of saikosaponin a for assay and saikosaponin b\(_2\) for assay in methanol to make 100 mL. When the procedure is run with 20 \( \mu L \) of the standard solution under the above operating conditions, saikosaponin a and saikosaponin b\(_2\) are eluted in this order with the resolution between these peaks being not less than 1.5.

**System repeatability** When the test is repeated 6 times with 20 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin a is not more than 1.0%.

**Saikosaponin a for component determination** See saikosaponin a for assay.

**Saikosaponin a for thin-layer chromatography** A white, crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 225–232°C (decomposition).

**Absorbance** \(<2.01>\) \( E_{1%}^{\text{cm}} \) (206 nm): 60–68 (15 mg, methanol, 200 mL). Previously dried in a desiccator (in vacuum, silica gel) for 24 hours.

**Purity** Related substances—Dissolve 1.0 mg of saikosaponin a for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with 10 \( \mu L \) of this solution as directed in the Identification (2) under Bupleurum Root: any spot other than the principal spot at the RF value of about 0.4 does not appear.

**Saikosaponin b\(_2\) for assay** C\(_{42}\)H\(_{68}\)O\(_{13}\) Saikosaponin b\(_2\) for thin-layer chromatography. It meets the following requirements.

**Purity** Related substances—Dissolve 5 mg in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of saikosaponin b\(_2\) and solvent obtained from the sample solution is not larger than the peak area of saikosaponin b\(_2\) from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Saireito Extract.

**Time span of measurement** About 6 times as long as the retention time of saikosaponin b\(_2\).

**System suitability**

Test for required detectability: To exactly 1 mL of the
standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of saikosaponin d obtained from 10 µL of this solution is equivalent to 3.5 to 6.5% of that from 10 µL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Saireito Extract.

**Saikosaponin b₂ for component determination** See saikosaponin b₂ for assay.

**Saikosaponin b₂ for thin-layer chromatography**

C₂₂H₄₀O₁₃ White crystals or crystalline powder. Freely soluble in ethanol (99.5), soluble in methanol, and practically insoluble in water. Melting point: about 240°C

Absorbance \( \leq 0.3 \) (252 nm): 35 – 424 [5 mg dried in a desiccator (in vacuum, silica gel) for 24 hours, methanol, 250 mL].

**Purity** Related substances—Dissolve 2 mg in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Proceed the test with 10 µL each of the sample solution and standard solution as directed in the Identification (1) under Saireito Extract: the spot other than the principal spot, having \( Rf \) value of about 0.3, obtained from the sample solution is not more intense than the spot from the standard solution.

**Saikosaponin d for assay** C₂₂H₄₀O₁₃ A white, crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 240°C.

Absorbance \( \leq 0.3 \) (206 nm): 63 – 71 (15 mg, methanol, 200 mL). Previously dried in a desiccator (in vacuum, silica gel) for 24 hours.

**Purity** Related substances—

(1) Dissolve 2.0 mg in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this as the standard solution. Proceed the test with 10 µL each of the sample solution and standard solution as directed in the Identification (1) under Saireito Extract: the spot other than the principal spot around \( Rf \) 0.4 is not larger and not more intense than the spot obtained from the sample solution.

(2) Dissolve 10 mg in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography \( < 2.01 \) according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than saikosaponin d and the solvent is not more than the peak area of saikosaponin d obtained with the standard solution.

Operating conditions

Detector, and column: Proceed as directed in the operating conditions in the Assay under Bupleurum Root.

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of saikosaponin d is about 13 minutes.

Time span of measurement: About 4 times as long as the retention time of saikosaponin d beginning after the solvent peak.

System suitability

Test for required detectability: Measure exactly 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of saikosaponin d obtained with 20 µL of this solution is equivalent to 3.5 to 6.5% of that with 20 µL of the standard solution.

System performance: Dissolve 6 mg each of saikosaponin d for assay and saikosaponin a for assay in methanol to make 100 mL. When the procedure is run with 20 µL of the standard solution under the above operating conditions, saikosaponin a and saikosaponin d are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin d is not more than 1.0%.

**Saikosaponin d for component determination** See saikosaponin d for assay.

**Salicylaldazine** C₁₄H₁₂N₂O₂ Dissolve 0.30 g of hydrazinium sulfate in 5 mL of water. To this solution add 1 mL of acetic acid (100) and 2 mL of a freshly prepared solution of salicylaldehyde in 2-propanol (1 in 5), shake well, and allow to stand until a yellow precipitate is produced. Extract with two 15 mL portions of dichloromethane, to the combined dichloromethane extracts add 5 g of anhydrous sodium sulfate, shake, decant or filter, and evaporate the dichloromethane in the supernatant liquid or filtrate. Dissolve the residue in a warmed mixture of toluene and methanol (3:2), and cool. Filter the crystals produced, and dry in a desiccator (in vacuum, silica gel) for 24 hours. It is a yellow, crystalline powder.

Melting point \( \leq 213 – 219°F \)

**Purity** Related substances—Dissolve 0.09 g of salicylaldehyde in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene to make exactly 100 mL, and perform the test with this solution as directed in the Purity (6) under Povidone: any spot other than the principal spot does not appear.

**Salicylaldehyde** HOC₆H₄CHO [K 8390, Special class]

**Salicylamide** C₅H₄NO₂ White crystals or crystalline powder, and it is odorless and tasteless. Very soluble in N,N-dimethylformamide, freely soluble in ethanol (95%), soluble in propylene glycol, sparingly soluble in diethyl ether, and slightly soluble in water and in chloroform. It dissolves in sodium hydroxide TS.

Melting point \( \leq 139 – 143°C \)

**Purity** Ammonium \( < 1.02 \)—Shake 1.0 g of salicylamide with 40 mL of water, and filter through filter paper previously washed well with water. Discard the first 10 mL of the filtrate, transfer the subsequent 20 mL to a Nessler tube, and add water to make 30 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: transfer 2.5 mL of Standard Ammonium Solution to a Nessler tube, and add water to make 30 mL.

Loss on drying \( \leq 0.40 \): not more than 0.5% (1 g, silica gel, 4 hours).

Residue on ignition \( \leq 0.44 \): not more than 0.1% (1 g).

Content: not less than 98.5%. Assay—Weigh accurately
about 0.2 g of salicylamide, previously dried, dissolve in 70 mL of N,N-Dimethylformamide, and titrate $<2.50$ with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination with a solution of 70 mL of N,N-Dimethylformamide in 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 13.71 mg of CH$_3$NO$_2$.

**Salicylic acid** HOC$_6$H$_4$COOH [K 8392, Special class]

**Salicylic acid for assay** HOC$_6$H$_4$COOH [K 8392, Special class]

**Salicylic acid TS** Dissolve 0.1 g of salicylic acid in 10 mL of sulfuric acid. Prepare before use.

**Santonin** C$_{15}$H$_{18}$O$_3$ [Same as the namesake monograph]

**Santonin for assay** [Same as the monograph Santonin. It contains not less than 99.0% of santonin (C$_{15}$H$_{18}$O$_3$).]

**Sarpogrelate hydrochloride** C$_{24}$H$_{31}$NO$_6$.HCl [Same as the namesake monograph]

**Schisandrin for thin-layer chromatography** C$_{24}$H$_{32}$O$_7$ [Same as the namesake monograph]

**Scopolamine hydrobromide** See scopolamine hydrobromide hydrate.

**Scopolamine hydrobromide for thin-layer chromatography** See scopolamine hydrobromide hydrate for thin-layer chromatography.

**Scopolamine hydrobromide hydrate** C$_{17}$H$_{21}$NO$_4$.HBr.3H$_2$O [Same as the namesake monograph]

**Scopolamine hydrobromide hydrate for thin-layer chromatography** C$_{17}$H$_{21}$NO$_4$.HBr.3H$_2$O [Same as the monograph Scopolamine Hydrobromide Hydrate. Proceed as directed in the Identification (3) under Opium Alkaloids and Atropine Injection: any spot other than the principal spot at the $R_f$ value of about 0.4 does not appear.]

**Scopolamine hydrobromide** See scopolamine hydrobromide hydrate.

**Scopolamine hydrobromide for thin-layer chromatography** See scopolamine hydrobromide hydrate for thin-layer chromatography.

**Scopolamine hydrobromide hydrate** C$_{17}$H$_{21}$NO$_4$.HBr.3H$_2$O [Same as the namesake monograph]

**Scopolamine hydrobromide hydrate for thin-layer chromatography** C$_{17}$H$_{21}$NO$_4$.HBr.3H$_2$O [Same as the monograph Scopolamine Hydrobromide Hydrate. Proceed as directed in the Identification (3) under Opium Alkaloids and Atropine Injection: any spot other than the principal spot at the $R_f$ value of about 0.7 does not appear.]

**Sea sand** A mixture of white, grey, brown or black grains, 0.3 to 1.0 mm in particle size.

**2nd Fluid for disintegration test** To 250 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 118 mL of 0.2 mol/L sodium hydroxide TS and water to make 1000 mL. It is clear and colorless, and has a pH about 6.8.

**2nd Fluid for dissolution test** A mixture of phosphate buffer solution, pH 6.8 and water (1:1).

**Selenious acid** H$_2$SeO$_3$ Colorless or white crystals. It is hygroscopic.

**Identification**—(1) Dissolve 0.2 g of selenious acid in 20 mL of water, and use this as the sample solution. To 10 mL of the sample solution add 2 mL of tin (II) chloride TS: a red precipitate is produced.

(2) To 10 mL of the sample solution obtained in (1) add 1 mL of diluted hydrochloric acid (2 in 3) and 1 mL of potassium iodide TS: a brown color is produced.

Preserve in a light-resistant tight container.

**Selenium** Se [K 8598, Special class]

**Selenium dioxide** SeO$_2$ White crystals or crystalline powder.

**Identification**—(1) To 10 mL of a solution of selenium dioxide (1 in 100) add 2 mL of tin (II) chloride TS: a red precipitate is produced.

(2) To 10 mL of a solution of selenium dioxide (1 in 100) add 1 mL of diluted hydrochloric acid (2 in 3) and 1 mL of potassium iodide TS: a brown color is produced.

**Content**: not less than 97.0%. Assay—Weigh accurately about 0.6 g of selenium dioxide, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution into an iodine bottle, add 80 mL of water, 3 g of potassium iodide and 5 mL of diluted hydrochloric acid (2 in 3), allow to stand at a dark place for 5 minutes, and titrate $<2.50$ with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS = 2.774 mg of SeO$_2$.

**Semicarbazide acetate TS** Place 2.5 g of semicarbazide hydrochloride, 2.5 g of anhydrous sodium acetate and 30 mL of methanol in a flask, heat on a water bath for 2 hours, cool to 20°C, and filter. To the filtrate add methanol to make 100 mL. Preserve in a cold place. Do not use the solution showing a yellow color.

**Semicarbazide hydrochloride** H$_2$NNHCONH$_2$.HCl White to light yellow crystals.

**Identification** (1) To 10 mL of a solution of semicarbazide hydrochloride (1 in 100) add 1 mL of silver nitrate TS: white precipitates appear.

(2) Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25$: it exhibits absorption at the wave numbers of about 3420 cm$^{-1}$, 3260 cm$^{-1}$, 2670 cm$^{-1}$, 1684 cm$^{-1}$, 1582 cm$^{-1}$, 1474 cm$^{-1}$, 1386 cm$^{-1}$, 1210 cm$^{-1}$, 1181 cm$^{-1}$, 770 cm$^{-1}$ and 719 cm$^{-1}$.

**Sennoside A for thin-layer chromatography** C$_{20}$H$_{18}$O$_{20}$ Yellow crystalline powder. Insoluble in water, in chloroform and in diethyl ether, and practically insoluble in methanol and in acetone. Melting point: 200 – 240°C (with decomposition).

**Purity** Related substances—Dissolve 1.0 mg of sennoside A for thin-layer chromatography in exactly 4 mL of a mixture of tetrahydrofuran and water (7:3), and perform the test with 80 µL of this solution as directed in the identification under Rhubarb: any spot other than the principal spot at the $R_f$ value of about 0.3 does not appear.

**L-Serine** C$_3$H$_7$NO$_3$ [K 9105, Special class]

**Sesamin for thin-layer chromatography** C$_{20}$H$_{18}$O$_6$
White crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Melting point** 2<sub>60</sub>: 122 - 124°C

**Identification** Determine the absorption spectrum of a solution of Sesame in methanol (3 in 200,000) as directed under Ultra violet-visible Spectrophotometry 2<sub>240</sub>**: it exhibits maxima between 235 nm and 239 nm and between 285 nm and 289 nm.

**Purity** Related substances—Dissolve 2.0 mg in 2 mL of methanol, and use this as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed in the Identification under Sesame: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**6]-Shogaol for assay** C<sub>17</sub>H<sub>24</sub>O<sub>3</sub> **[6]-Shogaol for thin-layer chromatography. It meets the following requirements. Absorbance 2<sub>240</sub>: E<sub>240</sub> (225 nm): 727 - 781 (5 mg, ethanol (99.5), 500 mL).**

**Purity** Related substances—Dissolve 5 mg of [6]-shogaol for assay in 10 mL of a mixture of acetonitrile and water (2:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (2:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2<sub>0.1</sub>** according to the following conditions. Determine the peak area of each solution by the automatic integration method: the total area of the peaks other than [6]-shogaol obtained form the sample solution is not larger than the peak area of [6]-shogaol from the standard solution.

**Operating conditions**
- Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions under Assay (2) of Mukoi-Daikenchuto Extract.
- Time span of measurement: 3 times as long as the retention time of [6]-shogaol, beginning after the solvent peak.

**System suitability**
- Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of acetonitrile and water (2:1) to make exactly 20 mL. Confirm that the peak area of [6]-shogaol obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.
- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol are not less than 5000 and not more than 1.5%, respectively.

**System repeatability:** When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-shogaol is not more than 1.5%.

**[6]-Shogaol for thin-layer chromatography** C<sub>17</sub>H<sub>24</sub>O<sub>3</sub> **A pale yellow oil. Miscible with methanol, ethanol (99.5) and with diethyl ether, and practically insoluble in water.**

**Purity** Related substances—Dissolve 1.0 mg of [6]-shogaol for thin-layer chromatography in 2 mL of methanol, and perform the test with this solution as directed under Thin-layer Chromatography 2<sub>60</sub>. Spot 10 μL on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: no spot other than the principal spot at around R<sub>f</sub> 0.5 appears.

**Silica gel** An amorphous, partly hydrated silicic acid occurring in glassy granules of various sizes. When used as a desiccant, it is frequently coated with a substance that changes color when the capacity to absorb water is exhausted. Such colored products may be regenerated by being heated at 110°C until the gel assumes the original color.

**Loss on ignition** 2<sub>43</sub>: not more than 5% (2 g, 950 ± 50°C).

**Water absorption:** not less than 31%. Weigh accurately about 10 g of silica gel, and allow to stand for 24 hours in a closed container in which the atmosphere is maintained at 80% relative humidity with sulfuric acid having a specific gravity of 1.19. Weigh again, and calculate the increase in mass.

**Siliceous earth** [K 8330, Diatomaceous earth, First class]

**Silicone oil** Colorless clear liquid, having no odor. **Viscosity** 2<sub>53</sub>: 50 - 100 mm²/s.

**Silicone resin** Light gray, half-clear, viscous liquid or a pasty material. It is almost odorless.

**Viscosity and refractive index**—Place 15 g of silicone resin in a Soxhlet extractor, then extract with 150 mL of carbon tetrachloride for 3 hours. The kinematic viscosity of the residual liquid, obtained by evaporating carbon tetrachloride from the extract on a water bath, is 100 to 1100 mm²/s (25°C). Its refractive index is 1.400 to 1.410 (25°C).

**Specific gravity** 2<sub>50</sub>: d<sub>25</sub> 0.98 – 1.02

**Loss on drying** 2<sub>43</sub>: 0.45 – 2.25 g with the extracted residue obtained in the Viscosity and refractive index (100°C, 1 hour).

**Silicic acid 26-water** SiO<sub>2</sub>·12WO<sub>3</sub>·26H<sub>2</sub>O
White to slightly yellowish, crystals. Deliquescent. Very soluble in water and in ethanol (95).

**Purity** Clarity and color of solution—a solution (1 in 20) is clear and colorless.

**Loss on ignition** 2<sub>43</sub>: 14 – 15% (2 g, dry at 110°C for 2 hours then 700 – 750°C, constant mass).

**Silver chromate-saturated potassium chromate TS** Dissolve 5 g of potassium chromate in 50 mL of water, add silver nitrate TS until a pale red precipitate is produced, and filter. To the filtrate add water to make 100 mL.

**Silver diethyldithiocarbamate** See silver N,N-diethyldithiocarbamate.

**Silver nitrate** AgNO<sub>3</sub> [K 8550, Special class]

**Silver nitrate-ammonia TS** Dissolve 1 g of silver nitrate in 20 mL of water, and add ammonia TS dropwise with stirring until the precipitate is almost entirely dissolved.

**Storage**—Preserve in tight, light-resistant containers.

**Silver nitrate TS** Dissolve 17.5 g of silver nitrate in water to make 1000 mL (0.1 mol/L). Preserve in light-resistant containers.

**Silver N,N-diethyldithiocarbamate** C<sub>10</sub>H<sub>16</sub>AgNS<sub>2</sub>
Sodium carbonate, anhydrous Na₂CO₃ [K 8625, Sodium carbonate, Special class]

Sodium carbonate dechydrate Na₂CO₃·10H₂O [K 8624, Special class]

Sodium carbonate for pH determination Na₂CO₃ [K 8625, for pH determination]

Sodium carbonate (standard reagent) Na₂CO₃ [K 8005, Standard reagent for volumetric analysis]

Sodium carbonate TS Dissolve 10.5 g of anhydrous sodium carbonate in water to make 100 mL (1 mol/L).

0.55 mol/L Sodium carbonate TS Dissolve 5.83 g of anhydrous sodium carbonate in water to make 100 mL.

Sodium chloride NaCl [K 8150, Special class]

Sodium chloride (standard reagent) NaCl [K 8005, Standard reagent for volumetric analysis]

Sodium chloride TS Dissolve 10 g of sodium chloride in water to make 100 mL.

0.1 mol/L Sodium chloride TS Dissolve 6 g of sodium chloride in water to make 1000 mL.

0.2 mol/L Sodium chloride TS Dissolve 11.7 g of sodium chloride in water to make 1000 mL.

1 mol/L Sodium chloride TS Dissolve 29.22 g of sodium chloride in water to make 500 mL.

Sodium citrate See sodium citrate hydrate.

Sodium citrate hydrate C₆H₅Na₃O₇·2H₂O [K 8288, Trisodium citrate dihydrate, or same as the namesake monograph]

Sodium cobaltinitrite See sodium hexanitrocobaltate (III).

Sodium cobaltinitrite TS See sodium hexanitrocobaltate (III) TS.

Sodium 1-decanesulfonate C₁₀H₂₁NaO₄ White powder.

Purity Clarity and color of solution—Dissolve 1.0 g in 20 mL of water: the solution is clear and colorless. Loss on drying <2.4%: not more than 3.0% (1 g, 105°C, 3 hours).

Content: not less than 98.0%. Assay—Weigh accurately 0.45 g of sodium 1-decanesulfonate, dissolve in 50 mL of water, and pass through a column, about 1.2 cm in inside diameter and about 25 cm in length, packed with about 20 mL of strongly acidic ion-exchange resin (0.3 to 1.0 mm, H type) at a flow rate of about 4 mL per minute. Wash with 150 mL of water at a flow rate of about 4 mL per minute. Combine the washing and the elute, and titrate <2.3% with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS = 0.4729 mg of NaBH₄

Sodium bromide NaBr [K 8514, Special class]

Sodium carbonate See sodium carbonate dechydrate.

Sodium carbonate, anhydrous Na₂CO₃ [K 8625, Sodium carbonate, Special class]

Sodium carbonate dechydrate Na₂CO₃·10H₂O [K 8624, Special class]

Sodium carbonate for pH determination Na₂CO₃ [K 8625, for pH determination]

Sodium carbonate (standard reagent) Na₂CO₃ [K 8005, Standard reagent for volumetric analysis]

Sodium carbonate TS Dissolve 10.5 g of anhydrous sodium carbonate in water to make 100 mL (1 mol/L).

0.55 mol/L Sodium carbonate TS Dissolve 5.83 g of anhydrous sodium carbonate in water to make 100 mL.

Sodium chloride NaCl [K 8150, Special class]

Sodium chloride (standard reagent) NaCl [K 8005, Standard reagent for volumetric analysis]

Sodium chloride TS Dissolve 10 g of sodium chloride in water to make 100 mL.

0.1 mol/L Sodium chloride TS Dissolve 6 g of sodium chloride in water to make 1000 mL.

0.2 mol/L Sodium chloride TS Dissolve 11.7 g of sodium chloride in water to make 1000 mL.

1 mol/L Sodium chloride TS Dissolve 29.22 g of sodium chloride in water to make 500 mL.

Sodium citrate See sodium citrate hydrate.

Sodium citrate hydrate C₆H₅Na₃O₇·2H₂O [K 8288, Trisodium citrate dihydrate, or same as the namesake monograph]

Sodium cobaltinitrite See sodium hexanitrocobaltate (III).

Sodium cobaltinitrite TS See sodium hexanitrocobaltate (III) TS.

Sodium 1-decanesulfonate C₁₀H₂₁NaO₄ White powder.

Purity Clarity and color of solution—Dissolve 1.0 g in 20 mL of water: the solution is clear and colorless. Loss on drying <2.4%: not more than 3.0% (1 g, 105°C, 3 hours).

Content: not less than 98.0%. Assay—Weigh accurately 0.45 g of sodium 1-decanesulfonate, dissolve in 50 mL of water, and pass through a column, about 1.2 cm in inside diameter and about 25 cm in length, packed with about 20 mL of strongly acidic ion-exchange resin (0.3 to 1.0 mm, H type) at a flow rate of about 4 mL per minute. Wash with 150 mL of water at a flow rate of about 4 mL per minute. Combine the washing and the elute, and titrate <2.3% with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS = 0.4729 mg of NaBH₄

Sodium bromide NaBr [K 8514, Special class]

Sodium carbonate See sodium carbonate dechydrate.
crystalline powder.

Identification—Determine the infrared absorption spectrum of sodium desoxycholate, previously dried, according to the potassium bromide disk method under Infrared Spectrophotometry $\leq 2.25\%$: it exhibits absorption at the wave numbers of about 3400 cm$^{-1}$, 2940 cm$^{-1}$, 1562 cm$^{-1}$ and 1408 cm$^{-1}$.

Purity Related substances—Dissolve 0.10 g of sodium desoxycholate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\leq 2.0\%$. Spot 10 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, methanol and acetic acid (100:80:40:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly concentrated sulfuric acid on the plate, and heat at 105°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Sodium 2,6-dichloroindophenol-sodium acetate TS Mix before use an equal volume of sodium 2,6-dichloroindophenol dehydrate solution (1 in 20) and acetic acid-sodium acetate TS, pH 7.0.

Sodium diethyldithiocarbamate See sodium $N,N$-diethyldithiocarbamate trihydrate.

Sodium $N,N$-diethyldithiocarbamate trihydrate $(C_2H_5)_2NCS_2Na_3H_2O$ [K 8454, Special class]

Sodium di-2-ethylhexyl sulfosuccinate $C_{18}H_{33}COOCH_2(C_9H_17COO)CHSO_3Na$ White or translucent white mucilaginous soft masses. Sparingly soluble in water.

Purity Clarity and color of solution—A solution prepared by dissolving 1.0 g in 100 mL of water is clear and colorless.

Loss on drying $\leq 2.4\%$: not more than 5.0% (1 g, 105°C, 2 hours).

Sodium dihydrogen phosphate See sodium dihydrogen phosphate dihydrate.

Sodium dihydrogen phosphate anhydrous $NaH_2PO_4$ A white, powder or crystalline powder. Freely soluble in water, and very slightly soluble in ethanol (99.5). It has a hygroscopic property.

A solution is acidic.

Sodium dihydrogen phosphate dihydrate $NaH_2PO_4\cdot2H_2O$ [K 9009, Special class]

Sodium dihydrogen phosphate TS, pH 2.2 Dissolve 1.56 g of sodium dihydrogen phosphate dihydrate in 800 mL of water, adjust the pH to 2.2 with phosphoric acid, and add water to make 1000 mL.

Sodium dihydrogen phosphate TS, pH 2.5 Dissolve 2.7 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid.

0.05 mol/L Sodium dihydrogen phosphate TS, pH 2.6 Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to exactly 2.6 and add water to make 1000 mL.

0.05 mol/L Sodium dihydrogen phosphate TS, pH 3.0 Dissolve 3.45 g of sodium dihydrogen phosphate dihydrate in 500 mL of water (solution A). Dilute 2.45 g of phosphoric acid with water to make 500 mL (solution B). To a volume of solution A add solution B until the mixture is adjusted to pH 3.0.

0.1 mol/L Sodium dihydrogen phosphate TS, pH 3.0 Dissolve 15.60 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 3.0 with phosphoric acid, and add water to make 1000 mL.

0.05 mol/L Sodium dihydrogen phosphate TS Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

0.1 mol/L Sodium dihydrogen phosphate TS Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 450 mL of water, adjust to a pH of 5.8 exactly with sodium hydroxide TS, and add water to make 500 mL.

2 mol/L Sodium dihydrogen phosphate TS Dissolve 312.02 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

Sodium disulfite $Na_2S_2O_5$ [K 8501, First class]

Sodium disulfite TS Dissolve 0.10 g of sodium disulfite in 10 mL of 1 mol/L hydrochloric acid TS, and add acetone to make 100 mL.

Sodium dithionite $Na_2S_2O_4$ A white to grayish white crystalline powder, having a strong irritating odor. It is decomposed with moisture or atmospheric oxygen.

Identification—(1) Dissolve 0.5 g of sodium dithionite in 50 mL of water, and use this as the sample solution. To 10 mL of the sample solution add 1 mL of copper (II) sulfate TS: a grayish brown color is produced.

(2) The sample solution obtained in (1) responds to the Qualitative Tests $\leq 1.09\%$ (1) for sodium salt. Preserve in a light-resistant tight container.

Sodium dodecylbenzene sulfonate $C_{18}H_{29}SO_3Na$ White, crystalline powder or mass. $\rho \geq 2.34\%$: The pH of a solution of 0.5 g of sodium dodecylbenzene sulfonate in 50 mL of freshly boiled and cooled water is between 5.0 and 7.0. Measure the pH at 25°C passing nitrogen with stirring.

Loss on drying $\leq 2.4\%$: not more than 0.5% (1 g, 105°C, 2 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 40 mg of sodium dodecylbenzene sulfonate, previously dried, and perform the test as directed in (4) Sulfur in the Procedure of determination under Oxygen Flask Combustion Method $\leq 1.06\%$, using a mixture of 20 mL of water and 2 mL of strong hydrogen peroxide water as absorbing solution.

Each mL of 0.01 mol/L barium perchlorate VS = 1.742 mg of $C_{18}H_{29}SO_3Na$

Sodium fluoride $NaF$ [K 8821, Special class]

Sodium fluoride (standard reagent) $NaF$ [K 8005, Standard reagent for volumetric analysis]

Sodium fluoride TS Dissolve 0.5 g of sodium fluoride in 100 mL of 0.1 mol/L hydrochloric acid TS. Prepare before use.
Sodium glycocholate for thin-layer chromatography

\( \text{C}_{26}\text{H}_{42}\text{NNaO}_{6}\cdot\text{xH}_{2}\text{O} \) White to pale brown crystalline powder or powder. Freely soluble in water and in methanol, and slightly soluble in ethanol (99.5). Melting point: about 260°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of sodium glycocholate for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25a), it exhibits absorption at the wave numbers of about 2940 cm\(^{-1}\), 1640 cm\(^{-1}\), 1545 cm\(^{-1}\), 1450 cm\(^{-1}\), 1210 cm\(^{-1}\), 1050 cm\(^{-1}\), and 600 cm\(^{-1}\).

Optical rotation \( <2.49^\circ \) [\( \alpha \text{D}^2 \)]: +25° to +35° (60 mg, methanol, 20 mL, 100 mm).

**Purity** Related substances—Dissolve 5 mg of sodium glycocholate for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03a). Proceed with 5 \( \mu \)L each of the sample solution and standard solution as directed in the Identification under Bear Bile: the spots other than the principal spot at the wave number of about 2940 cm\(^{-1}\) are not more intense than the spot from the standard solution more than about 0.2 obtained from the sample solution are not more intense than the spot from the standard solution.

**Sodium 1-heptane sulfonate** \( \text{C}_{7}\text{H}_{15}\text{NaO}_{3}\text{S} \) White, crystals or crystalline powder.

**Purity** Clarity and color of solution—Dissolve 1.0 g of sodium 1-heptane sulfonate in 10 mL of water: the solution is clear and colorless.

**Loss on drying** \( <2.41\% \): not more than 3.0% (1 g, 105°C, 3 hours).

**Content**: not less than 99.0%. Assay—Dissolve about 0.4 g of sodium 1-heptane sulfonate, previously dried and weighed accurately, in 50 mL of water, transfer to a chromatographic column, prepared by packing a chromatographic tube 9 mm in inside diameter and 160 mm in height with 10 mL of strongly acidic ion exchange resin for column chromatography (425 to 600 \( \mu \)m in particle diameter, \( \text{H}_2\text{O} \)), and flow at a flow rate of about 4 mL per minute. Wash the column at the same flow rate with 150 mL of type), and flow at a flow rate of about 4 mL per minute.

Each mL of 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS = 18.82 mg of \( \text{C}_{7}\text{H}_{15}\text{NaO}_{3}\text{S} \)

**Sodium hexanitrocobaltate (III) \( \text{Na}_3\text{Co(NO}_2\text{)}_6 \) [K 8347, Special class]**

**Sodium hexanitrocobaltate (III) TS** Dissolve 10 g of sodium hexanitrocobaltate (III) in water to make 50 mL, and filter if necessary. Prepare before use.

**Sodium hydrogen carbonate** \( \text{NaHCO}_3 \) [K 8622, Special class]

**Sodium hydrogen carbonate for pH determination** \( \text{NaHCO}_3 \) [K 8622, Sodium hydrogen carbonate, for pH determination]

**Sodium hydrogen carbonate TS** Dissolve 5.0 g of sodium hydrogen carbonate in water to make 100 mL.

7% Sodium hydrogen carbonate injection See 7% sodium bicarbonate injection.

**Sodium hydrogen sulfite** [K 8059, Special class]

**Sodium hydrogen sulfite TS** Dissolve 10 g of sodium hydrogen sulfite in water to make 30 mL. Prepare before use.

**Sodium hydrogen tartrate monohydrate** \( \text{NaHC}_4\text{H}_4\text{O}_6\cdot\text{H}_2\text{O} \) [K 8538, Sodium hydrogentartrate monohydrate, Special class]

**Sodium hydrogen tartrate TS** Dissolve 1 g of sodium bitartrate in water to make 10 mL (0.5 mol/L). Prepare before use.

**Sodium hydroxide** \( \text{NaOH} \) [K 8576, Special class]

**Sodium hydroxide-dioxane TS** Dissolve 0.80 g of sodium hydroxide in a mixture of 1,4-dioxane and water (3:1) to make 100 mL.

**Sodium hydroxide-methanol TS** Dissolve by thorough shaking 4 g of sodium hydroxide in methanol to make 100 mL. To the supernatant liquid obtained by centrifugation add methanol to make 500 mL. Prepare before use.

**Sodium hydroxide TS** Dissolve 4.3 g of sodium hydroxide in water to make 100 mL (1 mol/L). Preserve in polyethylene bottles.

**Sodium hydroxide TS, dilute** Dissolve 4.3 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare before use (0.1 mol/L).

0.01 mol/L Sodium hydroxide TS Dilute 10 mL of sodium hydroxide TS with water to make 1000 mL. Prepare before use.

0.05 mol/L Sodium hydroxide TS To 10 mL of 0.5 mol/L sodium hydroxide TS add water to make 100 mL.

0.2 mol/L Sodium hydroxide TS Dissolve 8.0 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare before use.

0.5 mol/L Sodium hydroxide TS Dissolve 22 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.
2 mol/L Sodium hydroxide TS  Dissolve 86 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

4 mol/L Sodium hydroxide TS  Dissolve 168 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

6 mol/L Sodium hydroxide TS  Dissolve 252 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

8 mol/L Sodium hydroxide TS  Dissolve 336 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

Sodium hypobromite TS  To 8 mL of bromine TS add 25 mL of water and 25 mL of sodium carbonate TS. Prepare before use.

Sodium hypochlorite-sodium hydroxide TS  To a volume of sodium hypochlorite TS for ammonium limit test, equivalent to 1.05 g of sodium hypochlorite (NaClO: 74.44), add 15 g of sodium hydroxide and water to make 1000 mL. Prepare before use.

Sodium hypoiodite sodium hydroxide TS  To 8 mL of iodine TS add 25 mL of water and 25 mL of sodium carbonate TS. Prepare before use.

Sodium hypochlorite TS  Prepare the solution by passing chlorine into sodium hydroxide or sodium carbonate solution, having the odor of chlorine.

Content: not less than 4.2 w/v% as sodium hypochlorite (NaClO: 74.44). Assay—Pipet 10 mL of sodium hypochlorite TS for ammonium limit test, and add water to make exactly 100 mL. Transfer exactly 10 mL of this solution to a glass-stoppered flask, add 90 mL of water, then add 2 g of potassium iodide and 6 mL of diluted acetic acid (1 in 2), stopper tightly, shake well, and allow to stand for 5 minutes in a dark place. Titrate <2.5d the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS = 3.722 mg of NaClO.

Sodium lauryl sulfate [Same as the namesake monograph]

Sodium lauryl sulfate TS  Dissolve 100 g of sodium lauryl sulfate in 900 mL of water, add 10 mL of 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

0.2% Sodium lauryl sulfate TS  Dissolve 0.1 g of sodium lauryl sulfate in 0.1 mol/L of pH 7.0 sodium phosphate buffer to make 50 mL.

Sodium metabisulfite  See sodium disulfite.

Sodium metabisulfite TS  See sodium disulfite TS.

Sodium, metallic Na  [K 8687, Sodium, Special class]

Sodium 1-methyl-1H-tetrazole-5-thiolate  See sodium 1-methyl-1H-tetrazole-5-thiolate dihydrate.

Sodium 1-methyl-1H-tetrazole-5-thiolate dihydrate  C₂H₃N₂NaS.2H₂O White, crystals or crystalline powder.

Melting point <2.60°: 90–94°C

Purity  Related substances—Dissolve 10 mg of sodium 1-methyl-1H-tetrazole-5-thiolate dihydrate in 10 mL of water, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography (2.68). Spot 5 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:2:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

Sodium molybdate  See sodium molybdate (VI) dihydrate.

Sodium molybdate (VI) dihydrate  Na₂MoO₄.2H₂O

[Sodium 2-naphthalenesulfonate  C₇H₆Na₂O₄S Pale brown, crystals or powder. Content: not less than 98.0%.

Sodium naphthoquinone sulfonate TS  Dissolve 0.25 g of sodium β-naphthoquinone sulfonate in methanol to make 100 mL.

Sodium β-naphthoquinone sulfonate  C₇H₆Na₂O₄S Yellow to orange-yellow, crystals or crystalline powder. Soluble in water, and practically insoluble in ethanol (95). Loss on drying <2.41°: Not more than 2.0% (1 g, in vacuum, 50°C).

Residue on ignition <2.44°: 26.5 – 28.0% (1 g, after drying).

Sodium nitrate  NaNO₃ [K 8562, Special class]

Sodium nitrite  NaNO₂ [K 8019, Special class]

Sodium nitrite TS  Dissolve 10 g of sodium nitrite in water to make 100 mL. Prepare before use.

Sodium nitroprusside  See sodium pentacyanonoitrosylferate (III) dihydrate.

Sodium nitroprusside TS  See sodium pentacyano-nitrosylferate (III) TS.

Sodium 1-nonanesulfonate  CH₃(CH₂)₈SO₃Na White crystalline powder. Freely soluble in water.

Loss on drying <2.41°: Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44°: 30 – 32% (0.5 g).

Sodium 1-octane sulfonate  CH₃(CH₂)₇SO₃Na White crystals or powder.

Residue on ignition <2.44°: 32.2 – 33.0% (1.0 g).

Sodium oxalate (standard reagent)  C₂O₄Na₂ [K 8005, Standard reagent for volumetric analysis]

Sodium pentacyanoammine ferrocate (II) n-hydrate  Na₃[Fe(CN)₅NH₃]·nH₂O A light yellow to light green-yellow crystalline powder.

Identification—(1) Dissolve 0.2 g of sodium pentacyanoammine ferrocate (II) n-hydrate in 5 mL of water, add 2 mL of sodium hydroxide solution (1 in 10), and heat: ammonia gas is evolved and a brown precipitate is produced.

(2) Dissolve 0.25 g of sodium pentacyanoammine ferro-
Sodium pentacyanotrisulfonate (III)-potassium hexacyanoferrate (III) TS

Mix an equal volume of a solution of sodium pentacyanotrisulfonate (III) dihydrate (in 50 mL) and a solution of potassium hexacyanoferrate (III) (1 in 100) and add water to make 25 mL, mix, and use after changing the color of the solution from a dark red to yellow. Prepare before use.

Sodium pentacyanotrisulfonate (III) dihydrate

\[ \text{Na}_2\text{Fe(CN)}_5\text{(NO)} \cdot 2\text{H}_2\text{O} \]

[Sodium perchlorate monohydrate] [K 8227, Special class]

\[ \text{NaClO}_3 \cdot \text{H}_2\text{O} \]

[Sodium perchlorate monohydrate] [K 8227, Special class]

[Sodium periodate] [K 8256, Special class]

\[ \text{NaIO}_4 \]

[Sodium periodate TS] Dissolve 60.0 g of sodium periodate in 120 mL of 0.05 mol/L sulfuric acid TS, and add water to make 1000 mL. If the solution is not clear, filter this through a glass-filter. Keep in a light-resistant vessel.

[Sodium peroxide] [NaO\(_2\)] [K 8231, Special class]

[Sodium p-phenol sulfonate] See sodium p-phenol sulfonate dihydrate.

Sodium p-phenol sulfonate dihydrate

C\(_6\)H\(_5\)O\(_4\)NaS\(_2\)H\(_2\)O

White to light yellow, crystals or crystalline powder, having a specific odor.

Identification

(1) To 10 mL of a solution of sodium p-phenol sulfonate (1 in 10) add 1 drop of iron (III) chloride TS: a purple color develops.

(2) Determine the absorption spectrum of a solution of sodium p-phenol sulfonate (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <\(2.24\)): it exhibits maxima between 269 nm and 273 nm and between 276 nm and 280 nm.

Purity

Clarity and color of solution—Dissolve 1.0 g of sodium p-phenol sulfonate in 25 mL of water: the solution is clear and colorless.

Content

Not less than 90.0%. Assay—Dissolve about 0.5 g of sodium p-phenol sulfonate, accurately weighed, in 50 mL of water. Transfer the solution to a chromatographic column, prepared by pouring strongly acidic ion exchange resin (H type) for column chromatography (150 to 300 \(\mu\)m in particle diameter) into a chromatographic tube about 1 cm in inside diameter and about 30 cm in height, and allow to flow. Wash the chromatographic column with water until the washing is no longer acidic, combine the washings with the above effluent solution, and titrate <\(2.50\) with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of bromocresol green-methyl red TS). Separately, dissolve 0.5 g of sodium p-phenol sulfonate, previously weighed accurately, in 50 mL of water and titrate with 0.1 mol/L sodium hydroxide VS, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 23.22 mg of C\(_6\)H\(_5\)O\(_4\)NaS\(_2\)H\(_2\)O

[Sodium phosphate] See trisodium phosphate dodecahydrate.

Sodium phosphate TS

Dissolve 5.68 g of disodium hydrogen phosphate and 6.24 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

0.1 mol/L Sodium phosphate buffer solution, pH 7.0

Dissolve 17.9 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL. Add to this solution to a 500 mL solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate in water until the pH becomes 7.0.

[Sodium pyruvate] Prepared for microbial test.

[Sodium salicylate] HOC\(_6\)H\(_4\)COONa [K 8397, Special class]

[Sodium salicylate-sodium hydroxide TS] Dissolve 1 g of sodium salicylate in 0.01 mol/L sodium hydroxide VS to make 100 mL.

[Sodium selenite] Na\(_2\)SeO\(_3\) A white crystalline powder.

Identification—(1) Dissolve 1 g of sodium selenite in 100 mL of water, and use this as the sample solution. To 10 mL of the sample solution add 2 mL of tin (II) chloride TS: a red precipitate is produced.

(2) The sample solution obtained in (1) responds to the Qualitative Tests <\(1.09\) (1) for sodium salt. Preserve in a light-resistant tight container.

[Sodium sulfate] See sodium sulfate monohydrate.
Sodium p-styrenesulfonate \( \text{C}_8\text{H}_8\text{NaO}_3\text{S} \) White crystals or crystalline powder. Freely soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Recrystallize from diluted ethanol (1 in 2), and dry in vacuum.

\textbf{Identification}—Determine the infrared absorption spectrum of sodium p-styrenesulfonate according to the potassium bromide disk method under Infrared Spectrophotometry \( <2.25> \); it exhibits absorption at the wave numbers of about 1236 cm\(^{-1} \), 1192 cm\(^{-1} \), 1136 cm\(^{-1} \), 1052 cm\(^{-1} \), 844 cm\(^{-1} \), and 688 cm\(^{-1} \).

\textbf{Optical rotation} \( <2.49> \) \( [\alpha]_D^10 + 40 - 50^o \) (40 mg, methanol, 20 mL, 100 mm).

\textbf{Purity}—Perform the test with 10 \( \mu \)L of a solution of sodium p-styrenesulfonate (1 in 1000) as directed in the Assay under Panipenem: Any obtrusive peaks for determination of panipenem are not observed.

\textbf{Sodium sulfate} See sodium sulfate decahydrate.

\textbf{Sodium sulfate, anhydrous} \( \text{Na}_2\text{SO}_4 \) [K 8987, Special class]

\textbf{Sodium sulfate decahydrate} \( \text{Na}_2\text{SO}_4.10\text{H}_2\text{O} \) [K 8986, Special class]

\textbf{Sodium sulfite} See sodium sulfite enneahydrate.

\textbf{Sodium sulfite enneahydrate} \( \text{Na}_2\text{S}_9\text{H}_2\text{O} \) [K 8949, Special class]

\textbf{Sodium sulfite TS} Dissolve 5 g of sodium sulfite enneahydrate in a mixture of 10 mL of water and 30 mL of glycerin. Or dissolve 5 g of sodium hydroxide in a mixture of 30 mL of water and 90 mL of glycerin, saturate a half volume of this solution with hydrogen sulfide, while cooling, and mix with the remaining half. Preserve in well-filled, light-resistant bottles. Use within 3 months.

\textbf{Sodium sulfite} See sodium sulfite heptahydrate.

\textbf{Sodium sulfite, anhydrous} \( \text{Na}_2\text{SO}_3 \) [K 8061, Sodium sulfite, Special class]

\textbf{Sodium sulfite heptahydrate} \( \text{Na}_2\text{SO}_3.7\text{H}_2\text{O} \) [K 8060, Special class]

\textbf{1 mol/L Sodium sulfite TS} Dissolve 1.26 g of anhydrous sodium sulfite in water to make 10 mL.

\textbf{Sodium-sulfite-sodium dihydrogen phosphate TS} Mix 1.5 mL of a solution dissolved 1.26 g of anhydrous sodium sulfite in 100 mL of water and 98.5 mL of a solution dissolved 1.56 g of sodium dihydrogen phosphate dihydrate in 100 mL of water. Prepare before use.

\textbf{Sodium tartrate} See sodium tartrate dihydrate.

\textbf{Sodium tartrate dihydrate} \( \text{C}_6\text{H}_4\text{Na}_2\text{O}_6.2\text{H}_2\text{O} \) [K 8540, Special class]

\textbf{Sodium toluenesulfonamide} \( \text{C}_8\text{H}_8\text{ClNNaO}_2\text{S.3H}_2\text{O} \) White. A white powder, or crystalline powder. Freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5).

\textbf{Identification}—Determine the infrared absorption spectrum of sodium toluenesulfonamide for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25> \); it exhibits the absorption at the wave numbers of about 2940 cm\(^{-1} \), 1600 cm\(^{-1} \), 1410 cm\(^{-1} \), 1305 cm\(^{-1} \), 1195 cm\(^{-1} \), 1080 cm\(^{-1} \), 1045 cm\(^{-1} \), 980 cm\(^{-1} \), 950 cm\(^{-1} \), 910 cm\(^{-1} \), and 860 cm\(^{-1} \).

\textbf{Optical rotation} \( <2.49> \) \( [\alpha]_D^10 + 40 - 50^o \) (40 mg, methanol, 20 mL, 100 mm).

\textbf{Purity}—Perform the test with 10 \( \mu \)L of sodium toluenesulfonamide for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.03> \). Perform the test with 5 \( \mu \)L each of the sample solution and standard solution as directed in the Identification under Bear Bile: the spots other than the principal spot at the RI value of about 0.2 obtained from the sample solution are not more intense than the spot from the standard solution.

\textbf{Sodium tetraborate-calcium chloride buffer solution, pH 8.0} Dissolve 0.572 g of sodium tetraborate decahydrate and 2.94 g of calcium chloride dihydrate in 800 mL of freshly boiled and cooled water, adjust the pH to 8.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

\textbf{Sodium tetraborate decahydrate} \( \text{Na}_2\text{B}_2\text{O}_5.10\text{H}_2\text{O} \) [K 8866, for pH standard solution]

\textbf{Sodium tetraborate-sulfuric acid TS} To 9.5 g of sodium tetraborate decahydrate add 1000 mL of purified sulfuric acid, and dissolve by shaking for a night.

\textbf{Sodium tetraphenylborate} \( (\text{C}_6\text{H}_5)_2\text{B} \) [K 9521]

\textbf{Sodium thioglycolate} \( \text{HSCH}_2\text{COONa} \) A white powder, having a characteristic odor.

\textbf{Identification} (1) To a solution (1 in 10) add 1 drop each of ammonia solution (28) and iron (III) chloride TS: a dark red-purple color appears.

(2) Perform the test as directed under Flame Coloration Test (1) \( <1.0> \): a yellow color appears.

\textbf{Purity} Clarity and color of solution—Dissolve 1 g in 10 mL of water: the solution is clear and colorless.

\textbf{Sodium thiosulfate} See sodium thiosulfate pentahydrate.

\textbf{Sodium thiosulfate pentahydrate} \( \text{Na}_2\text{S}_2\text{O}_3.5\text{H}_2\text{O} \) [K 8637, Special class]

\textbf{Sodium thiosulfate TS} Dissolve 26 g of sodium thiosulfate pentahydrate and 0.2 g of anhydrous sodium carbonate in freshly boiled and cooled water to make 1000 mL (0.1 mol/L).

\textbf{Sodium toluenesulfonchloramide trihydrate} \( \text{C}_8\text{H}_8\text{ClNNaO}_2\text{S.3H}_2\text{O} \) [K 8318, Sodium p-toluenesulfonchloramide trihydrate, Special class]

\textbf{Sodium toluenesulfonchloramide TS} Dissolve 1 g of sodium toluenesulfonchloramide trihydrate in water to make 100 mL. Prepare before use.

\textbf{Sodium tridecanesulfonate} \( \text{C}_{13}\text{H}_{27}\text{SO}_3\text{Na} \) White, crystalline powder or powder.

\textbf{Purity}—Perform the test as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \): the absorbances at 230 nm and 245 nm are not more than the standard absorption.
0.05 and 0.01, respectively.

**Sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy**  
(CH$_3$)$_3$SiCH$_2$CH$_2$SO$_3$Na  
Prepared for nuclear magnetic resonance spectroscopy.

**Sodium 3-trimethylsilylpropionate-d$_4$ for nuclear magnetic resonance spectroscopy**  
(CH$_3$)$_3$SiCD$_2$CD$_2$COONa  
Prepared for nuclear magnetic resonance spectroscopy.

**Sodium 2,4,6-trinitrobenzenesulfonate dihydrate**  
C$_6$H$_2$N$_3$NaO$_9$S.2H$_2$O  
White or pale yellowish crystals or powder.

**Sodium tungstate**  
See sodium tungstate (VI) dihydrate.

**Sodium tungstate (VI) dihydrate**  
Na$_2$WO$_4$.2H$_2$O  
[K 8612, Special class]

**Sodium valproate for assay**  
C$_8$H$_{15}$NaO$_2$  
[Same as the monograph Sodium Valproate. When dried, it contains not less than 99.0% of sodium valproate (C$_8$H$_{15}$NaO$_2$).]

**Solid plates**  
Dilute anti-E. coli protein antibody stock solution by adding 0.2 mol/L Tris hydrochloride buffer, pH 7.4, to a concentration of about 0.02 mg/mL. Add exactly 0.1 mL of this solution to each well in the microplates, cover with plate seal, and then shake gently. Centrifuge for 2 minutes if some solution sticks to the top of the microplate or elsewhere. Dissolve 0.5 g of bovine serum albumin in 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS (pH 7.4) to make the wash solution. After leaving the microplates for 16 to 24 hours at a constant temperature of about 25°C, remove the solution in each well by aspiration, add 0.25 mL of the wash solution, shake gently, and then remove this solution by aspiration. Repeat this procedure 2 more times for each well using 0.25 mL of the wash solution. Add 0.25 mL of the block buffer solution to each well, gently shake, and let stand for 16 to 24 hours at a constant temperature of about 25°C to make solid plates. When using, remove the solution from the wells by aspiration, add 0.25 mL of the wash solution to each well, shake gently, and then remove this solution by aspiration. Repeat this procedure 2 more times using 0.25 mL of the wash solution.

**Soluble starch**  
See starch, soluble.

**Soluble starch TS**  
Triturate 1 g of soluble starch in 10 mL of cooled water, pour gradually into 90 mL of boiled water while constantly stirring, boil gently for 3 minutes, and cool. Prepare before use.

**Sorbitan sesquioleate**  
[Same as the namesake monograph]

**d-Sorbitol**  
[Same as the namesake monograph]

**d-Sorbitol for gas chromatography**  
Prepared for gas chromatography.

**Soybean-casein digest medium**  
See Sterility Test 4.06.

**Soybean oil**  
[Same as the namesake monograph]

**Soybean peptone**  
See peptone, soybean.

**Stacking gel for celmoleukin**  
In 0.5 mol/L Tris buffer solution, pH 6.8, prepare stacking the gel using ammonium persulfate and N,N',N''-tetramethyldiethylenetriamine so the acrylamide concentration is 5.2% and the sodium lauryl sulfate concentration is 0.1%.

**Stannous chloride**  
See tin (II) chloride dihydrate.

**Stannous chloride-sulfuric acid TS**  
See tin (II) chloride-sulfuric acid TS.

**Stannous chloride TS**  
See tin (II) chloride TS.

**Stannous chloride TS, acidic**  
See tin (II) chloride TS, acidic.

**Starch**  
[K 8658, Special class]

**Starch-sodium chloride TS**  
Saturate starch TS with sodium chloride. Use within 5 to 6 days.

**Starch, soluble**  
[K 8659, Special class]

**Starch TS**  
Triturate 1 g of starch with 10 mL of cold water, and pour the mixture slowly, with constant stirring, into 200 mL of boiling water. Boil the mixture until a thin, translucent fluid is obtained. Allow to settle, and use the supernatant liquid. Prepare before use.

**Stearic acid for gas chromatography**  
C$_{18}$H$_{36}$O$_2$  
[K 8855, Special class]

**Stearyl alcohol**  
[Same as the namesake monograph]

**Sterile purified water**  
[Same as the monograph Sterile Purified Water in Containers. It is not necessary to confirm if they meet all of the requirement, provided that they are confirmed to be suitable for the purpose of the relevant test.]

**Strong ammonia water**  
See ammonia solution (28).

**Strong cupric acetate TS**  
See copper (II) acetate TS, strong.

**Strong hydrogen peroxide water**  
See hydrogen peroxide (30).

**Strongly acidic ion exchange resin**  
Contains strong acid ion exchange residues. Particle diameter is about 100 μm.

**Strongly basic ion exchange resin**  
Contains strong basic ion exchange residues. Particle diameter is about 100 μm.

**Strontium TS**  
Dissolve 76.5 g of strontium chloride in 100 mL of the mobile phase and use this solution, add the mobile phase to make exactly 100 mL, and use 100 mL of this solution.

**Strontium chloride**  
See strontium chloride hexahydrate.

**Strontium chloride hexahydrate**  
SrCl$_2$.6H$_2$O  
[K 8132, Special class]

**Strychnine nitrate for assay**  
C$_7$H$_{12}$N$_2$O$_2$.HNO$_3$  
To 1 g of strychnine nitrate add 14 mL of water and about 10 mg of active carbon, heat in a water bath for 10 minutes, filter while hot, cool the filtrate quickly to form crystals, and filter the crystals. Add 8 mL of water to the crystals, dissolve by heating in a water bath, filter while hot, cool quickly, and filter the crystals formed. Repeat this procedure with 8 mL of water, and dry the crystals in a desiccator (in vacuum, silica gel) for 24 hours. Colorless or white crystals or crystalline powdered. Sparingly soluble in water, in glycerin and in chloroform, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.  

**Purity**  
Related substances—Dissolve 35 mg of strychnine nitrate for assay in 100 mL of the mobile phase and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and
use this solution as the standard solution (1). Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions. Measure each peak area of these solutions by the automatic integration method: the total area of the peaks other than strychnine from the sample solution is not larger than the peak area of strychnine from the standard solution (1).

Operating conditions
Proceed the operating conditions in the Assay under Nux Vomica except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 40 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of strychnine obtained from 20 \(\mu\)L of the standard solution (2) can be measured by the automatic integration method and the peak height of strychnine from 20 \(\mu\)L of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of strychnine beginning after the solvent peak.

Loss on drying <2.4I>: not more than 0.5% (0.2 g, 105°C, 3 hours).

Content: not less than 99.0% calculated on the dried basis. Assay—Dissolve about 0.5 g of strychnine nitrate for assay, accurately weighed, in 40 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), heat if necessary, cool, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 39.74 mg of \(C_21H_22N_2O_2\cdotHNO_3\)

**Styrene** \(C_2H_4\) Colorless, clear liquid.

**Specific gravity** <2.56>: \(d: 0.902 – 0.910\)

**Purity**—Perform the test with 1 \(\mu\)L of styrene as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of styrene by the area percentage method: it shows the purity of not less than 99%.

Operating conditions
Detector: Thermal conductivity detector.
Column: A glass column, about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth (180 to 250 \(\mu\)m in particle diameter) coated with polyethylene glycol 20 M at the ratio of 10%.

Column temperature: A constant temperature of about 100°C.

Temperature of sample vaporization chamber: A constant temperature of about 150°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of styrene is about 10 minutes.

Time span of measurement: About twice as long as the retention time of styrene, beginning after the solvent peak.

**Styrene-maleic acid alternating copolymer partial butyl ester** A copolymer of styrene and maleic anhydride, polymerized using cumene as solvent and added 1-butanol or water to the maleic anhydride groups. Average molecular mass: about 1600. A white or pale yellowish white powder.

**Identification**—Dissolve 5 mg of the substance to be examined in sodium hydrogencarbonate solution (1 in 15) to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits a maximum between 256 nm and 260 nm, and a shoulder between 251 nm and 256 nm.

Absorbance <2.24> \(E_{1%}(258\text{ nm})\): 6.3 – 7.3 [5 mg calculated on the anhydrous basis, sodium hydrogencarbonate solution (1 in 15), 10 mL]

**Purity**—Proceed as directed in the Purity (3) under Zinostatin Stimalamer, with the exception of without using of (ii) Standard solution, and changing (iv) Sample solution, (v) Procedure and (vii) Determination as follows:

(iv) Sample solution Dissolve 3.0 mg of the substance to be examined in the buffer solution for sample to make 20 mL.

(v) Procedure Mount the gel in the electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the top reservoir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100 \(\mu\)L of the sample solution onto the surface of the gel, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as a bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached at 5 cm from the upper end of the gel.

(vii) Determination Determine the peak area, \(A_f\), of styrene-maleic acid alternating copolymer partial butyl ester and the total area, \(A\), of the peaks other than styrene-maleic acid alternating copolymer partial butyl ester, based on the absorbance at 600 nm of the gel determined by using a densitometer. Calculate the amount of styrene-maleic acid alternating copolymer partial butyl ester by the following formula: not less than 98.0%.

Amount (%) of styrene-maleic acid alternating copolymer partial butyl ester

\[
= \frac{A_f/(A_f + A)}{100}
\]

**Water <2.4H>:** Not more than 10.0% (10 mg, coulometric titration).

**Substrate buffer for celmoleukin** Dissolve 32.4 g of tripotassium citrate monohydrate in water to make 1000 mL, and add 1 mol/L citric acid TS for buffer solution to adjust the pH to 5.5. To 100 mL of this solution add and dissolve 0.44 g of o-phenylenediamine and then 60 \(\mu\)L of hydrogen peroxide (30). Prepare at the time of use.

**Substrate TS for lysozyme hydrochloride** To a suitable amount of dried cells of Micrococcus luteus add a suitable amount of phosphate buffer solution, pH 6.2, gently shake to make a suspension, and add the substrate cells or the same buffer solution so that the absorbance of the suspension at 640 nm is about 0.65. Prepare before use.

**Substrate TS for peroxidase determination** Dissolve 0.195 mL of hydrogen peroxidase (30), 8.38 g of disodium hydrogen phosphate dodecahydrate and 1.41 g of citric acid monohydrate in water to make 300 mL. To 15 mL of this solution add 13 mg of o-phenylenediamine dihydrochloride before use.

**Substrate TS for kallidinogenase assay (1)** Dissolve an
appropriate amount of H-D-valyl-L-leucyl-L-arginine \( p \)-nitroanilide dihydrochloride in 0.1 mol/L tris buffer solution, pH 8.0 to prepare a solution containing 1 mg of H-D-valyl-L-leucyl-L-arginine \( p \)-nitroanilide dihydrochloride in 5 mL.

**Substrate TS for kallidinogenase assay (2)** Dissolve 17.7 mg of \( N \)-\( \alpha \)-benzoyl-L-arginine ethyl ester hydrochloride in 0.1 mol/L tris buffer solution, pH 8.0 to make 100 mL.

**Substrate TS for kallidinogenase assay (3)** Suspend 0.6 g of milk casein purified by the Hammerstein's method in 80 mL of 0.05 mol/L sodium hydrogen phosphate TS, and dissolve by warming at 65°C for 20 minutes. After cooling, adjust to pH 8.0 with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 100 mL. Prepare before use.

**Substrate TS for kallidinogenase assay (4)** Dissolve 25 mg of H-D-valyl-L-leucyl-L-arginine-4-nitroanilide dihydrochloride in 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 \( \mu \)L each of these solutions as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_3 \), of the peak area of sulbactam to that of the internal standard.

\[
\text{Amount [mg (potency)] of sulbactam (C}_8\text{H}_{11}\text{NO}_5\text{S)} = M_S \times \frac{Q_1}{Q_3} \times 1000
\]

\( M_S \): amount [mg (potency)] of Sulbactam RS

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (7 in 1000).

**Operating conditions**
- **Detector:** Ultraviolet absorption photometer (wavelength: 220 nm)
- **Column:** A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 \( \mu \)m in particle diameter).
- **Column temperature:** A constant temperature of about 35°C.
- **Mobile phase:** To 750 mL of 0.005 mol/L tetrabutylammonium hydroxide TS add 250 mL of acetonitrile for liquid chromatography.
- **Flow rate:** Adjust the flow rate so that the retention time of sulbactam is about 6 minutes.

**System suitability**
- **System performance:** When the procedure is run with 10 \( \mu \)L of the standard solution according to the above operating conditions, sulbactam and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.
- **System repeatability:** When the test is repeated 6 times with 10 \( \mu \)L of the standard solution according to the above operating conditions, the relative standard deviation of the peak areas of sulbactam is not more than 2.0%.

**Sulfamic acid (standard reagent)** See amido sulfuric acid (standard reagent).

**Sulfanilamide** \( \text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2 \) [K 9066, Special class]

**Sulfanilamide for titration of diazotization** \( \text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2 \) [K 9066, For titration of diazotization]

**Sulfanilic acid** \( \text{H}_2\text{NC}_6\text{H}_4\text{SO}_3\text{H} \) [K 8586, Special class]

**Sulfathiazole** \( \text{C}_9\text{H}_9\text{N}_3\text{O}_3\text{S}_2 \) White crystalline powder.

**Melting point** 205–209°C

**Sulfathiazole for liquid chromatography** See 5-sulfosalicylic acid dihydrate.

**5-Sulfosalicylic acid dihydrate** \( \text{C}_7\text{H}_6\text{O}_6\text{S}.2\text{H}_2\text{O} \) [K 859, Special class]

**Sulfasalazine TS** Dissolve 5 g of 5-sulfosalicylic acid dihydrate in water to make 100 mL.

**Sulfur S** [K 8088, Special class]

**Sulfur dioxide** \( \text{SO}_2 \) Prepare by adding sulfuric acid dropwise to a concentrated solution of sodium bisulfite. Colorless gas, having a characteristic odor.

**Sulfuric acid** \( \text{H}_2\text{SO}_4 \) [K 895, Special class]

**Sulfuric acid, dilute** Cautiously add 5.7 mL of sulfuric acid to 10 mL of water, cool, and dilute with water to make
100 mL (10%).

**Sulfuric acid-ethanol TS** With stirring, add slowly 3 mL of sulfuric acid to 1000 mL of ethanol (99.5), and cool.

**Sulfuric acid for readily carbonizable substances** To sulfuric acid, the content of which has previously been determined by the following method, add water cautiously, and adjust the final concentration to 94.5% to 95.5% of sulfuric acid (H₂SO₄). When the concentration is changed owing to absorption of water during storage, prepare freshly.

*Assay—Weigh accurately about 2 g of sulfuric acid in a glass-stoppered flask rapidly, add 30 mL of water, cool, and titrate 0.250 M solution with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of bromothymol blue TS).

Each mL of 1 mol/L sodium hydroxide VS = 49.04 mg of H₂SO₄

**Sulfuric acid, fuming** H₂SO₄·nSO₃ [K 8741, Special class]

**Sulfuric acid-hexane-methanol TS** To 230 mL of a mixture of hexane and methanol (1:3) add cautiously 2 mL of sulfuric acid.

**Sulfuric acid-methanol TS** Prepare carefully by adding 60 mL of sulfuric acid to 40 mL of methanol.

**Sulfuric acid-methanol TS, 0.05 mol/L** Add gradually 3 mL of sulfuric acid to 1000 mL of methanol, while stirring, and allow to cool.

**Sulfuric acid-monobasic sodium phosphate TS** See sulfuric acid-sodium dihydrogenphosphate TS.

**Sulfuric acid, purified** Place sulfuric acid in a beaker, heat until white fumes are evolved, then heat for 3 minutes cautiously and gently. Use after cooling.

**Sulfuric acid-sodium dihydrogenphosphate TS** Add 6.8 mL of sulfuric acid to 500 mL of water, then dissolve 50 g of sodium dihydrogenphosphate dihydrate in this solution, and add water to make 1000 mL.

**Sulfuric acid-sodium hydroxide TS** With stirring add slowly 120 mL of sulfuric acid to 1000 mL of water, and cool (solution A). Dissolve 88.0 g of sodium hydroxide in 1000 mL of freshly boiled and cooled water (solution B). Mix equal volumes of solution A and solution B.

**Sulfuric acid TS** Cautiously add 1 volume of sulfuric acid to 2 volumes of water, and while warming on a water bath add dropwise potassium permanganate TS until a pale red color of the solution remains.

**0.05 mol/L sulfuric acid TS** Dilute 100 mL of 0.5 mol/L sulfuric acid TS with water to make 1000 mL.

**0.25 mol/L sulfuric acid TS** With stirring, add slowly 15 mL of sulfuric acid to 1000 mL of water, then cool.

**0.5 mol/L sulfuric acid TS** With stirring, add slowly 30 mL of sulfuric acid to 1000 mL of water, then cool.

**1 mol/L sulfuric acid TS** Add 60 mL of sulfuric acid in 1000 mL of water slowly with stirring, then allow to cool.

**2 mol/L sulfuric acid TS** To 1000 mL of water add gradually 120 mL of sulfuric acid with stirring, and cool.

**5 mol/L sulfuric acid TS** Add 300 mL of sulfuric acid in 1000 mL of water slowly with stirring, then allow to cool.

**Sulfurous acid** See sulfurous acid solution.

**Sulfurous acid solution** A clear and colorless liquid containing more than 5% of SO₂, having a pungent odor. Specific gravity: about 1.03 g/mL.

*Identification—To 1 mL of iodine TS add 20 mL of water, and add 1 mL of sulfuric acid solution: the color of the solution disappears, and this solution forms a white precipitate upon addition of 1 mL of barium chloride TS.

Preserve at a cold place.

**Sulpiride for assay** C₁₅H₂₃N₃O₄S [Same as the monograph Sulpiride. When dried, it contains not less than 99.0% of sulpiride (C₁₅H₂₃N₃O₄S).]

**Sulpyrine** See sulpyrine hydrate.

**Sulpyrine for assay** See sulpyrine hydrate for assay.

**Sulpyrine hydrate** C₁₅H₂₃N₃O₄S·H₂O [Same as the namesake monograph]

**Sulpyrine hydrate for assay** C₁₅H₂₃N₃O₄S·H₂O [Same as the monograph Sulpyrine Hydrate. Calculated on the dried basis, it contains not less than 99.0% of sulpyrine (C₁₅H₂₃N₃O₄S).]

**Suxamethonium chloride for thin-layer chromatography** See suxamethonium chloride hydrate for thin-layer chromatography.

**Suxamethonium chloride hydrate for thin-layer chromatography** C₁₄H₂₉Cl₂N₂O₄·2H₂O [Same as the monograph Suxamethonium Chloride Hydrate]

**Swertia herb** [Same as the namesake monograph]

**Swertiamarin for thin-layer chromatography** C₁₆H₂₂O₁₀ White, practically tasteless powder. Melting point <2.60: 113 – 114°C

**Purity** Related substances—Dissolve 2.0 mg of swertiamarin for thin-layer chromatography in exactly 1 mL of ethanol (95), and perform the test with 20 μL of this solution as directed in the Identification under Swertia Herb: any spot other than the principal spot at the Rf value of about 0.5 does not appear.

**Synthetic zeolite for drying** A mixture of 6(Na₂O)·6(Al₂O₃)·12(SiO₂) and 6(K₂O)·6(Al₂O₃)·12(SiO₂) prepared for drying. Usually, use the spherically molded form, 2 mm in diameter, prepared by adding a binder. White to grayish white, or color transition by adsorbing water. Average fine pore diameter is about 0.3 nm, and the surface area is 500 to 700 m² per g. Loss on ignition <2.45: not more than 2.0% [2 g, 550 – 600°C, 4 hours, allow to stand in a desiccator (phosphorus (V) oxide).]

**Talc** [Same as the namesake monograph]

**Tamsulosin hydrochloride** C₂₀H₂₈N₂O₅S·HCl [Same as the namesake monograph]

**Tamsulosin hydrochloride for assay** C₂₀H₂₈N₂O₅S·HCl [Same as the monograph Tamsulosin Hydrochloride. When dried, it contains not less than 99.0% of tamsulosin hydrochloride (C₂₀H₂₈N₂O₅S·HCl).]

**Tannic acid** [Same as the namesake monograph]
Tannic acid TS Dissolve 1 g of tannic acid in 1 mL of ethanol (95), and add water to make 10 mL. Prepare before use.

Tartaric acid See L-tartaric acid.

L-Tartaric acid C₄H₆O₆ [K 8532, l(+)-Tartaric acid, Special class].

Tartrate buffer solution, pH 3.0 Dissolve 1.5 g of L-tartaric acid and 2.3 g of sodium tartarate dihydrate in water to make 1000 mL.

Taurine H₂NCH₂CH₂SO₃H White crystals or crystalline powder.

Contents: not less than 95.0%. Assay—Weigh accurately about 0.2 g, dissolve in 50 mL of water, add 5 mL of formaldehyde solution, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 12.52 mg of C₂H₇NO₃S

Temocapril hydrochloride for assay C₂₂H₃₁O₄S₂.HCl [Same as the monograph Temocapril Hydrochloride. It contains not less than 99.5% of temocapril hydrochloride (C₂₂H₃₁O₄S₂.HCl: 513.07), calculated on the anhydrous basis.]

Terbinafine hydrochloride for assay C₂₁H₂₅N.HCl [Same as the monograph Terbinafine Hydrochloride]  

Terephthalic acid C₁₂H₈O₄ White crystals or crystalline powder. Slightly soluble in ethanol (95), and practically insoluble in water and ether.  

Residue on ignition <2.44%: not more than 0.3% (1 g).  

Content: not less than 95.0%. Assay—Weigh accurately about 2 g of terephthalic acid, dissolve in exactly 50 mL of 1 mol/L hydrochloric acid VS, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 83.07 mg of C₁₂H₈O₄

Terphenyl C₁₈H₁₄ White crystalline powder. 

Identification—Determine the absorption spectrum of a solution of terphenyl in methanol (1 in 250,000) as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.257: it exhibits the absorption at the wave numbers of about 3530 cm⁻¹, 3380 cm⁻¹, 1612 cm⁻¹, 1233 cm⁻¹, 1067 cm⁻¹ and 1056 cm⁻¹.

Testosterone propionate C₁₇H₂₃O₃ [Same as the name-sake monograph]

Tetra-bromophenolphthalein ethyl ester potassium salt C₂₅H₂₃O₄Br₄K [K 9042, Special class]

Tetra-bromophenolphthalein ethyl ester TS Dissolve 0.1 g of tetrabromophenolphthalein ethyl ester potassium salt in acetic acid (100) to make 100 mL. Prepare before use.

Tetra-n-butylammonium bromide [CH₃(CH₂)₃N]Br White, crystals or crystalline powder, having a slight, characteristic odor.

Melting point 2.60°: 101 – 105°C

Purity Clarity and color of solution—Dissolve 1.0 g of tetra-n-butylammonium bromide in 20 mL of water: the solution is clear and colorless.

Content: not less than 98.0%. Assay—Dissolve about 0.5 g of tetra-n-butylammonium bromide, accurately weighed, in 50 mL of water, add 5 mL of dilute nitric acid, and titrate with 0.1 mol/L silver nitrate VS while strongly shaking (potentiometric titration). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 27.79 mg of C₁₃H₂₅NBr

Tetra-n-butylammonium chloride C₁₅H₃₂ClN White crystals, and it is deliquescent.

Water <2.48°: not more than 6.0% (0.1 g).

Content: not less than 95.0%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.25 g of tetra-n-butylammonium chloride, dissolve in 50 mL of water, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS = 27.79 mg of C₁₅H₃₂ClN

Tetrabutylammonium hydrogensulfate C₁₃H₃₇NO₄S White crystalline powder.

Content: not less than 98.0%. Assay—Weigh accurately about 0.7 g of tetrabutylammonium hydrogensulfate, dissolve in 100 mL of freshly boiled and cooled water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromocresol green-methyl red TS).

Each mL of 0.1 mol/L sodium hydroxide VS = 33.95 mg of C₁₃H₃₇NO₄S

40% Tetrabutylammonium hydroxide TS A solution containing 40 g/dL of tetrabutylammonium hydroxide [(C₄H₉)₄NOH: 259.47].

Content: 36 – 44 g/dL. Assay—Pipet 10 mL of 40% tetrabutylammonium hydroxide TS, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 1 mol/L hydrochloric acid VS = 259.5 mg of C₄H₉NO

0.005 mol/L Tetrabutylammonium hydroxide TS To 10 mL of tetrabutylammonium hydroxide TS add 700 mL of
water, adjust to pH 4.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL.

Tetraethylammonium hydroxide-methanol TS Methanol solution containing 25 g/dL of tetraethylammonium hydroxide [(C2H5)4NOH: 259.47]. Colorless to pale yellow solution, having an ammonium-like odor.

Content: 22.5 – 27.5 g/dL. Assay—Pipet 15 mL of tetraethylammonium hydroxide-methanol TS and titrate <2.50< with 1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 1 mol/L hydrochloric acid VS

\[ = 259.5 \text{ mg of } (C_2H_5)_4NOH \]

10% Tetraethylammonium hydroxide-methanol TS A methanol solution containing 10 g/dL of tetraethylammonium hydroxide [(C2H5)4NOH: 259.47].

Content: 9.0 – 11.0 g/dL. Assay—Pipet 2 mL of 10% tetraethylammonium hydroxide-methanol TS, transfer to a glass-stoppered flask containing 20 mL of water, and titrate <2.50< with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS

\[ = 25.95 \text{ mg of } (C_2H_5)_4NOH \]

Tetraethylammonium hydroxide TS A solution containing 13 g/dL of tetraethylammonium hydroxide [(C2H5)4NOH: 259.47].

Content: 11.7 – 14.3 g/dL. Assay—Pipet a quantity, equivalent to about 0.3 g of tetraethylammonium hydroxide [(C2H5)4NOH], transfer to a glass-stoppered flask containing 15 mL of water, accurately weighed, and titrate <2.50< with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS

\[ = 25.95 \text{ mg of } (C_2H_5)_4NOH \]

Tetraethylammonium phosphate (C2H5)4NH2PO4 White powder. It is soluble in water.

Content: not less than 97.0%. Assay—Weigh accurately about 1.5 g of tetraethylammonium phosphate, dissolve in 80 mL of water, and titrate <2.50< with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS

\[ = 169.7 \text{ mg of } (C_2H_5)_4NH_2PO_4 \]

Tetracycline C22H24N2O8 Yellow to dark yellow, crystals or crystalline powder. Sparingly soluble in ethanol, and very slightly soluble in water.

Content: it contains not less than 870 µg (potency) per mg. Assay—Proceed as directed in the Assay under Tetracycline Hydrochloride. However, use the following formula.

\[ \text{Amount (µg (potency)) of tetracycline } (C_{22}H_{24}N_2O_8) = M_s \times \left( A_s / A_A \right) \times 1000 \]

\[ M_s: \text{ Amount [mg (potency)] of Tetracycline Hydrochloride RS} \]

Tetracycline Hydrochloride C22H24N2O8.HCl Yellow, crystals or crystalline powder.

Purity Related substances—Dissolve 20 mg of tetracycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20 µL of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than tetracycline is not more than 10%.

Tetradecyl trimethylammonium bromide CH3(CH2)13N(CH3)3Br A white powder.

Purity Clarity and color of solution—Dissolve 1.0 g in 20 mL of water: the solution is clear and colorless.

Content: not less than 98.0%. Assay—Weigh accurately about 0.5 g of tetradecyl trimethylammonium bromide, dissolve in 100 mL of water, add 5 mL of a mixture of water and nitric acid (2:1), and titrate <2.50< with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS

\[ = 33.64 \text{ mg of } C_{14}H_{33}NO \]

Tetraethylammonium hydroxide TS A solution containing 10% of tetraethylammonium hydroxide [C2H5)4NOH: 147.26]. A clear, colorless liquid, having a strong ammonia odor. It is a strong base and easily absorbs carbon dioxide from the air.

Content: 10.0 – 11.0% Assay—Weigh accurately about 3 g in a glass-stoppered flask containing 15 mL of water, and titrate <2.50< with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L hydrochloric acid VS

\[ = 14.73 \text{ mg of } C_2H_5NO \]

Tetra-n-heptylammonium bromide [CH(CH2)3]4NBr White, crystals or crystalline powder, having a slight, characteristic odor.

Melting point <2.60>: 87 – 89°C

Content: not less than 98.0%. Assay—Dissolve about 0.5 g of tetra-n-heptylammonium bromide, accurately weighed, in 50 mL of diluted acetonitrile (3 in 5), and 5 mL of dilute nitric acid, and titrate <2.50< with 0.1 mol/L silver nitrate VS while strongly shaking (potentiometric titration). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS

\[ = 49.07 \text{ mg of } C_{14}H_{29}NO \]

Tetrahydrofuran CH3(CH2)2CH2O [K 9705, Special class]

Tetrahydrofuran for gas chromatography Use tetrahydrofuran prepared by distilling with iron (II) sulfate heptahydrate.

Storage—Preserve in containers, in which the air has been displaced by nitrogen, in a dark, cold place.

Tetrahydrofuran for liquid chromatography C2H4O Clear and colorless liquid.

Refractive index <2.45>: nD20: 1.406 – 1.409

Density <2.50< 0.884 – 0.889 g/mL (20°C)

Purity Ultraviolet absorbing substances—Determine the absorption spectrum of tetrahydrofuran for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbences at
240 nm, 254 nm, 280 nm, 290 nm, and between 300 nm and 400 nm are not more than 0.35, 0.20, 0.05, 0.02 and 0.01, respectively.

Peroxide—Perform the test according to the method described in JIS K 9705: not more than 0.01%.

Tetrahydroxyquinone C₈H₈O₄ Dark blue crystals. Its color changes to yellow on exposure to light. Soluble in ethanol (95) and sparingly soluble in water.

Tetrahydroxyquinone indicator Mix 1 g of tetrahydroxyquinone with 100 g of sucrose homogeneously.

Tetrahydroxypropylenediamine for gas chromatography Prepared for gas chromatography.

Tetramethylammonium hydroxide (CH₃)₄NOH Ordinarily, available as an approximately 10% aqueous solution, which is clear and colorless, and has a strong ammonia-like odor. Tetramethylammonium hydroxide is a stronger base than ammonia, and rapidly absorbs carbon dioxide from the air. Use a 10% aqueous solution.

Purity Ammonia and other amines—Weigh accurately a quantity of the solution, corresponding to about 0.3 g of tetramethylammonium hydroxide [(CH₃)₄NOH], in a weighing bottle already containing 5 mL of water. Add a slight excess of 1 mol/L hydrochloric acid TS (about 4 mL), and evaporate on a water bath to dryness. The mass of the residue (tetramethylammonium chloride), dried at 105°C for 2 hours and multiplied by 0.8317, represents the quantity of tetramethylammonium hydroxide [(CH₃)₄NOH], and corresponds to ±0.2% of that found in the Assay.

Residue on evaporation: not more than 0.02% (5 mL, 105°C, 1 hour).

Content: not less than 98% of the labeled amount.

Assay—Accurately weigh a glass-stoppered flask containing about 15 mL of water. Add a quantity of the solution, equivalent to about 0.2 g of tetramethylammonium hydroxide [(CH₃)₄NOH], weigh again, and titrate <2.50 with 0.1 mol/L hydrochloric acid VS (indicator: methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS = 9.115 mg of C₄H₇NO

Tetramethylammonium hydroxide-methanol TS A methanol solution containing of 10 g/dL of tetramethylammonium hydroxide [(CH₃)₄NOH]; 91.15%

Content: 9.0 – 11.0 g/dL. Assay—Pipet 2 mL of tetramethylammonium hydroxide-methanol TS, transfer to a glass-stoppered flask containing 20 mL of water, and titrate <2.50 with 0.1 mol/L hydrochloric acid VS (indicator: bromocresol green-methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS = 9.115 mg of C₄H₇NO

Tetramethylammonium hydroxide TS Pipet 15 mL of tetramethylammonium hydroxide, and add dehydrated ethanol (99.5) to make exactly 100 mL.

Tetramethylammonium hydroxide TS, pH 5.5 To 10 mL of tetramethylammonium hydroxide add 990 mL of water, and adjust the pH to 5.5 with diluted phosphoric acid (1 in 10).

3,3',5,5'-Tetramethylbenzidine dihydrochloride dihydrate C₁₆H₂₂Cl₂N₂·H₂O White to slightly reddish-white crystalline powder.

N,N',N',N'-Tetramethylethlenediamine (CH₃)₂NCH₂CH₂N(CH₃)₂ Pale yellow clear liquid.

Specific gravity 2.560 ± 0.020; 0.774 – 0.799

Content: not less than 99.0%.

Tetramethylsilane for nuclear magnetic resonance spectroscopy (CH₃)₄Si Prepared for nuclear magnetic resonance spectroscopy.

Tetra-n-pentylammonium bromide [CH₃(CH₂)₄NH]Br White, crystals or crystalline powder. It is hygroscopic.

Melting point <2.60°C: 100 – 101°C

Tetraphenylboron potassium TS Add 1 mL of acetic acid (31) to a solution of potassium biphthalate (1 in 500), then to this solution add 20 mL of a solution of tetraphenylboron sodium (7 in 1000), shake well, and allow to stand for 1 hour. Collect the produced precipitate on filter paper, and wash it with water. To 1/3 quantity of the precipitate add 100 mL of water, warm, with shaking, at about 50°C for 5 minutes, cool quickly, allow to stand for 2 hours with occasional shaking, and filter, discarding the first 30 mL of the filtrate.

Tetraphenylboron sodium See sodium tetraphenylborate.

Tetra-n-propylammonium bromide [CH₃CH₂CH₂NH]Br White, crystals or crystalline powder.

Purity Clarity and color of solution—Dissolve 1.0 g of tetra-n-propylammonium bromide in 20 mL of water: the solution is clear and colorless.

Content: not less than 98.0%. Assay—Weigh accurately about 0.4 g of tetra-n-propylammonium bromide, dissolve in 50 mL of water, add 5 mL of dilute nitric acid, and titrate <2.50 with 0.1 mol/L silver nitrate VS while shaking strongly (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS = 26.63 mg of C₃H₅Br

Theophylline C₇H₈N₄O₂ White powder. Slightly soluble in water.

Melting point <2.60°C: 269 – 274°C

Purity Caffeine, theobromine or paraxanthine—To 0.20 g of theophylline add 5 mL of potassium hydroxide TS or 5 mL of ammonia TS: each solution is clear.

Loss on drying <2.41%: not more than 0.5% (1 g, 105°C, 4 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 0.25 g of theophylline, previously dried, dissolve it in 40 mL of N,N-dimethylformamide, and titrate <2.50 with 0.1 mol/L sodium methoxide VS (indicator: 3 drops of thymol blue-N,N-dimethylformamide TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS = 18.02 mg of C₇H₈N₄O₂

Theophylline for assay C₇H₈N₄O₂ [Same as the monograph Theophylline meeting the following additional specifications.]

Purity Related substances—Dissolve 50 mg of theophylline for assay in water to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL of the
sample solution and standard solution as directed under Liquid Chromatography. Determine each peak from both solutions by the automatic integration method: the total area of peaks other than the peak of theophylline obtained from the sample solution is not larger than the peak area of theophylline from the standard solution.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 270 nm).
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted acetic acid (100) (1 in 100) and methanol (4:1).
Flow rate: Adjust the flow rate so that the retention time of theophylline is about 10 minutes.
Time span of measurement: About 3 times as long as the retention time of theophylline.
System suitability
Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 25 mL. Confirm that the peak area of theophylline obtained from 20 μL of this solution is equivalent to 15 to 25% of that of theophylline from 20 μL of the standard solution.
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of theophylline are not less than 3000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of theophylline is not more than 3.0%.

Thiamine nitrate C₁₂H₁₇N₅O₄S [Same as the namesake monograph]
Thianthol [Same as the monograph Thianthol. Proceed as directed in the Identification (3) under Sulfur, Salicylic Acid and Thianthol Ointment: any spot other than the principal spot does not appear.]

3-Thienylethylpenicillin sodium C₁₄H₁₅N₂O₂S₂ White to pale yellowish white powder. Very soluble in water, freely soluble in methanol, and sparingly soluble in ethanol (95%).

Optical rotation \(\left< 2.49^\circ \right> [\alpha]^{20}_D + 265 + 290^\circ\) (0.5 g calculated on the anhydrous bases, water, 50 mL, 100 mm).

Water \(\left< 2.48^\circ\right>:\) Not more than 10.0% (0.2 g, volumetric titration, direct titration).

Content: not less than 90% calculated on the anhydrous basis. Assay—Weigh accurately about 0.1 g of 3-thienylethylpenicillin sodium, dissolve in 35 mL of water, add 0.75 mL of 0.1 mol/L hydrochloric acid TS, and adjust to pH 8.5 with 0.1 mol/L sodium hydroxide TS. To this solution add 2 mL of a penicillinsae solution prepared by dissolving penicillinsae, equivalent to 513,000 Levy units, in 25 mL of water and neutralizing with dilute sodium hydroxide TS until a pale red color appears with 1 drop of a solution of phenolphthalein in ethanol (95) (1 in 1000) as indicator, and allow to stand at 25°C for 5 minutes. Titrate \(\left< 2.50^\circ\right>\) this solution with 0.1 mol/L sodium hydroxide VS until the solution reaches to pH 8.5 (potentiometric titration). Use the water freshly boiled and cooled.

Each mL of 0.1 mol/L sodium hydroxide VS = 36.24 mg of C₁₄H₁₅N₂NaO₄S₂

Thimerosal C₁₂H₁₅N₂NaO₂ S White or yellowish crystalline powder. Freely soluble in water.

Melting point \(\left< 2.6^\circ\right>: 107 – 114°C.

Thioacetamide C₃H₇NS A white crystalline powder or colorless crystals, having a characteristic odor. Freely soluble in water and in ethanol (99.5). Melting point: 112 – 115°C

Thioacetamide-alkaline glycerin TS To 0.2 mL of a solution of thioacetamide (1 in 25) add 1 mL of alkaline glycerin TS, and heat for 20 seconds in a water bath. Prepare before use.

Thioacetamide TS To 0.2 mL of a solution of thioacetamide (1 in 25) add 1 mL of a mixture of 15 mL of sodium hydroxide TS, 5 mL of water and 20 mL of 85% glycerin, and heat in a water bath for 20 seconds. Prepare before use.

Thiodiglycol S(CH₂CH₂OH)₂ [β-Thiodiglycol for amino acid autoanalysis] Colorless or pale yellow, clear liquid.

Specific gravity \(\left< 2.56^\circ\right> d_{0}^{20}: 1.180 – 1.190\)

Water \(\left< 2.48^\circ\right>:\) not more than 0.7%.

Thioglycolate medium I for sterility test See fluid thioglycolate medium.

Thioglycolate medium II for sterility test See alternative thioglycolate medium.

Thioglycolic acid See mercapto acetic acid.

Thionyl chloride SOCl₂ A colorless or light yellow, clear liquid, having a pungent odor.

Specific gravity \(\left< 2.56^\circ\right> d_{0}^{20}:\) about 1.65 (Method 3)

Content: not less than 95.0%. Assay—Weigh accurately 0.1 g of thionyl chloride in a weighing bottle, put the bottle in a glass-stoppered conical flask containing 50 mL of water cooled to about 5°C, stopper immediately, dissolve the sample thoroughly, and transfer the solution to a 200-mL beaker. Wash the conical flask and the weighing bottle in it with 30 mL of water, and combine the washings and the solution in the beaker. Add 1 drop of an aqueous solution of polyvinyl alcohol (100 g/L), and titrate \(\left< 2.50^\circ\right>\) with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 5.949 mg of SOCl₂

Thiopental for assay C₃H₇N₂O₃S₄ Dissolve 10 g of Thiopental Sodium in 300 mL of water. To this solution add slowly 50 mL of dilute hydrochloric acid with stirring. Take the produced crystals by filtration, wash with water until the filtrate indicates no reaction to chloride, and air-dry. Add diluted ethanol (99.5) (3 in 5), dissolve by heating in a water bath, allow to stand, and take the produced crystals by filtration. Air-dry the crystals in air, and dry again at 105°C for 4 hours. White, odorless crystals.

Melting point \(\left< 2.6^\circ\right>: 159 – 162°C\)
Purity (1) Clarity and color of solution—Dissolve 1.0 g of thioptenal for assay in dehydrated ethanol: the solution is clear and light yellow.

(2) Related substances—Dissolve 0.05 g of thioptenal for assay in 15 mL of acetonitrile, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase in the Purity (4) under Thiopental Sodium to make exactly 200 mL, and use this solution as the standard solution. Proceed as directed in Purity (4) under Thiopental Sodium.

Loss on drying (2.47): not more than 0.20% (1 g, 105°C, 3 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 0.35 g of thioptenal for assay, previously dried, dissolve in 5 mL of dehydrated ethanol and 50 mL of chloroform, and titrate with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 24.23 mg of \( \text{C}_{11} \text{H}_{18} \text{N}_{2} \text{O}_{2} \)S

Thiopental sodium \( \text{C}_{11} \text{H}_{18} \text{N}_{2} \text{NaO}_{2} \)S [Same as the namesake monograph]

Thiosemicarbazide \( \text{H}_{2}\text{NCSHNH}_{2} \) White crystals or crystalline powder.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry (2.25): it exhibits absorption at the wave numbers of about 3370 cm\(^{-1}\), 3180 cm\(^{-1}\), 1648 cm\(^{-1}\), 1622 cm\(^{-1}\), 1535 cm\(^{-1}\), 1288 cm\(^{-1}\), 1167 cm\(^{-1}\), 1003 cm\(^{-1}\), and 803 cm\(^{-1}\).

Thiourea \( \text{H}_{2}\text{NCSNH}_{2} \) [K 8635, Special class]

Thiourea TS Dissolve 10 g of thiourea in water to make 100 mL.

L-Threonine \( \text{C}_{4}\text{H}_{9}\text{NO}_{3} \) [Same as the namesake monograph]

Threoprocaterol hydrochloride \( \text{C}_{10}\text{H}_{22}\text{N}_{2}\text{O}_{5}\text{HCl} \) To procaterol hydrochloride add 10 volumes of 3 mol/L hydrochloric acid TS, heat, and reflux for 3 hours. After cooling, neutralize (pH 8.5) with sodium hydroxide TS, and collect the crystals produced. Suspend the crystals in water, dissolve by acidifying the solution at pH 1 to 2 with addition of hydrochloric acid, neutralize (pH 8.5) by adding sodium hydroxide TS, and separate the crystals produced. Suspend the crystals in 2-propanol, and acidify the solution at pH 1 to 2 by adding hydrochloric acid. The crystals are dissolved and reproduced. Collect the crystals, dry at about 60°C while passing air. White to pale yellowish white, odorless crystals or crystalline powder. Melting point: about 207°C (with decomposition).

Purity—Dissolve 0.10 g of threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 2 \( \mu \)L of the sample solution as directed under Liquid Chromatography according to the operating conditions in the Purity (3) under Procaterol Hydrochloride Hydrate. Measure each peak area by the automatic integration method, and calculate the amount of threoprocaterol by the area percentage method: it shows the purity of not less than 95.0%. Adjust the detection sensitivity so that the peak height of threoprocaterol obtained from 2 \( \mu \)L of the solution prepared by diluting 5.0 mL of the sample solution with diluted methanol (1 in 2) to make 100 mL, is 5 to 10% of the full scale, and the time span of measurement is about twice as long as the retention time of threoprocaterol beginning after the peak of solvent.

Thrombin [Same as the namesake monograph]

Thymine \( \text{C}_{5}\text{H}_{6}\text{N}_{2}\text{O}_{2} \) Occurs as a white powder.

Purity—Dissolve 10 mg of the substance to be examined in 100 mL of methanol, add the mobile phase to make exactly 250 mL, and use this solution as the sample solution. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 \( \mu \)L each of these solutions and perform the test as directed in the Purity (3) under Zudivudine. Determine the area of each peak in the sample and standard solutions by the automatic integration method: the total area of peaks other than thymine from the sample solution is not larger than that from the standard solution. However, the time span of measurement is about 10 times the retention time of thymine, beginning after the solvent peak.

Thymol \( \text{C}_{8}\text{H}_{8}\text{O} \)Occurs

Thymol blue \( \text{C}_{27}\text{H}_{30}\text{O}_{5}\text{S} \) [K 8643, Special class]

Thymol blue-N,N-dimethylformamide TS Dissolve 0.1 g of thymol blue in 100 mL of N,N-dimethylformamide.

Thymol blue-dioxane TS Dissolve 0.05 g of thymol blue in 100 mL of 1,4-dioxane, and filter if necessary. Prepare before use.

Thymol blue TS Dissolve 0.1 g of thymol blue in 100 mL of ethanol (95), and filter if necessary.

Thymol blue TS, dilute Dissolve 0.05 g of thymol blue in 100 mL of ethanol (99.5), and filter if necessary. Prepare before use.

Thymol for assay [Same as the monograph Thymol. It contains not less than 99.0% of thymol (\( \text{C}_{10}\text{H}_{10}\text{O} \).)]

Thymol for spraying test solution \( \text{C}_{9}\text{H}_{10}\text{O} \) White crystals or crystalline powder, having an aromatic odor. Very soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25): it exhibits absorp-
Thymolphthalein C_{2}H_{18}N_{2}O_{4} \ [K \ 8642, \ Special \ class]

Thymolphthalein TS Dissolve 0.1 g of thymolphthalein in 100 mL of ethanol (95), and filter if necessary.

Thymol-sulfuric acid-methanol TS for spraying Dissolve 1.5 g of thymol for spraying test solution in 100 mL of methanol, and add 5.7 mL of sulfuric acid.

Tiaramide hydrochloride for assay C_{1}2H_{2}5CIN_{2}O_{4}.HCl [Same as the monograph Tiaramide Hydrochloride. When dried, it contains not less than 99.0% of tiaramide hydrochloride (C_{1}2H_{2}5CIN_{2}O_{4}.HCl).]

Tiapride hydrochloride for assay C_{1}5H_{17}N_{2}O_{4}.HCl [Same as the monograph Tiapride Hydrochloride]

Tin Sn [K 8580, Special class]

Tin (II) chloride dihydrate SnCl_{2}.2H_{2}O [K 8136, Special class]

Tin (II) chloride-hydrochloric acid TS To 20 g of tin add 85 mL of hydrochloric acid, heat until hydrogen gas no longer are evolved, and allow to cool. Mix 1 volume of this solution and 10 volume of dilute hydrochloric acid. Prepare before use.

Tin (II) chloride-sulfuric acid TS Dissolve 10 g of tin (II) chloride dihydrate in diluted sulfuric acid (3 in 200) to make 100 mL.

Tin (II) chloride TS Dissolve 1.5 g of Tin (II) chloride hydrate in 10 mL of water containing a small amount of hydrochloric acid. Preserve in glass-stoppered bottles in which a fragment of tin has been placed. Use within 1 month.

Tin (II) chloride TS, acidic Dissolve 8 g of Tin (II) chloride dihydrate in 500 mL of hydrochloric acid. Preserve in glass-stoppered bottles. Use within 3 months.

Tipepidine hibenzate for assay C_{1}2H_{17}NS_{2}.C_{1}3H_{16}O_{4} [Same as the monograph Tipepidine Hibenzate. When dried, it contains not less than 99.0% of C_{1}2H_{17}NS_{2}.C_{1}3H_{16}O_{4}]

Titanium dioxide See titanium (IV) oxide.

Titanium dioxide TS See titanium (IV) oxide TS.

Titanium (III) chloride (20) TiCl_{3} [K 8401, Titanium (III) chloride solution, Special class] Store in light-resistant, glass-stoppered containers.

Titanium (III) chloride-sulfuric acid TS Mix carefully 20 mL of titanium (III) chloride TS and 13 mL of sulfuric acid, add carefully hydrogen peroxide (30) in small portions until a yellow color develops, and heat until white fumes evolve. After cooling, add water, heat again in the same manner, repeat this procedure until the solution is colorless, and add water to make 100 mL.

Titanium (III) chloride TS To titanium (III) chloride (20) add dilute hydrochloric acid to obtain a solution containing 15 g/dL of titanium (III) chloride (TiCl_{3}). Prepare before use.

Content: 14.0 – 16.0 g/dL. Assay—To exactly 2 mL of titanium (III) chloride add 200 mL of water and 5 mL of a hydrochloric acid solution (2 in 3), and titrate <2.50 with 0.1 mol/L ferric ammonium sulfate VS under carbon dioxide until a slight red color develops in the solution (indicator: 5 mL of ammonium thiocyanate TS).

Each mL of 0.1 mol/L ferric ammonium sulfate VS = 15.42 mg of TiCl_{3}

Titanium (IV) oxide TiO_{2} [K 8703, Special class]

Titanium (IV) oxide TS To 100 mL of sulfuric acid add 0.1 g of titanium (IV) oxide, and dissolve by gradually heating on a flame with occasional gentle shaking.

Titanium trichloride See titanium (III) chloride.

Titanium trichloride-sulfuric acid TS See titanium (III) chloride-sulfuric acid TS.

Titanium trichloride TS See titanium (III) chloride TS.

Titanium yellow C_{2}8H_{19}N_{2}O_{4}.S_{4} A dark yellow to dark yellow-brown powder or masses.

Identification—Determine the infrared absorption spectrum of titanium yellow, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry (2.25); it exhibits its absorption at the wave numbers of about 1603 cm\(^{-1}\), 1467 cm\(^{-1}\), 1394 cm\(^{-1}\), 1306 cm\(^{-1}\), 1040 cm\(^{-1}\), 988 cm\(^{-1}\), 820 cm\(^{-1}\) and 644 cm\(^{-1}\).

Preserve in a light-resistant tight container.

Tocopherol C_{15}H_{17}O_2 [Same as the namesake monograph]

Tocopherol acetate C_{3}1H_{52}O_3 [Same as the namesake monograph]

Tocopherol calcium succinate C_{6}6H_{10}6CaO_{10} [Same as the namesake monograph]

Tocopherol succinate C_{1}2H_{14}O_5 Wet 0.5 g of tocopherol calcium succinate with 5 mL of acetic acid (100), add 10 mL of toluene, and warm at 70°C for 30 minutes with occasional shaking. After cooling, add 30 mL of water, shake thoroughly, and allow to stand. Remove the water layer, wash the toluene layer with several 30-mL portions of water until the washings become neutral, and allow to stand. Shake the toluene extract with 3 g of anhydrous sodium sulfate, decant the toluene layer, distil the toluene under reduced pressure, and obtain a light-yellow, viscous liquid. When preserved at room temperature for a long time, it becomes a pale yellowish solid.

Absorbance <2.24\(^{*}\) E\(_{1}^{1}\)\(_{1}\)\(_{0}\) (286 nm): 38.0 – 42.0 (10 mg, chloroform, 100 mL).

Tolbutamide C_{12}H_{18}N_{2}O_{5} S [Same as the namesake monograph]

Toluene C_{6}H_{12}CH_{3} [K 8680, Special class]

\(\alpha\)-Toluene sulfonamide C_{6}H_{12}NO_{3}S Colorless crystals or white crystalline powder. Soluble in ethanol (95), and sparingly soluble in water.

Melting point <2.60\(^{*}\): 157 – 160°C
Purity p-Toluene sulfonamide—Use a solution of o-toluene sulfonamide in ethyl acetate (1 in 5000) as the sample solution. Perform the test with 10 μL of the sample solution as directed under Gas Chromatography <2.02> according to the operating conditions in the Purity (5) under Saccharin Sodium Hydrate: any peak other than the peak of o-toluene sulfonamide does not appear. Adjust the flow rate so that the retention time of o-toluene sulfonamide is about 10 minutes, and adjust the detection sensitivity so that the peak height of o-toluene sulfonamide obtained from 10 μL of the sample solution is about 50% of the full scale. Time span of measurement is about twice as long as the retention time of o-toluene sulfonamide beginning after the solvent peak.

Water <2.48>: not more than 0.5% (4 g, use 25 mL of methanol for Karl Fischer method and 5 mL of pyridine for Karl Fischer method).

Content: not less than 98.5%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.025 g of o-toluene sulfonamide, and perform the test as directed under Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS = 1.712 mg of C6H5NO2S

O-Toluene sulfonamide CH3C6H4SO2NH2 White, crystals or crystalline powder. Melting point: about 137°C.

Purity Related substances—Dissolve 30 mg of p-toluene sulfonamide in acetone to make exactly 200 mL. Proceed with 10 μL of this solution as directed in the Purity (3) under Tolazamide: any peak other than the principal peak at the RI value of about 0.6 does not appear.

p-Toluene sulfonic acid See p-toluenesulfonic acid monohydrate.

p-Toluenesulfonic acid monohydrate CH3C6H4SO2H·H2O [K 8681, Special class]

O-Toluic acid C6H5CO2 White, crystals or crystalline powder.

Melting point <2.60>: 102 – 105°C

Content: not less than 98.0%.

Toluidine blue See toluidine blue O

Toluidine blue O C15H10ClN6S Dark green powder, soluble in water, and slightly soluble in ethanol (95).

Identification—

(1) A solution (1 in 100) shows a blue to purple color.

(2) A solution in ethanol (95) (1 in 200) shows a blue color.

(3) A solution shows a maximum absorption at around 630 nm.

Triamcinolone acetonide C24H31FO6 Same as the namesake monograph

Trichloroacetic acid CCl3COOH [K 8667, Special class]

Trichloroacetic acid-gelatin-tris buffer solution To 1 volume of a solution of trichloroacetic acid (1 in 5) add 6 volume of gelatin-tris buffer solution, pH 8.0 and 5 volume of water.

Trichloroacetic acid TS Dissolve 1.80 g of trichloroacetic acid, 2.99 g of sodium acetate trihydrate and 1.98 g of acetic acid (31) in water to make 100 mL.

Trichloroacetic acid TS for serrapeptase Dissolve 1.80 g of trichloroacetic acid and 1.80 g of anhydrous sodium acetate in 5.5 mL of 6 mol/L acetic acid TS and water to make 100 mL.

Trichlorofluoromethane CCl3F A colorless liquid or gas.

Specific gravity <2.50> d17.5: 1.494

Boiling point <2.57>: 23.7°C

1,1,2-Trichloro-1,2,2-trifluoroethane CFC12 CF2Cl Colorless volatile liquid. Miscible with acetone and with diethyl ether, and not with water.

Purity Related substances—Perform the test with 0.1 μL of 1,1,2-trichloro-1,2,2-trifluoroethane as directed under Gas Chromatography <2.02> according to the operating conditions in the Purity (5) under Halothane: any peak other than the peak of 1,1,2-trichloro-1,2,2-trifluoroethane does not appear.

Tricine C6H12NO3 White crystalline powder. Melting point: 182 to 184°C (with decomposition).

Triethanolamine See 2,2,2’-nitrilotriethanol.

Triethylamine C2H5N Clear colorless liquid, having a pungent odor. Miscible with methanol, with ethanol (95) and with diethyl ether.

Specific gravity <2.60> d20: 0.722 – 0.730

Boiling point <2.57>: 89 – 90°C

Triethylamine buffer solution, pH 3.2 To 4 mL of triethylamine add 2000 mL of water, and adjust the pH to 3.2 with phosphoric acid.

Triethylamine-phosphate buffer solution, pH 5.0 To 1.0 mL of triethylamine add 900 mL of water, adjust the pH to 5.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL.

1% Triethylamine-phosphate buffer solution, pH 3.0 Dissolve 10 g of triethylamine in 950 mL of water, adjust the pH to 3.0 with phosphoric acid, and make exactly 1000 mL.

Trifluoroacetic acid CF3COOH Colorless, clear liquid, having a pungent odor. Miscible well with water.

Specific gravity <2.50> d20: 1.535

Boiling point <2.57>: 72 – 73°C

Trifluoroacetic acid for nuclear magnetic resonance spectroscopy CF3COOH Prepared for nuclear magnetic resonance spectroscopy.

Trifluoroacetic acid TS To 1 mL of trifluoroacetic acid add water to make 1000 mL.

Trifluoroacetic anhydride for gas chromatography (CF3CO)2O Colorless, clear liquid, having a pungent odor. Boiling point <2.57>: 40 – 45°C

Trimetazidine hydrochloride for assay C14H22N2O3·2HCl [Same as the monograph Trimetazidine Hydrochloride. It contains not less than 99.0% of trimetazidine hydrochloride (C14H22N2O3·2HCl), calculated on the anhydrous basis.]

Trimethylsilyl imidazole C6H12N3Si Clear, colorless to pale yellow liquid.

Refractive index <2.45> nD: 1.4744 – 1.4764

2,4,6-trinitrobenzenesulfonic acid See 2,4,6-trinitroben-
2.4,6-Trinitrobenzenesulfonic acid dihydrate
\(\text{C}_6\text{H}_5\text{K}_3\text{O}_7\cdot\text{H}_2\text{O}\) Pale yellow to light yellow powder.

Water \(\leq 2.4\%\): 11–15% (0.1 g, volumetric titration, direct titration).

**Content:** not less than 98%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.3 g of 2,4,6-trinitrobenzenesulfonic acid, dissolve in 50 mL of a mixture of water and ethanol (99.5) (1:1), and titrate \(\leq 2.5\%\) with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 29.32 mg of \(\text{C}_6\text{H}_5\text{K}_3\text{O}_7\cdot\text{H}_2\text{O}\)

2,4,6-Trinitrophenol \(\text{HOC}_6\text{H}_2(\text{NO}_2)_3\) Light yellow to yellow, moist crystals. It is added 15 to 25% of water for the sake of safety, because it might explode by heating, mechanical shocking and friction when it is dried.

**Identification:**—To 0.1 g add 10 mL of water, dissolve by warming, and add 12 mL of a mixture of 1% copper (II) sulfate solution and ammonia TS (5:1): green precipitates appear.

**Content:** not less than 99.5%. Assay—Weigh accurately about 0.25 g, previously dried in a desiccator (silica gel) for warming, and add 12 mL of a mixture of 1% copper (II) sulfate solution and ammonia TS (5:1): green precipitates appear.

Each mL of 0.1 mol/L sodium hydroxide VS = 22.91 mg of \(\text{HOC}_6\text{H}_2(\text{NO}_2)_3\)

**Identification:**—To 0.1 g of triphenylmethanol for thin-layer chromatography in 100 mL of methanol and perform the chromatography in 100 mL for the sake of safety, because it might explode by heating, mechanical shocking and friction when it is dried.

**Identification:**—To 0.1 g add 10 mL of water, dissolve by warming, and add 12 mL of a mixture of 1% copper (II) sulfate solution and ammonia TS (5:1): green precipitates appear.

**Content:** not less than 99.5%. Assay—Weigh accurately about 0.25 g, previously dried in a desiccator (silica gel) for warming, and add 12 mL of a mixture of 1% copper (II) sulfate solution and ammonia TS (5:1): green precipitates appear.

Each mL of 0.1 mol/L sodium hydroxide VS = 22.91 mg of \(\text{HOC}_6\text{H}_2(\text{NO}_2)_3\)

2,4,6-Trinitrophenol-ethanol TS Dissolve 1 g of 2,4,6-trinitrophenol in 50 mL of diluted ethanol (99.5) (9 in 10) and adjust the pH to 7.0 with 0.1 mol/L hydrochloric acid TS. Use within 2 days.

2,4,6-Trinitrophenol TS, alkaline Mix 20 mL of 2,4,6-trinitrophenol TS with 10 mL of a solution of sodium hydroxide (1 in 20), and add water to make 100 mL. Use within 2 days.

Triphenylmethanol for thin-layer chromatography
\(\text{C}_9\text{H}_8\text{O}_2\) Occurs as a white powder.

**Purity:** Dissolve 0.1 g of triphenylmethanol for thin-layer chromatography in 100 mL of methanol and perform the test as directed in the Purity (2) under Zidovudine: spots other than the principal spot with an Rf value of about 0.73 are not observed.

Triphenyltetrazolium chloride See 2,3,5-triphenyl-2H-tetrazolium chloride.

2,3,5-Triphenyl-2H-tetrazolium chloride TS See 2,3,5-triphenyl-2H-tetrazolium chloride TS.

2,3,5-Triphenyl-2H-tetrazolium chloride \(\text{C}_{19}\text{H}_{15}\text{ClN}_4\) [K 8214, Special class]
0.05 mol/L Tris buffer solution, pH 7.0  Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol in about 750 mL of water, adjust to pH 7.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

0.05 mol/L Tris buffer solution, pH 8.6  Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 950 mL of water, add 2 mol/L hydrochloric acid TS to adjust the pH to 8.6, and then add water to make 1000 mL.

Tris(4-butyphenyl)phosphate $[(CH_3)_3C_6H_4O]_3PO$ White crystals or crystalline powder. Melting point $<2.60^\circ$: 100 – 104°C

0.2 mol/L Tris-hydrochloride buffer solution, pH 7.4  Dissolve 6.61 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 0.97 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 250 mL.

0.05 mol/L Tris-hydrochloride buffer solution, pH 7.5  Dissolve 6.35 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 1.18 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 1000 mL.

Tris(hydroxymethyl)aminomethane  See 2-amino-2-hydroxymethyl-1,3-propanediol.

Trisodium citrate dihydrate  See sodium citrate dihydrate.

0.1 mol/L Trisodium citrate TS  Dissolve 29.4 g of trisodium citrate dihydrate in water to make 1000 mL.

Trisodium ferrous pentacyanoamine TS  See iron (II) trisodium pentacyanoamine TS.

Trisodium phosphate dodecahydrate

Trypsin for liquid chromatography  An enzyme obtained from the bovine pancreas. This one part digests 250 parts of casein in the following reaction system.

Casein solution—To 0.1 g of milk casein add 30 mL of water, disperse the casein well, add 1.0 mL of diluted sodium hydroxide TS (1 in 10) to dissolve, and add water to make 50 mL. Prepare before use.

Sample solution—Dissolve 0.01 g of trypsin for liquid chromatography in 500 mL of water.

Procedure—To 5 mL of the casein solution add 2 mL of the sample solution and 3 mL of water, mix, then allow to stand at 40°C for 1 hour, and add 3 drops of a mixture of 95% water, and slightly soluble in cold water. Prepare before use.

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Trypsin for test of elcatonin  Dissolve 5 mg of trypsin for liquid chromatography in 20 mL of solution of ammonium hydrogen carbonate (1 in 100). Prepare before use.

Trypsin for test of ulinastatin  Dissolve crystalline trypsin for ulinastatin assay in ice-cooled 1 mmol/L hydrochloric acid TS containing 1 mmol/L calcium chloride dihydrate so that each mL of the solution contains 180 μg of trypsin. Prepare before use, and preserve in an ice-cooled water bath.

L-Tryptophan  C$_6$H$_5$N$_2$O$_2$ [Same as the namesake monograph]

Ubenimex for assay  C$_8$H$_8$N$_4$O$_4$ [Same as the monograph Ubenimex. When dried, it contains not less than 99.9% of ubenimex (C$_8$H$_8$N$_4$O$_4$)].

Ubiquinone-9  Yellow to orange, crystalline powder. Odorless and tasteless. Freely soluble in formic acid, very slightly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether. It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Optical rotation $<2.49 > [\alpha]_D^2$: $-10.5$ to $-12.5^\circ$ (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Loss on drying $<2.47 >$: not more than 0.30% (1 g, 105°C, 3 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 0.3 g of L-tyrosine, previously dried, dissolve in 6 mL of formic acid, add 50 mL of acetic acid (100), and titrate $<2.50$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 18.12 mg of C$_6$H$_5$N$_2$O$_2$

Ubenimex for assay  C$_8$H$_8$N$_4$O$_4$ [Same as the monograph Ubenimex. When dried, it contains not less than 99.9% of ubenimex (C$_8$H$_8$N$_4$O$_4$)].

Urea  H$_2$NCONH$_2$ [K 8731, Special class]

Urethane  See ethyl carbamate.

Ursodeoxycholic acid  C$_24$H$_40$O$_4$ [Same as the namesake monograph]

Ursodeoxycholic acid for assay  C$_24$H$_40$O$_4$ [Same as the namesake monograph. However, when dried, it contains not less than 99.0% of ursodeoxycholic acid (C$_24$H$_40$O$_4$) meeting the following additional specifications.]

Purity  Related substances—Dissolve 0.15 g of ursodeoxycholic acid for assay in 5 mL of methanol for liquid chromatography, and use this solution as the sample solution. Pipet 0.2 mL of this solution and add methanol for liquid chromatography to make exactly 50 mL. Pipet 2.5 mL of this solution, add methanol for liquid chromatography to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 0.5 mL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01 >$ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 2.5 with respect to ursodeoxycholic acid, obtained from the sample solution is not larger...
than the peak area of ursodeoxycholic acid from the standard solution, and the area of the peak, having the relative retention time of about 3.5, obtained from the sample solution is not larger than 1/5 times the peak area of ursodeoxycholic acid from the standard solution. Furthermore, the total area of the peaks other than the peak of ursodeoxycholic acid and other than the peaks mentioned above is not larger than 1/5 times the peak area of ursodeoxycholic acid from the standard solution.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 3 mm in inside diameter and 7.5 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of methanol for liquid chromatography, diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (96:69:35).
Flow rate: Adjust the flow rate so that the retention time of ursodeoxycholic acid is about 2.3 minutes.
Time span of measurement: About 7 times as long as the retention time of ursodeoxycholic acid.
System suitability
Test for required detectability: Pipet 2 mL of the standard solution, and add methanol for liquid chromatography to make exactly 20 mL. Confirm that the peak area of ursodeoxycholic acid obtained from 5 μL of this solution is equivalent to 8 to 12% of that of ursodeoxycholic acid from 5 μL of the standard solution.
System performance: To 30 mg of chenodeoxycholic acid for thin-layer chromatography and 30 mg of lithocholic acid for thin-layer chromatography, add 1 mL of the sample solution, dissolve in methanol for liquid chromatography to make 50 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, ursodeoxycholic acid, chenodeoxycholic acid, and lithocholic acid are eluted in this order with the resolution between these peaks being not less than 7, respectively.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0%.

\( n \)-Valerianic acid \( \text{CH}_3(\text{CH}_2)_3\text{COOH} \) Clear, colorless to pale yellow liquid, having a characteristic odor. Miscible with ethanol (95) and with diethyl ether, and soluble in water.

Specific gravity \(< 2.56\) \( d^2_{40} \): 0.936 – 0.942
Distilling range \(< 2.57\) : 186 – 188°C, not less than 98 vol%.

\( L \)-Valine \( \text{C}_5\text{H}_9\text{NO}_2 \) [Same as the namesake monograph]

\( L \)-Valine for assay \( \text{C}_5\text{H}_9\text{NO}_2 \) [Same as the monograph \( L \)-Valine. When dried, it contains not less than 99.0% of \( L \)-valine (\( \text{C}_5\text{H}_9\text{NO}_2 \)).]

\( \text{H}-\text{d}-\text{Valyl-t-Leucyl-\text{L}-arginine n-nitroanilide d} \) dihydrochloride \( \text{C}_9\text{H}_{14}\text{N}_4\text{O}_2\cdot2\text{HCl} \) White to pale yellow, powder or masses. Sparingly soluble in water.

Absorbance \(< 2.24\) \( E^\%_{1%\text{m}} \) (316 nm): 214 – 236 (0.01 g, water, 500 mL).

**Vanadium pentoxide** See vanadium (V) oxide.

**Vanadium pentoxide TS** See vanadium (V) oxide TS.

**Vanadium pentoxide, dilute** See vanadium (V) oxide TS, dilute.

**Vanadium (V) oxide** \( \text{V}_2\text{O}_5 \) Orangish yellow to yellow-brown powder.

Identification—Dissolve 0.3 g in 10 mL of ammonia TS and 15 mL of water. To 2 mL of this solution add 20 mL of water, mix, and add gently 1 mL of copper (II) sulfate TS: yellow precipitates appear.

**Vanadium (V) oxide TS** Add vanadium (V) oxide to phosphoric acid, saturate with vanadium (V) oxide by shaking vigorously for 2 hours, and filter through a glass filter.

**Vanadium (V) oxide TS, dilute** Dilute 10 mL of vanadium (V) oxide TS with water to make 100 mL. Prepare before use.

**Vanillin** \( \text{C}_6\text{H}_5\text{CHO(OCH}_3\text{)(OH)} \) A white to light yellow crystalline powder, having a characteristic odor.

Melting point \(< 2.60\) : 80.5 – 83.5°C.

Preserve in a light-resistant tight container.

**Vanillin-hydrochloric acid TS** Dissolve 5 mg of vanillin in 0.5 mL of ethanol (95), and to this solution add 0.5 mL of water and 3 mL of hydrochloric acid. Prepare before use.

**Vanillin-sulfuric acid-ethanol TS** Dissolve 3 g of vanillin in 30 mL of ethanol (95.9), and add 100 mL of sulfuric acid.

**Vanillin-sulfuric acid-ethanol TS for spraying** Dissolve 3 g of vanillin in 30 mL of ethanol (95.9), and add 100 mL of dilute sulfuric acid.

**Vanillin-sulfuric acid TS** Add cautiously 75 mL of sulfuric acid to 25 mL of ice-cold ethanol (95). After cooling, add 1 g of vanillin to dissolve. Prepare before use.

**Vasopressin** \( \text{C}_{46}\text{H}_{65}\text{N}_{15}\text{O}_{12}\text{S}_2 \) A white powder.

Constituent amino acids—Perform the test as directed in the Constituent amino acids under Oxytocin, and calculate the respective molar ratios with respect to glycine: 0.9 – 1.1 for aspartic acid, 0.9 – 1.1 for glutamic acid, 0.9 – 1.1 for proline, 0.8 – 1.1 for tyrosine, 0.9 – 1.1 for phenylalanine, 0.9 – 1.1 for arginine and 0.8 – 1.1 for cystine, and not more than 0.03 for other amino acids.

**Verapamil hydrochloride for assay** \( \text{C}_{36}\text{H}_{58}\text{N}_4\text{O}_9\text{HCl} \) [Same as the monograph Verapamil Hydrochloride. When dried, it contains not less than 99.0% of verapamil hydrochloride (\( \text{C}_{36}\text{H}_{58}\text{N}_4\text{O}_9\text{HCl} \)).]

**Vinblastine sulfate** \( \text{C}_{46}\text{H}_{56}\text{N}_4\text{O}_10\text{H}_2\text{SO}_4 \) [Same as the namesake monograph]

**Vincristine sulfate** \( \text{C}_{46}\text{H}_{58}\text{N}_4\text{O}_{10}\text{H}_2\text{SO}_4 \) [Same as the namesake monograph]

**Vinyl acetate** \( \text{C}_2\text{H}_4\text{O}_2 \) Clear, colorless liquid.

Specific gravity \(< 2.56\) \( d^2_{40} \): 0.932 – 0.942
Distilling range \(< 2.57\) : 80.5 – 83.5°C.

**Water** \( \leq 2.48\) : not more than 0.2%.

**Vinyl chloride** \( \text{C}_2\text{H}_3\text{Cl} \) Colorless gas.

Boiling point \(< 2.57\) : –14°C
Melting point \(< 2.60\) : –160°C
2-Vinylpyridine C₇H₇N A clear, colorless or dark brown liquid.

Refractive index <2.45> nD²⁰: 1.546 – 1.552

Specific gravity <2.56> d₄³: 0.975 – 0.982

1-Vinyl-2-pyrrolidone C₅H₈NO Clear liquid.

Purity—Perform the test with 0.5 μL of 1-vinyl-2-pyrrolidone as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area of the solutions by the automatic integration method, and calculate the amount of 1-vinyl-2-pyrrolidone by the area percentage method: it is not less than 99.0%.

Operating conditions
Detector: A hydrogen flame-ionization detector.
Column: A hollow, capillary glass column about 0.53 mm in inside diameter and about 30 m in length, having an about 1.0-μm layer of polyethylene glycol 20 M for gas chromatography on the inner side.

Column temperature: Maintain the temperature at 80°C for 1 minute, then raise at the rate of 10°C per minute to 190°C, and hold constant to the temperature for 20 minutes.

Temperature of sample vaporization chamber: A constant temperature of about 190°C.
Carrier gas: Helium.
Flow rate: Adjust the flow rate so that the retention time of 1-vinyl-2-pyrrolidone is about 15 minutes.
Detection sensitivity: Adjust the detection sensitivity so that the peak height of 1-vinyl-2-pyrrolidone from 0.5 μL of 1-vinyl-2-pyrrolidone is about 70% of the full scale.

Time span of measurement: About twice as long as the retention time of 1-vinyl-2-pyrrolidone beginning after the solvent peak.

Water <2.48>—Take 50 mL of methanol for Karl Fischer method and 10 mL of butyrolactone in a dry titration flask, and titrate with Karl Fischer TS until end point. Weigh accurately about 2.5 g of 1-vinyl-2-pyrrolidone, transfer immediately to a titration flask, and perform the test: water is not more than 0.1%.

V₈ protease A protease obtained from Staphylococcus aureus strain. When an amount of the enzyme hydrolyzes 1 mg/mL. Keep at a cold place and use within 6 days after preparation.

Voglibose for assay C₁₀H₁₂NO₇ [Same as the monograph Voglibose]

Warfarin potassium for assay [Same as the monograph Warfarin Potassium. When dried, it contains not less than 99.0% of warfarin potassium (C₁₉H₁₅KO₄).]

25% Water containing benzoil peroxide See Benzoil peroxide, 25% water containing.

Water for ammonium limit test To 1500 mL of water add cautiously 4.5 mL of sulfuric acid, distil using a hard glass distiller, discard the sufficient volume of first distillate, and use the remaining distillate (ammonium-free water) as the water for ammonium limit test.

Purity—Mix 40 mL of water for ammonium limit test with 6.0 mL of phenol-sodium pentacyanonitrosylferrate (III) TS. Add 4.0 mL of sodium hypochlorite-sodium hydroxide TS, mix, and allow to stand for 60 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbance at 640 nm is not more than 0.010.

Water for bacterial endotoxins test [Use the water described under the monographs of Water for Injection or Sterile Water for Injection in Containers, or the water produced by other procedures that shows no reaction with the lysate reagent employed, at the detection limit of the reagent, and is suitable for bacterial endotoxins test.]

Water for injection [Use the water described under the monographs of Water for Injection or Sterile Water for Injection in Containers. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of relevant test.]

Water, sterile purified [Use the water described by the monograph of Sterile Purified Water in Containers. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of relevant test.]

Wijs’ TS Transfer 7.9 g of iodine trichloride and 8.9 g of iodine to separate flasks, dissolve each with acetic acid (100), mix both solutions, and add acetic acid (100) to make 1000 mL. Preserve in light-resistant, glass containers.

Wogonin for thin-layer chromatography C₂₀H₂₀O₆ Yellow crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 204 – 208°C

Identification—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits maxima between 207 nm and 211 nm, and between 273 nm and 277 nm.

Purity Related substances—Dissolve 1 mg in 1 mL of methanol, and perform the test with 10 μL of this solution as directed in the Identification (3) under Saireito Extract: no spot other than the principal spot (Rf value is about 0.4) appears.

Xanthene C₁₃H₁₀O White to light yellow crystals or crystalline powder, having a slight, characteristic odor.

Melting point <2.48>: 98 – 102°C

Water <2.48>: not more than 0.5% (0.15 g).

Xanthene-9-carboxylic acid C₁₅H₁₂O₃ Dissolve 0.25 g of propantheline bromide in 5 mL of water and 10 mL of sodium hydroxide TS, heat the mixture to boiling, then continue to heat for 2 minutes. Cool to 60°C, add 5 mL of dilute sulfuric acid, cool, filter the precipitate, and wash thoroughly with water. Recrystallize the residue from dilute ethanol, and dry for 3 hours in a desiccator (in vacuum, silica gel).

Melting point <2.60>: 217 – 222°C

Xanthone C₁₃H₁₆O₂ Light yellow powder. Freely soluble in chloroform, and slightly soluble in hot water and in diethyl ether.

Melting point <2.60>: 174 – 176°C

Purity Related substances—Dissolve 0.050 g of xanthone in chloroform to make exactly 10 mL. Perform the test with 5 μL of this solution as directed in the Purity under Propan-
Xanthodyl \( \text{C}_9\text{H}_2\text{O}_2 \) White to pale yellow powder. Dissolves in ethanol (95%), in diethyl ether, in chloroform, and in acetic acid (100), and is practically insoluble in water.

Melting point 〈2.60〉: 121 - 124°C
Residue on ignition 〈2.44〉: not more than 2.0% (0.5 g).

Xylene \( \text{C}_6\text{H}_4(\text{CH}_3)_2 \) 〈K 8271, First class〉

\( \alpha \)-Xylene \( \text{C}_6\text{H}_4(\text{CH}_3)_2 \) Colorless, clear liquid.
Refractive index 〈2.45〉: \( n_\rho^2 \): 1.501 - 1.506
Specific gravity 〈2.56〉: \( d_\rho^2 \): 0.875 - 0.885
Distilling range 〈2.57〉: 143 - 146°C, not less than 95 vol%.

Xylene cyanol FF \( \text{C}_3\text{H}_2\text{N}_2\text{Na}_2\text{O}_8\text{Zn}.4\text{H}_2\text{O} \) White powder. The pH of a solution of 0.01 mol/L bismuth nitrate VS until the color of the solution changes from yellow to red (indicator: 2 drops of xylenol orange TS).

\( \alpha \)-Xylene \( \text{C}_6\text{H}_4(\text{CH}_3)_2 \) Colorless, clear liquid.
Refractive index 〈2.45〉: \( n_\rho^2 \): 1.501 - 1.506
Specific gravity 〈2.56〉: \( d_\rho^2 \): 0.875 - 0.885
Distilling range 〈2.57〉: 143 - 146°C, not less than 95 vol%.

Xylene cyanol FF

Zinc acetate dihydrate \( \text{Zn(CH}_3\text{COO})_2.2\text{H}_2\text{O} \) 〈K 8356, Special class〉

Zinc, arsenic-free See zinc for arsenic analysis.

Zinc chloride \( \text{ZnCl}_2 \) 〈K 8111, Special class〉

Zinc chloride TS Dissolve 10 g of zinc chloride and 10 g of potassium hydrogen phthalate in 900 mL of water, adjust the pH to 4.0 with sodium hydroxide TS, and add water to make 1000 mL.

0.04 mol/L Zinc chloride TS Dissolve 5.452 g of zinc chloride in water to make 1000 mL.

Zinc diethylidithiocarbamate See Test Methods for Plastic Containers 〈7.02〉.

Zinc dibutylidithiocarbamate See Test Methods for Plastic Containers 〈7.02〉.

Zinc disodium ethylenediamine tetraacetate See zinc disodium ethylenediamine tetraacetate tetrahydrate.

Zinc disodium ethylenediamine tetraacetate tetrahydrate \( \text{C}_9\text{H}_2\text{N}_2\text{Na}_2\text{O}_4\text{Zn}.4\text{H}_2\text{O} \) White powder. The pH of a solution of zinc disodium ethylenediamine tetraacetate (1 in 100) is between 6.0 and 9.0.

Purity Clarity and color of solution—Dissolve 0.1 g of zinc disodium ethylenediamine tetraacetate tetrahydrate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

Content: not less than 98.0%. Assay—Dissolve about 0.5 g of zinc disodium ethylenediamine tetraacetate tetrahydrate, accurately weighed, in water to make exactly 100 mL. Pipet 10 mL of this solution, adjust the pH to about 2 with 80 mL of water and dilute nitric acid, and titrate 〈2.50〉 with 0.01 mol/L bismuth nitrate VS until the color of the solution changes from yellow to red (indicator: 2 drops of xylene orange TS).

Each mL of 0.01 mol/L bismuth nitrate VS = 4.716 mg of \( \text{C}_9\text{H}_2\text{N}_2\text{Na}_2\text{O}_4\text{Zn}.4\text{H}_2\text{O} \).

Zinc dust See zinc powder.

Zinc for arsenic analysis \( \text{Zn} \) 〈K 8012〉 Use granules of about 800 \( \mu \)m.

Zinc iodide-starch TS To 100 mL of boiling water add a solution of 0.75 g of potassium iodide in 5 mL of water, a solution of 2 g of zinc chloride in 10 mL of water and a smooth suspension of 5 g of starch in 30 mL of water, with stirring. Continue to boil for 2 minutes, then cool.

Sensitivity—Dip a glass rod into a mixture of 1 mL of 0.1 mol/L sodium nitrite VS, 500 mL of water and 10 mL of hydrochloric acid, and touch on zinc iodide-starch paste TS: an apparently blue color appears.

Storage—Preserve in tightly stoppered bottles, in a cold place.

Zincon \( \text{C}_{20}\text{H}_{15}\text{N}_2\text{Na}_2\text{O}_4 \) A dark red to purple powder.

Identification—Determine the infrared absorption spectrum of zincon, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry 〈2.25〉: it exhibits absorption at the wave numbers of about 1604 cm\(^{-1}\), 1494 cm\(^{-1}\), 1294 cm\(^{-1}\), 1194 cm\(^{-1}\), 1110 cm\(^{-1}\), 1046 cm\(^{-1}\) and 764 cm\(^{-1}\).
Preserve in a light-resistant tight container.

**Zinc**

**Zinc sulfate** See zinc sulfate heptahydrate.

**Zinc sulfate for volumetric analysis** See zinc sulfate heptahydrate.

**Zinc sulfate heptahydrate** ZnSO$_4$·7H$_2$O [K 8953, Special class]

**Zinc sulfate TS** Dissolve 10 g of zinc sulfate heptahydrate in water to make 100 mL.

**Zirconyl-alizarin red S TS** Dissolve 0.2 g of zirconyl nitrate in 5 mL of dilute hydrochloric acid, add 10 mL of alizarin red S TS, and then add water to make 30 mL.

**Zirconyl-alizarin S TS** See zirconyl-alizarin red S TS.

**Zirconyl nitrate** See zirconyl nitrate dihydrate.

**Zirconyl nitrate dihydrate** ZrO(NO$_3$)$_2$·2H$_2$O A white crystalline powder. Freely soluble in water.

*Identification*—(1) To 5 mL of a solution (1 in 20) add 5 mL of sodium hydroxide TS: a white, milky precipitate is formed.

(2) To 10 mL of a solution (1 in 20) add 10 mL of sulfuric acid, cool, and superimpose 2 mL of iron (II) sulfate TS: a brown ring is produced at the zone of contact.

**Zolpidem tartrate for assay** (C$_{19}$H$_{21}$N$_3$O)$_2$·C$_4$H$_6$O$_6$ [Same as the monograph Zolpidem Tartrate. It contains not less than 99.5% of zolpidem tartrate [(C$_{19}$H$_{21}$N$_3$O)$_2$·C$_4$H$_6$O$_6$], calculated on the anhydrous basis.]

### 9.42 Solid Supports/Column Packings for Chromatography

**Aminopropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Carbamoyl group bound silica gel for liquid chromatography** Prepared for liquid chromatography.

**Cellulose for thin-layer chromatography** Use a high-grade cellulose prepared for thin-layer chromatography.

**Cellulose with fluorescent indicator for thin-layer chromatography** Use cellulose for thin-layer chromatography containing a suitable fluorescent substance.

**Cyanopropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**DEAE-cross-linking dextran anion exchanger (Cl type), slightly alkaline** Slightly alkaline anion exchanger prepared by introducing diethylaminoethyl group into cross-linking dextran of gel filtration carrier.

**Diethylaminoethyl cellulose for column chromatography** Prepared for column chromatography.

**Diethylaminomethyl group bound to synthetic polymer for liquid chromatography** Produced by binding diethylaminomethyl group to a hydrophilic synthetic polymer, for liquid chromatography. Exchange volume is about 0.1 mg equivalents/cm$^3$.

**Dimethylaminopropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Dimethyilsilanized silica gel with fluorescent indicator for thin-layer chromatography** Dimethyilsilanized silica gel for thin-layer chromatography to which a fluorescent indicator is added.

**Diol silica gel for liquid chromatography** Prepared for liquid chromatography.

**Divinylbenzene-methacrylate co-polymer for liquid chromatography** Prepared for liquid chromatography.

**Divinylbenzene-N-vinyl pyrrolidone copolymer for column chromatography** Prepared for column chromatography.

**Fluorosilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Gel-type strong acid cation-exchange resin for liquid chromatography (degree of cross-linkage: 8%)** Prepared for liquid chromatography.

**Gel type strong acid ion-exchange resin for liquid chromatography (degree of cross-linkage: 6%)** Prepared for liquid chromatography.

**Glycol etherifized silica gel for liquid chromatography** Glycol group is bound to silica gel for liquid chromatography.

**Graphite carbon for gas chromatography** Prepared for gas chromatography.

**Hexasilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Hydrophilic silica gel for liquid chromatography** Diolized porous silica gel prepared for liquid chromatography (5-10 μm in particle diameter).

**Hydroxypropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Neutral alumina for chromatography** Prepared for chromatography (75 – 180 μm in particle diameter).

**Neutral alumina for column chromatography** Prepared for column chromatography.

**Octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography** Prepared for liquid chromatography.

**Octadecylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Octadecylsilanized silica gel for thin-layer chromatography** Octadecylsilanized silica gel prepared for thin-layer chromatography.

**Octadecylsilanized silica gel with fluorescent indicator for thin-layer chromatography** Octadecylsilanized silica gel for thin-layer chromatography containing fluorescent indicator.
Octadecylsilanized silicone polymer coated silica gel for liquid chromatography Prepared for liquid chromatography.

Octylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Pentaethylenehexamminated polyvinyl alcohol polymer bead for liquid chromatography Prepared for liquid chromatography.

Phenyalted silica gel for liquid chromatography Prepared for liquid chromatography.

Phenylisilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Polyamide for column chromatography Prepared for column chromatography.

Polyamide for thin-layer chromatography Prepared for thin-layer chromatography.

Polyamide with fluorescent indicator for thin-layer chromatography Add a fluorescent indicator to polyamide for thin-layer chromatography.

Porous acrylonitrile-divinylbenzene copolymer for gas chromatography (pore diameter: 0.06 – 0.08 \(\mu m\), 100 – 200 \(m^2/g\)) A porous acrylonitrile-divinylbenzene copolymer prepared for gas chromatography. The average pore diameter is 0.075 \(\mu m\), and surface area is 500 to 600 \(m^2/g\).

Porous ethyl vinybenzene-divinylbenzene copolymer for gas chromatography Prepared for gas chromatography.

A porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0075 \(\mu m\), 500 – 600 \(m^2/g\)) A porous ethylvinylbenzene-divinylbenzene copolymer prepared for gas chromatography. The average pore diameter is 0.0075 \(\mu m\), and surface area is 500 to 600 \(m^2/g\).

Porous polymer beads for gas chromatography Prepared for gas chromatography.

Porous silica gel for liquid chromatography A porous silica gel prepared for liquid chromatography.

Porous styrene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0085 \(\mu m\), 300 – 400 \(m^2/g\)) A porous styrene-divinylbenzene copolymer prepared for gas chromatography. The average pore diameter is 0.0085 \(\mu m\), and surface area is 300 to 400 \(m^2/g\).

Porous styrene-divinylbenzene copolymer for liquid chromatography A porous styrene-divinylbenzene copolymer prepared for liquid chromatography.

Quaternary alkylaminated styrene-divinylbenzene copolymer for liquid chromatography Prepared for liquid chromatography.

Silica gel for gas chromatography A silica gel prepared for gas chromatography.

Silica gel for liquid chromatography A silica gel prepared for liquid chromatography.

Silica gel for liquid chromatography with attached carbamoyl groups Prepared for liquid chromatography.

Silica gel for thin-layer chromatography A silica gel prepared for thin-layer chromatography.

Silica gel for thin-layer chromatography (particle size 5 – 7 \(\mu m\), with fluorescent indication) Prepared for high-performance thin-layer chromatography.

Silica gel with complex fluorescent indicator for thin-layer chromatography A silica gel for thin-layer chromatography containing suitable complex fluorescent indicators.

Silica gel with fluorescent indicator for thin-layer chromatography A silica gel for thin-layer chromatography containing a suitable fluorescent indicator.

Siliceous earth for chromatography A siliceous earth prepared for chromatography.

Siliceous earth for gas chromatography A siliceous earth prepared for gas chromatography.

Slightly acidic ion-exchange silica gel for liquid chromatography Prepared for liquid chromatography.

Strongly acidic ion-exchange resin for column chromatography Prepared for column chromatography.

Strongly acidic ion-exchange resin for liquid chromatography Prepared for liquid chromatography.

Strongly acidic ion-exchange silica gel for liquid chromatography Prepared for liquid chromatography.

Strongly basic ion-exchange resin for column chromatography Prepared for column chromatography.

Styrene-divinylbenzene copolymer for liquid chromatography Prepared for liquid chromatography.

Sulfonamide group bound to hexadecylsilanized silica gel for liquid chromatography Prepared for column chromatography.

Synthetic magnesium silicate for column chromatography Prepared for column chromatography (150 – 250 \(\mu m\) in particle diameter).

Teflon for gas chromatography See Tetrafluoroethylene polymer for gas chromatography.

Terephthalic acid for gas chromatography \(C_8H_6(COOH)_2\) Prepared for gas chromatography.

Tetrafluoroethylene polymer for gas chromatography Prepared for gas chromatography.

Trimethylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Weakly acidic CM-bridged cellulose cation exchanger (H type) Weakly acidic cation exchanger, intensified by crosslinking porous spherical cellulose, into which carboxymethyl groups have been introduced.

Weakly acidic ion exchange resin for liquid chromatography Prepared for liquid chromatography.

Weakly acidic ion exchange silica gel for liquid chromatography Prepared for liquid chromatography.

Zeolite for gas chromatography (0.5 nm in pore diameter) Zeolite prepared for gas chromatography.
9.43 Filter Papers, Filters for filtration, Test Papers, Crucibles, etc.

Filter paper  [R 3801, Filter paper (for chemical analysis), Filter paper for qualitative analysis]
No.1: For bulky gelatinous precipitate
No.2: For moderate-sized precipitate
No.3: For fine precipitate
No.4: Hardened filter paper for fine precipitate

Filter paper for quantitative analysis  [R 3801, Filter paper (for chemical analysis), Filter paper for quantitative analysis]
No. 5A: For bulky gelatinous precipitate
No. 5B: For moderate-sized precipitate
No. 5C: For fine precipitate
No. 6: Thin filter paper for fine precipitate

Porcelain crucible  [R 1301, Porcelain crucible for chemical analysis]

Sintered glass filter  [R 3503, Glass appliance for chemical analysis, Buchner funnel glass filter]
G3: 20–30 μm in pore size
G4: 5–10 μm in pore size

Blue litmus paper  See litmus paper, blue.

Congo red paper  Immerse filter paper in congo red TS, and air-dry.

Glass fiber  See glass wool.

Glass wool  [K 8251, Special class]

Lead acetate paper  See lead (II) acetate paper.

Lead (II) acetate paper  Usually, immerse strips of filter paper, 6 cm × 8 cm in size, in lead (II) acetate TS, drain off the excess liquid, and dry the paper at 100°C, avoiding contact with metals.

Litmus paper, blue  [K 9071, Litmus paper, Blue litmus paper]

Litmus paper, red  [K 9071, Litmus paper, Red litmus paper]

Phosgene test paper  Dissolve 5 g of 4-dimethylaminobenzaldehyde and 5 g of diphenylamine in 100 mL of ethanol (99.5). Immerse a filter paper 5 cm in width in this solution, and allow to dry spontaneously while the paper is suspended in a dark place under clear air. Then cut off the 5-cm portions from the upper side and lower side of the paper, and cut the remaining paper to a length of 7.5 cm.

Preserve in tight, light-resistant containers. Do not use the paper, which has changed to a yellow color.

Potassium iodate-starch paper  Impregnate filter paper with a mixture of equivalent volumes of a solution of potassium iodate (1 in 20) and freshly prepared starch TS, and dry in a clean room.

Storage—Preserve in a glass-stoppered bottle, protected from light and moisture.

Red litmus paper  See litmus paper, red.

Turmeric paper  Macerate 20 g of powdered dried rhizome of Curcuma longa Linné with four 100 mL-portions of cold water, decant the supernatant liquid each time, and discard it. Dry the residue at a temperature not over 100°C. Macerate the dried residue with 100 mL of ethanol (95) for several days, and filter. Immerse filter paper in this ethanol decoction, and allow the ethanol (95) to evaporate spontaneously in clean air.

Sensitivity—Dip a strip of turmeric paper, about 1.5 cm length, in a solution of 1 mg of boric acid in a mixture of 1 mL of hydrochloric acid and 4 mL of water, after 1 minute remove the paper from the liquid, and allow it to dry spontaneously: the yellow color changes to brown. When the strip is moistened with ammonia TS, the color of the strip changes to greenish black.

Zinc iodide-starch paper  Impregnate the filter paper for volumetric analysis with freshly prepared zinc iodide-starch TS, and dry in the clean room. Preserve in a glass-stoppered bottle, protected from light and moisture.

9.44 Standard Particles, etc.

α-Alumina for specific surface area determination
α-Al2O3  Prepared for specific surface area determination.

α-Alumina for thermal analysis  α-Al2O3  Prepared for thermal analysis.

Calibration ball for particle density measurement
Calibration ball with a known volume prepared for measurement of particle density. The volume of the calibration ball must be accurately determined to the nearest 0.001 cm³.

Indium for thermal analysis  In  Prepared for thermal analysis.

Content: not less than 99.99%.

Nickel for thermal analysis  [K 9062 (Nickel). Content: not less than 99.99%]

Standard particles for calibrating light-shielded automatic fine particle counter
Use plastic spherical particles of known size and number.

Sn  [K 8580 (Tin). Content: not less than 99.99%]
Measuring Instruments and Appliances, Thermometers, etc.

9.61 Optical Filters for Wavelength and Transmission Rate Calibration

Use optical filters for wavelength calibration and those for transmission rate calibration shown in Table 9.61-1 and Table 9.61-2, respectively. The optical filters for transmission rate calibration are also used for the calibration of absorbances.

### Table 9.61-1 Optical Filters for Wavelength Calibration

<table>
<thead>
<tr>
<th>Type of filter</th>
<th>Range of wavelength calibration (nm)</th>
<th>Product name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neodymium optical filter for wavelength calibration</td>
<td>400 – 750</td>
<td>JCRM 001</td>
</tr>
<tr>
<td>Holmium optical filter for wavelength calibration</td>
<td>250 – 600</td>
<td>JCRM 002</td>
</tr>
</tbody>
</table>

### Table 9.61-2 Optical Filters for Transmission Rate Calibration

<table>
<thead>
<tr>
<th>Type of filter</th>
<th>Transmission rate for calibration (%)</th>
<th>Product name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical filter for calibration within the visible wavelength range</td>
<td>1</td>
<td>JCRM 101</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>JCRM 110</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>JCRM 120</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>JCRM 130</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>JCRM 140</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>JCRM 150</td>
</tr>
<tr>
<td>Optical filter for calibration within the ultraviolet wavelength range</td>
<td>10</td>
<td>JCRM 210 A</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>JCRM 250 A</td>
</tr>
<tr>
<td>Optical filter for calibration within the near-ultraviolet wavelength range</td>
<td>10</td>
<td>JCRM 310</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>JCRM 330</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>JCRM 350</td>
</tr>
</tbody>
</table>

9.62 Measuring Instruments, Appliances

Measuring Instruments are the instruments or machines used for measuring mass or volume in the JP tests, and Appliances are the instruments specified in order to make test conditions as consistent as possible in those tests.

**Balances and weights**

1. Chemical balances—Use balances readable to the extent of 0.1 mg.
2. Semimicrobalances—Use balances readable to the extent of 10 µg.
3. Microbalances—Use balances readable to the extent of 1 µg.
4. Weights—Use calibrated weights.

**Carbon dioxide measuring detector tube** [Gas detector tube measurement system K 0804] Packed with measurement packing for carbon dioxide.

**Carbon monoxide measuring detector tube** [Gas detector tube measurement system K 0804] Packed with measurement packing for carbon monoxide.

**Cassia flask** Use glass-stoppered flasks, shown in Fig. 9.62-1, made of hard glass and having graduation lines of volume on the neck.

**Gas mixer** Use the apparatus, shown in Fig. 9.62-3, made of hard glass.

**Nessler tube** Use colorless, glass-stoppered cylinders 1.0 to 1.5 mm in thickness, shown in Fig. 9.62-2, made of hard glass. The difference of the height of the graduation line of 50 mL from the bottom among cylinders does not exceed 2 mm.

**Sieves** Sieves conform to the specifications in Table 9.62-1. Use the sieve number of nominal size as the designation.

**Volumetric measures** Use volumetric flasks, transfer pipets, push-button micropipets, burets and measuring cylinders conforming to the Japanese Industrial Standard.
9.63 Thermometers

Ordinarily, use calibrated thermometers with an immersion line (rod) or calibrated total immersion mercury-filled thermometers according to the Japanese Industrial Standards. Use the thermometers with the immersion line (rod), shown in Table 9.63-1, for the tests in Congealing Point, Melting Point (Method 1), Boiling Point and Distilling Range.

A: Gas buret (capacity of 100 mL, about 13.7 mm in inside diameter, graduated in 0.2 mL divisions, and graduated in 0.1 mL divisions at the lower, narrow part).

B: Gas buret (capacity of 100 mL, about 4.2 mm in inside diameter at the upper stem with graduation in 0.02 mL division, about 28.5 mm in inside diameter at the lower stem with graduation in 1 mL divisions).

C: (C₁, C₂, C₃ and C₄): Three-way stopcock.

D: Inlet of sample (bent forward at 20 mm in length).

E: Outlet of mixed gas (bent forward at 20 mm in length).

F: Jacket (about 770 mm in length, about 40 mm in outside diameter, almost completely filled with water at room temperature).

G: Rubber pressure tubing, about 4 mm in inside diameter (G₁: about 80 cm in length; G₂ and G₃: about 120 cm in length).

H: Heavy-wall capillary tube (about 1 mm in inside diameter).

K: Receiver.

L₁: Leveling bulb (L₁: filled with about 50 mL of mercury; L₂ and L₃: filled with about 150 mL of mercury).

Fig. 9.62-3
Table 9.62-1  Specification of Sieves

<table>
<thead>
<tr>
<th>Sieve number</th>
<th>Nominal size (μm)</th>
<th>Nominal opening (mm)</th>
<th>Permissible variation of opening (mm)</th>
<th>Diameter of wire (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average</td>
<td>Maximum</td>
</tr>
<tr>
<td>3.5</td>
<td>5600</td>
<td>5.60</td>
<td>±0.18</td>
<td>0.47</td>
</tr>
<tr>
<td>4</td>
<td>4750</td>
<td>4.75</td>
<td>±0.15</td>
<td>0.41</td>
</tr>
<tr>
<td>4.7</td>
<td>4000</td>
<td>4.00</td>
<td>±0.13</td>
<td>0.37</td>
</tr>
<tr>
<td>5.5</td>
<td>3350</td>
<td>3.35</td>
<td>±0.11</td>
<td>0.32</td>
</tr>
<tr>
<td>6.5</td>
<td>2800</td>
<td>2.80</td>
<td>±0.09</td>
<td>0.29</td>
</tr>
<tr>
<td>7.5</td>
<td>2360</td>
<td>2.36</td>
<td>±0.08</td>
<td>0.25</td>
</tr>
<tr>
<td>8.6</td>
<td>2000</td>
<td>2.00</td>
<td>±0.07</td>
<td>0.23</td>
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<tr>
<td>10</td>
<td>1700</td>
<td>1.70</td>
<td>±0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>12</td>
<td>1400</td>
<td>1.40</td>
<td>±0.05</td>
<td>0.18</td>
</tr>
<tr>
<td>14</td>
<td>1180</td>
<td>1.18</td>
<td>±0.04</td>
<td>0.16</td>
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<tr>
<td>16</td>
<td>1000</td>
<td>1.00</td>
<td>±0.03</td>
<td>0.14</td>
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<tr>
<td>18</td>
<td>850</td>
<td>0.850</td>
<td>±0.029</td>
<td>0.127</td>
</tr>
<tr>
<td>22</td>
<td>710</td>
<td>0.710</td>
<td>±0.025</td>
<td>0.112</td>
</tr>
<tr>
<td>26</td>
<td>600</td>
<td>0.600</td>
<td>±0.021</td>
<td>0.101</td>
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<tr>
<td>30</td>
<td>500</td>
<td>0.500</td>
<td>±0.018</td>
<td>0.089</td>
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<tr>
<td>36</td>
<td>425</td>
<td>0.425</td>
<td>±0.016</td>
<td>0.081</td>
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<tr>
<td>42</td>
<td>355</td>
<td>0.355</td>
<td>±0.013</td>
<td>0.072</td>
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<td>50</td>
<td>300</td>
<td>0.300</td>
<td>±0.012</td>
<td>0.065</td>
</tr>
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<td>60</td>
<td>250</td>
<td>0.250</td>
<td>±0.0099</td>
<td>0.058</td>
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<tr>
<td>70</td>
<td>212</td>
<td>0.212</td>
<td>±0.0087</td>
<td>0.052</td>
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<tr>
<td>83</td>
<td>180</td>
<td>0.180</td>
<td>±0.0076</td>
<td>0.047</td>
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<td>100</td>
<td>150</td>
<td>0.150</td>
<td>±0.0066</td>
<td>0.043</td>
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<td>119</td>
<td>125</td>
<td>0.125</td>
<td>±0.0058</td>
<td>0.038</td>
</tr>
<tr>
<td>140</td>
<td>106</td>
<td>0.106</td>
<td>±0.0052</td>
<td>0.035</td>
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<tr>
<td>166</td>
<td>90</td>
<td>0.090</td>
<td>±0.0046</td>
<td>0.032</td>
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<tr>
<td>200</td>
<td>75</td>
<td>0.075</td>
<td>±0.0041</td>
<td>0.029</td>
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<tr>
<td>235</td>
<td>63</td>
<td>0.063</td>
<td>±0.0037</td>
<td>0.026</td>
</tr>
<tr>
<td>282</td>
<td>53</td>
<td>0.053</td>
<td>±0.0034</td>
<td>0.024</td>
</tr>
<tr>
<td>330</td>
<td>45</td>
<td>0.045</td>
<td>±0.0031</td>
<td>0.022</td>
</tr>
<tr>
<td>391</td>
<td>38</td>
<td>0.038</td>
<td>±0.0029</td>
<td>0.020</td>
</tr>
</tbody>
</table>
### Thermometers with Immersion Line

<table>
<thead>
<tr>
<th>No.</th>
<th>Liquid</th>
<th>Gas filled above liquid</th>
<th>Temperature range</th>
<th>Minimum graduation</th>
<th>Longer graduation lines at</th>
<th>Graduation numbered at</th>
<th>Total length (mm)</th>
<th>Stem diameter (mm)</th>
<th>Bulb length (mm)</th>
<th>Distance from bottom of bulb to graduation at the lowest temperature (mm)</th>
<th>Distance from top of thermometer to graduation at the highest temperature (mm)</th>
<th>From of top of thermometer loop</th>
<th>Test temperature</th>
<th>Maximum scale error at any point</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>Mercury</td>
<td>Nitrogen or Argon</td>
<td>−17 – 50°C</td>
<td>0.2°C</td>
<td>each 1°C</td>
<td>280 – 300</td>
<td>6.0 ± 0.3</td>
<td>12 – 18</td>
<td>75 – 90</td>
<td>35 – 65</td>
<td>−15°C, 15°C, 45°C</td>
<td>0.2°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 2</td>
<td>Mercury</td>
<td>Nitrogen or Argon</td>
<td>40 – 100°C</td>
<td>0.2°C</td>
<td>each 1°C</td>
<td>280 – 300</td>
<td>6.0 ± 0.3</td>
<td>12 – 18</td>
<td>75 – 90</td>
<td>35 – 65</td>
<td>45°C, 70°C, 95°C</td>
<td>0.2°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 3</td>
<td>Mercury</td>
<td>Nitrogen or Argon</td>
<td>90 – 150°C</td>
<td>0.2°C</td>
<td>each 1°C</td>
<td>280 – 300</td>
<td>6.0 ± 0.3</td>
<td>12 – 18</td>
<td>75 – 90</td>
<td>35 – 65</td>
<td>95°C, 120°C, 145°C</td>
<td>0.2°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 4</td>
<td>Mercury</td>
<td>Nitrogen or Argon</td>
<td>140 – 200°C</td>
<td>0.2°C</td>
<td>each 1°C</td>
<td>280 – 300</td>
<td>6.0 ± 0.3</td>
<td>12 – 18</td>
<td>75 – 90</td>
<td>35 – 65</td>
<td>145°C, 170°C, 195°C</td>
<td>0.2°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 5</td>
<td>Mercury</td>
<td>Nitrogen or Argon</td>
<td>190 – 250°C</td>
<td>0.2°C</td>
<td>each 1°C</td>
<td>280 – 300</td>
<td>6.0 ± 0.3</td>
<td>12 – 18</td>
<td>75 – 90</td>
<td>35 – 65</td>
<td>195°C, 220°C, 245°C</td>
<td>0.2°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 6</td>
<td>Mercury</td>
<td>Nitrogen or Argon</td>
<td>240 – 320°C</td>
<td>0.2°C</td>
<td>each 1°C</td>
<td>280 – 300</td>
<td>6.0 ± 0.3</td>
<td>12 – 18</td>
<td>75 – 90</td>
<td>35 – 65</td>
<td>220°C, 245°C, 280°C, 315°C</td>
<td>0.2°C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The values in the table are approximate and subject to slight variations based on the manufacturer's specifications.
Absorptive Cream

吸水クリーム

Method of preparation

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Petrolatum</td>
<td>400 g</td>
</tr>
<tr>
<td>Cetanol</td>
<td>100 g</td>
</tr>
<tr>
<td>White Beeswax</td>
<td>50 g</td>
</tr>
<tr>
<td>Sorbitan Sesquiole</td>
<td>50 g</td>
</tr>
<tr>
<td>Lauromacrogol</td>
<td>5 g</td>
</tr>
<tr>
<td>Ethyl Parahydroxybenzoate or Methyl Parahydroxybenzoate</td>
<td>1 g</td>
</tr>
<tr>
<td>Butyl Parahydroxybenzoate or Propyl Parahydroxybenzoate</td>
<td>1 g</td>
</tr>
<tr>
<td>Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Melt White Petroleum, Cetanol, White Beeswax, Sorbitan Sesquiole and Lauromacrogol by heating on a water bath, mix and maintain at about 75°C. Add Ethyl Parahydroxybenzoate or Butyl Parahydroxybenzoate to Purified Water or Purified Water in Containers, dissolve by warming at 80°C. Combine both solutions, mix to make emulsion, cool, and stir thoroughly until it congeals.

Description

Absorptive Cream is white in color and is lustrous. It has a slightly characteristic odor.

Containers and storage

Containers—Tight containers.

Acebutolol Hydrochloride

アセプトロール塩酸塩

\[
\text{C}_{18}\text{H}_{28}\text{N}_{2}\text{O}_{4}\cdot\text{HCl}: 372.89
\]

\[\text{N-\{3-Acetyl-4-\{(2RS)-2-hydroxy-3-(1-methylethyl)aminopropoxy\}phenyl\}butanamide monohydrochloride}
\]

[34381-68-5]

Acebutolol Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of \(\text{C}_{18}\text{H}_{28}\text{N}_{2}\text{O}_{4}\cdot\text{HCl}\).

Description

Acebutolol Hydrochloride occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Acebutolol Hydrochloride (1 in 20) shows no optical rotation.

Identification

(1) Determine the absorption spectrum of a solution of Acebutolol Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\) and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Acebutolol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Acebutolol Hydrochloride (1 in 100) responds to the Qualitative Tests \(<1.00\) for chloride.

Melting point

\(<2.60\) 141 – 145°C

Purity

(1) Heavy metals \(<1.07\)—Proceed with 1.0 g of Acebutolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.11\)—Prepare the test solution with 1.0 g of Acebutolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 40 mg of Acebutolol Hydrochloride in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 25 mL, and pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\). Spot 5 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the upper layer of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying \(<2.41\) Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition \(<2.44\) Not more than 0.2% (1 g).

Assay

Weigh accurately about 0.25 g of Acebutolol Hydrochloride, previously dried, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate \(<2.50\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 37.29 mg of \(\text{C}_{18}\text{H}_{28}\text{N}_{2}\text{O}_{4}\cdot\text{HCl}\)

Containers and storage

Containers—Well-closed containers.
Aceglutamide Aluminum

アセグルタミドアルミニウム

\[
\text{C}_{35}\text{H}_{59}\text{Al}_3\text{N}_{10}\text{O}_{24}: 1084.84
\]

Pentakis[(2S)-2-acetylamoino-4-carbamoylbutanoato]tetrahydroxotrialuminium [12607-92-0]

Aceglutamide Aluminum contains not less than 85.4% and not more than 87.6% of aceglutamide \((\text{C}_7\text{H}_7\text{N}_2\text{O}_4: 188.18)\), and not less than 7.0% and not more than 8.0% of aluminum (Al: 26.98), calculated on the dried basis.

**Description** Aceglutamide Aluminum occurs as a white powder, having an astringent bitter taste.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It is hygroscopic.

**Identification**

(1) Dissolve 0.03 g each of Aceglutamide Aluminum and Aceglutamide RS in 5 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 \(\mu\)L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of I-propanol, water and acetic acid (100) (16:8:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of bromocresol green in ethanol (95) (1 in 100), then spray evenly diluted ammonia solution (28) (1 in 100): the spots from the sample solution and the standard solution show a light yellow and have the same \(R_f\) value.

(2) A solution of Aceglutamide Aluminum in dilute hydrochloric acid (1 in 20) responds to the Qualitative Tests <1.09> for aluminum salt.

**Optical rotation** <2.49> \(\alpha\)D20 \(= -5.5 - 7.5^\circ\) (2 g calculated on the dried basis, water, 50 mL, 100 mm).

**Purity**

(1) Heavy metals <1.07>—Put 1.0 g of Aceglutamide Aluminum in a porcelain crucible, cover the crucible loosely, and heat gently to carbonize. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat gently until the white fumes no more evolve, and heat to incinerate at 500 to 600°C. If the incineration is not accomplished, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat in the same manner as above, then ignite at 500 to 600°C to incinerate. After cooling, add 2 mL of hydrochloric acid, proceed with this solution according to Method 2, and perform the test. Prepare the control solution as follows: proceed in the same manner as the preparation of the test solution with the same amount of the reagents, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Aceglutamide Aluminum according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Aceglutamide Aluminum in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Separately, dissolve 10 mg of 2-acetamidoglutaramide in the mobile phase to make exactly 100 mL. Pipet 3 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 2-acetamidoglutaramide from the sample solution is not larger than that from the standard solution (2), the peak areas other than aceglutamide and 2-acetamidoglutaramide from the sample solution are not larger than 10/10 times the peak area of aceglutamide from the sample solution (1), and the total of the peak areas other than aceglutamide and 2-acetamidoglutaramide from the sample solution is not larger than the peak area of aceglutamide from the standard solution (1).

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of aceglutamide.

**System suitability**—

Test for required detection: To exactly 5 mL of the standard solution (1) add the mobile phase to make exactly 50 mL. Confirm that the peak area of aceglutamide obtained from 20 \(\mu\)L of this solution is equivalent to 7 to 13% of that of aceglutamide obtained from 20 \(\mu\)L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay (1).

System repeatability: When the test is repeated 6 times with 20 \(\mu\)L of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of aceglutamide is not more than 2.0%.

**Loss on drying** <2.41> Not more than 5.0% (1 g, 130°C, 5 hours).

**Assay**

(1) Aceglutamide—Weigh accurately about 50 mg of Aceglutamide Aluminum, dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 45 mg of Aceglutamide RS, dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, \(Q_s\) and \(Q_d\), of the peak area of aceglutamide to that of the internal standard.

\[
\text{Amount (mg) of Aceglutamide (C}_7\text{H}_7\text{N}_2\text{O}_4\text{)} = M_s \times Q_t/Q_s
\]

\(M_s\): Mass of Aceglutamide (C7H7N2O4) sampled

\(Q_t\): peak area of aceglutamide

\(Q_s\): peak area of internal standard
Mₙ: Amount (mg) of Aceglutamide RS

Internal standard solution—A solution of thymine in methanol (1 in 4000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of diluted perchloric acid (1 in 1000) and methanol (99:1).
Flow rate: Adjust the flow rate so that the retention time of aceglutamide is about 5 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, aceglutamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of aceglutamide to that of the internal standard is not more than 1.0%.

(2) Aluminum—Weigh accurately about 3.0 g of Aceglutamide Aluminum, add 20 mL of dilute hydrochloric acid, and heat on a water bath for 60 minutes. After cooling, add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.8, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate <2.5D> with 0.05 mol/L zinc acetate VS until the color of the solution changes from light dark green to light red (indicator: 2 mL of dithizone TS). Perform a blank determination in the same manner.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.349 mg of Al

Containers and storage—Containers—Tight containers.

Acemetacin

Acemetacin occurs as a light yellow crystalline powder.

It is soluble in acetone, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) To 1 mg of Acemetacin add 1 mL of concentrated chromotropic acid TS, and heat in a water bath for 5 minutes: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Acemetacin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Acemetacin as directed in the potassium bromide disk method under Infrared Spectrometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Acemetacin as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.6D> 151 – 154°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Acemetacin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.40 g of Acemetacin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 50 mL. Pipet 1 mL of this solution, add acetone to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin Layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin layer chromatography. Develop the plate with a mixture of hexane, 4-methyl-2-pentanone and acetic acid (100) (3:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 2 spots other than the principal spot appear from the sample solution, and these spots are not more intense than the spot obtained from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Acemetacin, previously dried, dissolve in 20 mL of acetone, add 10 mL of water, and then titrate <2.5D> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same method, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 41.58 mg of C₂₁H₁₈ClNO₆

Containers and storage—Containers—Tight containers.

Acemetacin, when dried, contains not less than 99.0% and not more than 101.0% of C₂₁H₁₈ClNO₆.
Acemetacin Capsules

アセメタシンカプセル

Acemetacin Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of acemetacin (C$_{21}$H$_{18}$ClNO$_6$). 415.82).

**Method of preparation** Prepare as directed under Capsules, with Acemetacin.

**Identification** To an amount of powdered contents of Acemetacin Capsules, equivalent to 0.1 g of Acemetacin according to the labeled amount, add 100 mL of methanol, shake well, and filter. Take 10 mL of the filtrate, and distil the methanol under reduced pressure. To the residue add 1 mL of methanol, shake well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of acemetacin in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03).

Spot 2 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, 4-methyl-2-pentanone and acetic acid (100) (3:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the sample solution and standard solution show the same $R_f$ value.

**Uniformity of dosage units** (6.02) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Acemetacin Capsules, add 40 mL of methanol, shake well, and add methanol to make exactly $V$ mL so that each mL contains about 0.6 mg of acemetacin (C$_{21}$H$_{18}$ClNO$_6$). Filter this solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 2 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

**Dissolution** (6.10) When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Acemetacin Capsules is not less than 70%.

Start the test with 1 capsule of Acemetacin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add the dissolution medium to make exactly $V$ mL so that each mL contains about 33 µg of acemetacin (C$_{21}$H$_{18}$ClNO$_6$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of acemetacin for assay, previously dried at 105°C for 2 hours, dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 319 nm as directed under Ultraviolet-visible Spectrophotometry (2.24).

Dissolution rate (%) with respect to the labeled amount of acemetacin (C$_{21}$H$_{18}$ClNO$_6$) = $M_S \times A_T / A_S \times V / V \times 1 / C \times 180$

$M_S$: Amount (mg) of acemetacin for assay

C: Labeled amount (mg) of acemetacin (C$_{21}$H$_{18}$ClNO$_6$) in 1 capsule

**Assay** Take out the contents of no less than 20 Acemetacin Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 30 mg of acemetacin (C$_{21}$H$_{18}$ClNO$_6$), add 40 mL of methanol, shake well, and add methanol to make exactly 50 mL. Filter this solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 2 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of acemetacin for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of acemetacin to that of the internal standard.

Amount (mg) of acemetacin (C$_{21}$H$_{18}$ClNO$_6$) = $M_S \times Q_T / Q_S$

$M_S$: Amount (mg) of acemetacin for assay

**Internal standard solution**—A solution of hexyl parahydroxybenzoate in methanol (1 in 1000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 6 g of acetic acid (100) add water to make 1000 mL, and adjust the pH to 3.2 with a solution of 1.36 g of sodium acetate trihydrate in 100 mL of water. To 200 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of acemetacin is about 7 minutes.

**System suitability**—

System performance: Dissolve 75 mg of acemetacin and 75 mg of indometacin in 50 mL of methanol. To 2 mL of this solution add 2 mL of the internal standard solution, and add methanol to make 50 mL. When the procedure is run with 20 µL of this solution under the above operating conditions, acemetacin, indometacin and the internal standard are eluted in this order with the resolutions between the peaks of acemetacin and indometacin and between the peaks of in-
dometacin and the internal standard being not less than 3, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acemetacin to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Tight containers.

Acemetacin Tablets

アセメタシン錠

Acemetacin Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of acemetacin (C₂₁H₁₈ClNO₆: 415.82).

Method of preparation  Prepare as directed under Tablets, with Acemetacin.

Identification  To a quantity of powdered Acemetacin Tablets, equivalent to 0.1 g of Acemetacin according to the labeled amount, add 100 mL of methanol, shake well, and filter. Take 10 mL of the filtrate, and distill the methanol under reduced pressure. Dissolve the residue in 1 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of acemetacin in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, 4-methyl-2-pentanone and acetic acid (100) (3:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the sample solution and standard solution show the same Rf value.

Uniformity of dosage units <6.02>  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Acemetacin Tablets add 3 mL of water, and shake until the tablet is disintegrated. Add 15 mL of methanol, shake for 20 minutes, and add methanol to make exactly 50 mL so that each mL contains about 33 μg of acemetacin (C₂₁H₁₈ClNO₆). Centrifuge this solution, filter the supernatant liquid, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, and use this solution as the sample solution. Proceed as directed in the Assay.

\[
M_S = \frac{M_T \times A_T / A_S \times V/V \times 1/C \times 100}{M_S}
\]

M₅: Amount (mg) of acemetacin for assay

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (1 in 250).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To 6 g of acetic acid (100) add water to make 1000 mL, and adjust the pH to 3.2 with a solution of

medium, the dissolution rate in 45 minutes of Acemetacin Tablets is not less than 80%.

Start the test with 1 tablet of Acemetacin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 33 μg of acemetacin (C₂₁H₁₈ClNO₆) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of acemetacin for assay, previously dried at 105°C for 2 hours, dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A₇₅ and A₃₁₉ of the sample solution and standard solution at 319 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of acemetacin (C₂₁H₁₈ClNO₆)

\[
M_S = \frac{M_T \times A_T / A_S \times V/V \times 1/C \times 100}{M_S}
\]

M₅: Amount (mg) of acemetacin for assay

C: Labeled amount (mg) of acemetacin (C₂₁H₁₈ClNO₆) in 1 tablet

Assay  Weigh accurately the mass of not less than 20 Acemetacin Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.6 g of acemetacin (C₂₁H₁₈ClNO₆), add 120 mL of methanol, shake for 20 minutes, and add methanol to make exactly 200 mL. Centrifuge this solution, filter the supernatant liquid, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of acemetacin for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q₇₅ and Q₃₁₉, of the peak area of acemetacin to that of the internal standard.

Amount (mg) of acemetacin (C₂₁H₁₈ClNO₆)

\[
M_S = \frac{M_T \times A_T / A_S \times V/V \times 1/C \times 100}{M_S}
\]

M₅: Amount (mg) of acemetacin for assay

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (1 in 250).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To 6 g of acetic acid (100) add water to make 1000 mL, and adjust the pH to 3.2 with a solution of
1.36 g of sodium acetate trihydrate in 100 mL of water. To 200 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of acetaminophen is about 7 minutes.

System suitability—
System performance: Dissolve 75 mg of acetaminophen and 75 mg of indomethacin in 50 mL of methanol. To 4 mL of this solution add 1 mL of the internal standard solution, and add methanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, acetaminophen, indomethacin and the internal standard are eluted in this order with the resolutions between the peaks of acetaminophen and indomethacin and between the peaks of indomethacin and the internal standard being not less than 3, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acetaminophen to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Acetaminophen

Paracetamol

Acetaminophen, when dried, contains not less than 98.0% of C₇H₉NO₂.

Description Acetaminophen occurs as white crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), sparingly soluble in water, and very slightly, soluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification Determine the infrared absorption spectra of Acetaminophen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Acetaminophen RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 169 – 172°C

Purity (1) Chloride <1.03>—Dissolve 4.0 g of Acetaminophen in 100 mL of water by heating, cool with shaking in ice water, allow to stand until ordinary temperature is attained, add water to make 100 mL, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Sulfate <1.14>—To 25 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Acetaminophen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Acetaminophen according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 50 mg of Acetaminophen in 1 mL of methanol, add the mobile phase to make 200 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the peak area of acemetacin from the sample solution is not larger than the peak area of acetaminophen from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogenphosphate, pH 4.7 and methanol (4:1)

Flow rate: Adjust the flow rate so that the retention time of acetaminophen is about 5 minutes.

Selection of column: Dissolve 0.01 g each of Acetaminophen and p-aminophenol in 1 mL of methanol, add the mobile phase to make 50 mL, to 1 mL of this solution add the mobile phase to make 10 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of p-aminophenol and acetaminophen in this order with the resolution between these peaks being not less than 7.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of acetaminophen obtained from 10 μL of the standard solution is about 15% of the full scale.

Time span of measurement: About 6 times as long as the retention time of acetaminophen beginning after the solvent peak.

Loss on drying <2.41> Not more than 0.3% (0.5 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Acetaminophen and Acetaminophen RS, previously dried, dissolve in 2 mL of methanol, and add water to make exactly 100 mL. Pipet 3 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Determine the absor-
Acetazolamide

\[
\text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2: 222.25
\]

\(\text{N}-(5\text{-Sulfamoyl-1,3,4-thiadiazol-2-yl})\text{acetamide} [59-66-5]\)

Acetazolamide contains not less than 98.0% and not more than 102.0% of \(\text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2\), calculated on the dried basis.

**Description** Acetazolamide occurs as a white to pale yellowish white, crystalline powder. It is odorless, and has a slight bitter taste.

It is slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

**Melting point:** About 255°C (with decomposition).

**Identification (1)** To 0.1 g of Acetazolamide add 5 mL of sodium hydroxide TS, then add 5 mL of a solution of 0.1 g of hydroxyaminomethylicloride and 0.05 g of copper (II) sulfate pentahydrate in 10 mL of water: a light yellow color develops. Then heat this solution for 5 minutes: a deep yellow color is produced gradually.

(2) To 0.02 g of Acetazolamide add 2 mL of dilute hydrochloric acid, boil for 10 minutes, cool, and add 8 mL of water: this solution responds to the Qualitative Tests \(<1.09\) for primary aromatic amines.

(3) To 0.2 g of Acetazolamide add 0.5 g of granulated zinc and 5 mL of dilute hydrochloric acid (1 in 2): the gas evolved darkens moistened lead (II) acetate paper.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Acetazolamide in 10 mL of sodium hydroxide TS: the solution is clear and colorless to pale yellow.

(2) Chloride \(<1.07\)—To 1.5 g of Acetazolamide add 75 mL of water, and warm at 70°C for 20 minutes with occasional shaking. After cooling, filter, and to 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.038%).

(3) Sulfate \(<1.14\)—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Prepare the test solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals \(<1.07\)—Proceed with 1.0 g of Acetzolamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Silver-reducing substances—Wet 5 g of Acetazolamide with 5 mL of aldehyde-free ethanol, and add 125 mL of water, 10 mL of nitric acid and exactly 5 mL of 0.1 mol/L silver nitrate VS. Stir for 30 minutes by protecting from light, filter through a glass filter (G3), and wash the residue on the glass filter with two 10-mL portions of water. Combine the filtrate with the washings, to the solution add 5 mL of ferric ammonium sulfates, and titrate \(<2.50\) with 0.1 mol/L ammonium thiocyanate VS: not less than 4.8 mL of 0.1 mol/L ammonium thiocyanate VS is consumed.

**Loss on drying** \(<2.41\) Not more than 0.5% (0.5 g, 105°C, 3 hours).

**Residue on ignition** \(<2.44\) Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 0.15 g of Acetazolamide, and dissolve in 400 mL of water in a water bath by heating. After cooling, add water to make exactly 1000 mL. Pipet 5 mL of the solution, add 10 mL of 1 mol/L hydrochloric acid TS, and then add water to make exactly 100 mL. Determine the absorbance \(A\) of this solution at the wavelength of maximum absorption at about 265 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24\).

Amount (mg) of \(\text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2\) = \(A/474 \times 200,000\)

**Containers and storage** Containers—Well-closed containers.

**Storage**—Light-resistant.

**Acetic Acid**

酢酸

Acetic Acid contains not less than 30.0 w/v% and not more than 32.0 w/v% of \(\text{C}_2\text{H}_4\text{O}_2\): 60.05.

**Description** Acetic Acid is a clear, colorless liquid. It has a pungent, characteristic odor and an acid taste.

It is miscible with water, with ethanol (95) and with glycerin.

**Specific gravity** \(d_2^{20}:\) about 1.04

**Identification** Acetic Acid changes blue litmus paper to red, and responds to the Qualitative Tests \(<1.09\) for acetate.

**Purity (1)** Chloride—To 20 mL of Acetic Acid add 40 mL of water, and use this solution as the sample solution. To 10 mL of the sample solution add 5 drops of silver nitrate TS: no opalescence is produced.

(2) Sulfate—To 10 mL of the sample solution obtained in (1) add 1 mL of barium chloride TS: no turbidity is produced.

(3) Heavy metals \(<1.07\)—Evaporate 10 mL of Acetic Acid on a water bath to dryness, and to the residue add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution by adding 2 mL of dilute acetic acid and water to make 50 mL (not
more than 3 ppm).

(4) Potassium permanganate-reducing substances—To 20 mL of the sample solution obtained in (1) add 0.02 mol/L potassium permanganate VS: the red color does not disappear within 30 minutes.

(5) Non-volatile residue—Evaporate 30 mL of Acetic Acid on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

**Assay** Measure exactly 5 mL of Acetic Acid, add 30 mL of water, and titrate 2.50 μL with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS = 60.05 mg of C₂H₄O₂

**Containers and storage** Containers—Tight containers.

### Glacial Acetic Acid

**氷酢酸**

C₂H₄O₂: 60.05

**Acetic acid**

[C₆H₄O₂]

**Description** Glacial Acetic Acid is a clear, colorless, volatile liquid, or colorless or white, crystalline masses. It has a pungent, characteristic odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

Boiling point: about 118°C

Specific gravity d₂₀: about 1.049

**Identification** A solution of Glacial Acetic Acid (1 in 3) changes blue litmus paper to red, and responds to the Qualitative Tests (1.090) for acetate.

**Congealing point** 2.42°C Not below 14.5°C.

**Purity (1)** Chloride—To 10 mL of Glacial Acetic Acid add water to make 100 mL, and use this solution as the sample solution. To 10 mL of the sample solution add 5 drops of silver nitrate TS: no turbidity is produced.

(2) Sulfate—To 10 mL of the sample solution obtained in (1) add 1 mL of barium chloride TS: no turbidity is produced.

(3) Heavy metals <1.07μg—Evaporate 2.0 mL of Glacial Acetic Acid on a water bath to dryness. Dissolve the residue in 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution by adding 2.0 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Potassium permanganate-reducing substances—To 20 mL of the sample solution obtained in (1) add 0.10 mL of 0.1 mol/L potassium permanganate VS: the red color does not disappear within 30 minutes.

(5) Non-volatile residue—Evaporate 10 mL of Glacial Acetic Acid on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

**Assay** Place 10 mL of water in a glass-stoppered flask, and weigh accurately. Add about 1.5 g of Glacial Acetic Acid, weigh accurately again, then add 30 mL of water, and titrate 2.50 μL with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS = 60.05 mg of C₂H₄O₂

**Containers and storage** Containers—Tight containers.

### Acetohexamide

**アセトヘキサミド**

C₁₅H₂₀N₂O₄S: 324.40

4-Acetyl-N-(cyclohexylcarbamoyl)benzenesulfonamide [968-81-0]

Acetohexamide, when dried, contains not less than 98.0% and not more than 101.0% of C₁₅H₂₀N₂O₄S.

**Description** Acetohexamide occurs as a white to yellowish white powder.

It is freely soluble in N,N-dimethylformamide, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point: about 185°C (with decomposition).

**Identification** (1) Dissolve 0.10 g of Acetohexamide in 100 mL of methanol. To 5 mL of the solution add 20 mL of 0.5 mol/L hydrochloric acid TS and 75 mL of methanol, and use the solution as the sample solution (1). Determine the absorption spectrum of the sample solution (1) as directed under Ultraviolet-visible Spectrophotometry (2.24), using methanol as the blank, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to exactly 10 mL of the sample solution (1) add methanol to make exactly 50 mL, and use the solution as the sample solution (2). Determine the absorption spectrum of the sample solution (2) as directed under Ultraviolet-visible Spectrophotometry (2.24), using methanol as the blank, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Acetohexamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity (1)** Chloride <1.0μg—Dissolve 1.5 g of Acetohexamide in 40 mL of N,N-dimethylformamide, add 6 mL of dilute nitric acid and N,N-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and N,N-dimethylformamide to make 50 mL (not more than
(2) Sulfate—Dissolve 2.0 g of Acetohexamide in 40 mL of N,N-dimethylformamide, and add 1 mL of dilute hydrochloric acid and N,N-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and N,N-dimethylformamide to make 50 mL (not more than 0.010%).

(3) Heavy metals—Proceed with 1.0 g of Acetohexamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances (i) Cyclohexylamine—Dissolve exactly 1.0 g of Acetohexamide in exactly 30 mL of 0.5 mol/L sodium hydroxide TS, add exactly 5 mL of hexane, shake vigorously for 60 minutes, allow to stand for 5 minutes, and use the upper layer as the sample solution. Separately, dissolve exactly 50 mg of cyclohexylamine in 0.5 mol/L sodium hydroxide TS to make exactly 50 mL. Pipet 2 mL of this solution, and add 0.5 mol/L sodium hydroxide TS to make exactly 300 mL. Pipet 30 mL of this solution, add exactly 5 mL of hexane, shake vigorously for 60 minutes, allow to stand for 5 minutes, and use the upper layer as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography according to the following conditions, and determine the peak area of cyclohexylamine by the automatic integration method: the peak area of cyclohexylamine is not more than that with the standard solution.

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A fused-silica column 0.53 mm in inside diameter and 30 m in length, coated the inner surface with methylsilicone polymer for gas chromatography 1.5 μm in thickness.
Column temperature: A constant temperature of about 90°C.
Injection port temperature: A constant temperature of about 150°C.
Detector temperature: A constant temperature of about 210°C.
Carrier gas: Helium
Flow rate: Adjust the flow rate so that the retention time of cyclohexylamine is about 4 minutes.
Split ratio: 1:1

System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of diclohexylurea is not less than 10,000.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cyclohexylamine is not more than 5%.

(ii) Dicyclohexylurea—Dissolve exactly 1.0 g of Acetohexamide in exactly 10 mL of 0.5 mol/L sodium hydroxide TS, add exactly 20 mL of methanol, shake, then add exactly 5 mL of dilute hydrochloric acid (1 in 10), shake vigorously for 15 minutes, and centrifuge. Filter 10 mL or more of the supernatant liquid through a membrane filter with pore size of not larger than 0.5 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dissolve exactly 50 mg of dicyclohexylurea in methanol to make exactly 100 mL. Pipet 2 mL of this solution, and add methanol to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 10 mL of 0.5 mol/L sodium hydroxide TS, shake, then add exactly 5 mL of dilute hydrochloric acid (1 in 10), shake, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak area of dicyclohexylurea by the automatic integration method: the peak area of dicyclohexylurea is not more than that with the standard solution.

Related substances (iii) Other related substances—Dissolve 0.10 g of Acetohexamide in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 20 mL. Pipet two 1 mL portions of this solution, add acetone to make exactly 10 mL and 25 mL, respectively, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography (3) Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, ammonia solution (28) and cyclohexane (6:2:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than spot from the standard solution (1), and the number of them which are more intense than the spot from the standard solution (2) is not more than 4.

Loss on drying—Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition—Not more than 0.1% (1 g).

Assay—Weigh accurately about 0.3 g of Acetohexamide, previously dried, dissolve in 30 mL of N,N-dimethylfor-
Acetylcholine Chloride for Injection / Official Monographs

Acetylcholine Chloride for Injection

Acetylcholine Chloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 98.0% and not more than 102.0% of acetylcholine chloride (C₇H₁₆ClNO₂), and not less than 19.3% and not more than 19.8% of chlorine (Cl: 35.45), calculated on the dried basis.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of acetylcholine chloride (C₇H₁₆ClNO₂).

Method of preparation

Prepare as directed under Injection.

Description

Acetylcholine Chloride for Injection occurs as white crystals or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95).

It is extremely hygroscopic.

Identification

(1) Determine the infrared absorption spectrum of Acetylcholine Chloride for Injection, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Acetylcholine Chloride for Injection (1 in 10) responds to the Qualitative Tests <1.09> (2) for chloride.

Melting point

149 – 152°C. Seal Acetylcholine Chloride for Injection in a capillary tube for melting point immediately after drying both of the sample and the tube at 105°C for 3 hours, and determine the melting point.

Purity

(1) Clarity and color of solution—Dissolve 1.0 g of Acetylcholine Chloride for Injection in 10 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.10 g of Acetylcholine Chloride for Injection in 10 mL of freshly boiled and cooled water, and add 1 drop of bromothymol blue TS, and 0.30 mL of 0.01 mol/L sodium hydroxide VS: the solution is blue in color.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Acetylcholine Chloride for Injection according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.05> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay

(1) Acetylcholine chloride—Weigh accurately the contents of not less than 10 Acetylcholine Chloride for Injections. Weigh accurately about 0.5 g of the contents, dissolve in 15 mL of water, then add exactly 40 mL of 0.1 mol/L sodium hydroxide VS, stopper loosely, and heat on a water bath for 30 minutes. Cool quickly, and titrate <2.50> the excess sodium hydroxide with 0.05 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS = 18.17 mg of C₇H₁₆ClNO₂

(2) Chlorine—Titrate <2.50> the solution, which has been titrated in (1), with 0.1 mol/L silver nitrate VS (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS = 3.545 mg of Cl

Containers and storage

Containers—Well-closed containers.

Containers and storage

Containers—Hermetic containers.

Acetylcysteine

Acetylcysteine occurs as white crystals or crystalline powder.

It is freely soluble in water and in ethanol (99.5).

It dissolves in sodium hydroxide TS.

Acetylcysteine contains not less than 99.0% and not more than 101.0% of C₅H₉NO₃S, calculated on the dried basis.

Description

Acetylcysteine occurs as white, crystals or crystalline powder.

It is very soluble in water, and in ethanol (99.5).

It is extremely hygroscopic.

Identification

(1) Determine the infrared absorption spectrum of Acetylcysteine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and com-
pare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \( \angle \alpha.D. +21.0° \pm 27.0° \) — Weigh accurately an amount of Acetylcysteine, equivalent to about 2.5 g calculated on the dried basis, and dissolve with 2 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 100) and 15 mL of a solution of sodium hydroxide (1 in 25). To this solution add a solution, prepared by adjusting the pH to 7.0 of 500 mL of a solution of potassium dihydrogen phosphate (17 in 125) with sodium hydroxide TS and adding water to make 1000 mL, to make exactly 50 mL. Determine the optical rotation of this solution using a 100-mm cell.

**Melting point** \( \angle 2.49° \) — 107–111°C

**Purity** (1) Chloride \( \angle 1.03° \) — Dissolve 0.40 g of Acetylcysteine in 25 mL of sodium hydroxide TS, add 4 mL of hydrogen peroxide (30%), heat in a water bath for 45 minutes, and cool. Then add 5 mL of nitric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.040%).

(2) Sulfate \( \angle 1.14° \) — Perform the test with 0.8 g of Acetylcysteine. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).

(3) Ammonium \( \angle 1.02° \) — Perform the test with 0.10 g of Acetylcysteine, using the dilution under reduced pressure. Prepare the control solution with 2.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals \( \angle 1.07° \) — Dissolve 1.0 g of Acetylcysteine in 40 mL of water, add 3 mL of sodium hydroxide TS, 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(5) Iron \( \angle 1.10° \) — Prepare the test solution with 1.0 g of Acetylcysteine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(6) Related substances — Dissolve 50 mg of Acetylcysteine in 25 mL of the mobile phase, and use this solution as the sample solution. The sample solution is prepared before using. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography \( \angle 2.01° \) according to the following conditions, determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the area of the peak other than acetylcysteine is not more than 0.3%, and the total area of the peak other than acetylcysteine is not more than 0.6%.

**Operating conditions** —

Detector: An ultraviolet absorption photometer (wave-length: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 2500) and acetonitrile (19:1).

Flow rate: Adjust the flow rate so that the retention time of acetylcysteine is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of acetylcysteine, beginning after the solvent peak.

System suitability —

Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 10 mL. To 1 mL of this solution, add the mobile phase to make 20 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 25 mL. Confirm that the peak area of acetylcysteine obtained with 10 μL of this solution is equivalent to 15 to 25% of that with 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acetylcysteine are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of acetylcysteine is not more than 2.0%.

(7) Residual solvent — Being specified separately.

**Loss on drying** \( \angle 2.41° \) — Not more than 0.5% (1 g, 80°C, 3 hours).

**Residue on ignition** \( \angle 2.44° \) — Not more than 0.3% (1 g).

**Assay** — Weigh accurately about 0.2 g of Acetylcysteine, place it in a stoppered flask, dissolve in 20 mL of water, add 4 g of potassium iodide and 5 mL of dilute hydrochloric acid, then add exactly 25 mL of 0.05 mol/L iodine VS, stopper tightly, allow to stand for 20 minutes in an ice cold water in the dark, and titrate \( \angle 2.50° \) the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

Each mL of 0.05 mol/L iodine VS = 16.32 mg of C₇H₇NO₅S

**Containers and storage** — Containers — Tight containers.

**Aciclovir**

Aciclovir occurs as a white to pale yellowish
white crystalline powder.

It is slightly soluble in water and very slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS and in dilute sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Aciclovir in 0.1 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.2ζ>, and compare the spectrum with the Reference Spectrum or the spectrum of Aciclovir RS: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aciclovir as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.2ζ>, and compare the spectrum with the Reference Spectrum or the spectrum of Aciclovir RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Aciclovir in 20 mL of dilute sodium hydroxide TS: the solution is clear and is not more colored than the following control solution.

Control solution: To 2.5 mL of Matching Fluid for Color F add diluted dilute hydrochloric acid (1 in 10) to make 100 mL.

(2) Heavy metals <1.0ζ>—Proceed with 1.0 g of Aciclovir according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Use the sample solution obtained in the Assay as the sample solution. Separately, weigh accurately about 25 mg of guanine, dissolve in 50 mL of dilute sodium hydroxide TS, and add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0ζ>, according to the following conditions, and determine the peak areas, A<sub>T</sub> and A<sub>S</sub>, of aciclovir.

Amount (mg) of C<sub>8</sub>H<sub>11</sub>N<sub>5</sub>O<sub>3</sub> = M<sub>S</sub> × A<sub>T</sub>/A<sub>S</sub>

M<sub>S</sub>: Amount (mg) of Aciclovir RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 1.0 g of sodium 1-decanesulfonate and 6.0 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To this solution add 40 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of aciclovir is about 3 minutes.

System suitability—

System performance: Dissolve 0.1 g of Aciclovir in 5 mL of dilute sodium hydroxide TS, add 2 mL of a solution of guanine in dilute sodium hydroxide TS (1 in 4000), and add the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, aciclovir and guanine are eluted in this order with the resolution between these peaks being not less than 17.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aciclovir is not more than 1.0%.

Containers and storage Containers—Well-closed containers.
Aciclovir Injection

Aciclovir Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of aciclovir (C₈H₁₁N₅O₃: 225.20).

Method of preparation Prepare as directed under Injections, with Aciclovir.

Description Aciclovir Injection occurs as a colorless or pale yellow, clear liquid.

Identification To a volume of Aciclovir Injection, equivalent to 25 mg of aciclovir according to the labeled amount, add 0.5 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.5 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits a maximum between 254 nm and 258 nm.

Bacterial endotoxins 4.01 Less than 0.5 EU/mg.

Extractable volume 6.05 It meets the requirement.

Foreign insoluble matter 6.06 Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter 6.07 It meets the requirement.

Sterility 4.06 Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exact volume of Aciclovir Injection, equivalent to about 25 mg of aciclovir (C₈H₁₁N₅O₃), add 0.1 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separate, weigh accurately about 25 mg of Aciclovir RS (separately determine the water 2.40 in the same manner as Aciclovir), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Aciclovir RS (separately determine the water 2.40 in the same manner as Aciclovir), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q₇ and Q₈, of the peak area of aciclovir to that of the internal standard.

Amount (mg) of aciclovir (C₈H₁₁N₅O₃)
= Mₛ × Q₇/Q₈

Mₛ: Amount (mg) of Aciclovir RS, calculated on the anhydrous basis

Internal standard solution—A solution of nicotinic acid in 0.1 mol/L hydrochloric acid TS (3 in 20,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Aciclovir for Syrup

Aciclovir for Syrup is a preparation for syrup, which is suspended before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of aciclovir (C₈H₁₁N₅O₃: 225.20).

Method of preparation Prepare as directed under Preparations for Syrup, with Aciclovir.

Identification Dissolve an amount of Aciclovir for Syrup, equivalent to 12 mg of Aciclovir according to the labeled amount, in 0.1 mol/L hydrochloric acid TS to make 50 mL. To 2 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits a maximum between 254 nm and 258 nm.

Uniformity of dosage units 6.02 Perform the test according to the following method: Aciclovir for Syrup in single-unit container meets the requirement of the Content uniformity test.

To the total content of 1 container of Aciclovir for Syrup add 27/25 mL of diluted sodium hydroxide TS (1 in 10), and treat with ultrasonic waves to disintegrate, add water to make exactly 5 mL so that each mL contains about 0.8 mg of aciclovir (C₈H₁₁N₅O₃), and filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 1.45 g of phosphoric acid and 25 mL of dilute acetic acid add water to make 900 mL. Adjust this solution to pH 2.5 with 1 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of aciclovir is about 7 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and aciclovir are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of aciclovir to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.
Dissolution

**Assay**

Weigh accurately an amount of Aciclovir for Syrup, equivalent to about 0.2 g of aciclovir (C₈H₁₁N₅O₃) according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution.

**Identification**

To a volume of thoroughly shaked Aciclovir Syrup, equivalent to 80 mg of Aciclovir according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL. Centrifuge this solution, to 1 mL of the supernatant liquid add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 254 nm and 258 nm.

**Containers and storage**

Containers—Tight containers.

**Aciclovir Syrup**

アシクロビルシロップ

Aciclovir Syrup is a suspension syrup. It contains not less than 93.0% and not more than 107.0% of the labeled amount of aciclovir (C₈H₁₁N₅O₃: 225.20).

**Method of preparation**

Prepare as directed under Syrups, with Aciclovir.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Flow rate: Adjust the flow rate so that the retention time of aciclovir is about 5 minutes.

System suitability—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, aciclovir and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of aciclovir to that of the internal standard is not more than 1.0%.

**Containers and storage**

Containers—Tight containers.
<2.48> in the same manner as Aciclovir), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_1 \) and \( A_5 \), at 252 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.26>.

Dissolution rate (%) with respect to the labeled amount of aciclovir (\( C_8H_{11}N_5O_3 \))

\[
M_S = \frac{M_T}{V_T} \times \frac{A_1}{A_5} \times 1800
\]

\( M_S \): Amount (mg) of Aciclovir RS, calculated on the anhydrous basis

\( V_T \): Amount (mL) of sample

\( C \): Labeled amount (mg) of aciclovir (\( C_8H_{11}N_5O_3 \)) in 1 mL

**Assay**  Shake thoroughly Aciclovir Syrup. To an exact volume as the standard solution. Perform the test with 20 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution.

Separately, weigh accurately about 40 mg of Aciclovir RS (separately determine the water <2.48> in the same manner as Aciclovir), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Aciclovir RS and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.20> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_5 \), of the peak area of aciclovir to that of the internal standard.

Amount (mg) of aciclovir (\( C_8H_{11}N_5O_3 \))

\[
M_S = \frac{M_T \times Q_1}{Q_5} \times 2
\]

\( M_S \): Amount (mg) of Aciclovir RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of nicotinic acid in 0.1 mol/L hydrochloric acid TS (1 in 2000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 1.45 g of phosphoric acid and 25 mL of dilute acetic acid add water to make 900 mL. Adjust this solution to pH 2.5 with 1 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of aciclovir is about 7 minutes.

**System suitability**—

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the internal standard and aciclovir are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of aciclovir to that of the internal standard is not more than 1.0%.

**Containers and storage**  Containers—Tight containers.

### Aclarubicin Hydrochloride

Aclarubicin Hydrochloride is the hydrochloride of an anthracycline substance having antitumor activity produced by the growth of *Streptomyces galilaeus*.

It contains not less than 920 \( \mu \)g (potency) and not more than 975 \( \mu \)g (potency) per mg, calculated on the anhydrous basis. The potency of Aclarubicin Hydrochloride is expressed as mass (potency) of aclarubicin (\( C_{32}H_{37}NO_{15} \): 811.87).

**Description**  Aclarubicin Hydrochloride occurs as a yellow to pale orange-yellow powder.

It is very soluble in chloroform and in methanol, freely soluble in water, and slightly soluble in ethanol (95%).

**Identification** (1)  Determine the absorption spectrum of a solution of Aclarubicin Hydrochloride in diluted methanol (4 in 5) (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)  Determine the infrared absorption spectrum of Aclarubicin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3)  A solution of Aclarubicin Hydrochloride in methanol (1 in 200) responds to the Qualitative Tests <1.09> (2) for chloride.
Acrinol Hydrate / Official Monographs

Optical rotation $< 2.49$° [α]D$^0$: $-146 - 162°$ (50 mg calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH $< 2.54$° The pH of a solution obtained by dissolving 0.05 g of Aclarubicin Hydrochloride in 10 mL of water is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Aclarubicin Hydrochloride in 10 mL of water: the solution is clear and yellow to pale orange-yellow.

(2) Heavy metals $< 1.07$°—Proceed with 1.0 g of Aclarubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Aclarubicin Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography $< 2.01$° according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the area percentage method: the amount of aclavinone having the relative retention time of about 0.6 to aclarubicin is not more than 0.2%, aclacinomycin L1 having the relative retention time of about 0.75 to aclarubicin is not more than 0.5%, 1-deoxyxypyrromycin having the relative retention time of about 1.7 to aclarubicin is not more than 1.5% and aclacinomycin S1 having the relative retention time of about 2.3 to aclarubicin is not more than 0.5%, and the total amount of the peaks other than aclarubicin and the peaks mentioned above is not more than 1.0% of the peak area of aclarubicin.

Operating conditions—
Detector: A visible absorption photometer (wavelength: 436 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of chloroform, methanol, acetic acid (100), and water (680:2000:1000:200:1).
Flow rate: Adjust the flow rate so that the retention time of aclarubicin is about 5 minutes.
Time span of measurement: As long as about 4 times of the retention time of aclarubicin beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of aclarubicin obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the solution for system suitability test.

System performance: Dissolve 5 mg of Aclarubicin Hydrochloride in 10 mL of 0.1 mol/L hydrochloric acid TS, and allow to stand for 60 minutes. To 1.0 mL of this solution add 1.0 mL of 0.2 mol/L sodium hydroxide TS, 1.0 mL of phosphate buffer solution, pH 6.0 and 1.0 mL of chloroform, shake vigorously, and take the chloroform layer. When the procedure is run with 20 μL of the chloroform under the above operating conditions, aclarubicin and 1 are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 5 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of aclarubicin is not more than 2.0%.

Water $< 2.45$° Not more than 3.5% (0.1 g, volumetric titration, direct titration).

Residue on ignition $< 2.44$° Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Aclarubicin Hydrochloride, equivalent to about 20 mg (potency), and dissolve in dilute methanol (4 in 5) to make exactly 100 mL. Pipet 15 mL of this solution, add dilute methanol (4 in 5) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh an accurately amount of Aclarubicin RS, equivalent to about 20 mg (potency), add 0.6 mL of diluted hydrochloric acid (1 in 250) and dilute methanol (4 in 5) to make exactly 100 mL. Pipet 15 mL of this solution, add dilute methanol (4 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry $< 2.24°$, and determine the absorbances, $A_T$ and $A_S$, at 433 nm.

Amount [μg (potency)] of aclarubicin (C42H53NO15) $= M_S \times A_T/A_S \times 1000$

$M_S$: Amount [mg (potency)] of Aclarubicin RS

Containers and storage Containers—Tight containers.
Storage—Light-resistant and at 5°C or below.

Acrinol Hydrate

Ethacridine Lactate

アクセリノール水和物

\[ \text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_2\cdot\text{C}_2\text{H}_5\text{O}_2\cdot\text{H}_2\text{O}: 361.39 \]

2-Ethoxy-6,9-diaminoacridine monolactate monohydrate [1837-57-6]

Acrinol Hydrate contains not less than 98.5% and not more than 101.0% of acrinol (C15H15N3O.C3H6O3: 343.38), calculated on the anhydrous basis.

Description Acrinol Hydrate occurs as a yellow, crystalline powder. It is sparingly soluble in water, in methanol and in ethanol (99.5).

Melting point: about 245°C (with decomposition).
The pH of a solution of Acrinol Hydrate (1 in 100) is between 5.5 and 7.0.

Identification (1) Determine the absorption spectrum of a solution of Acrinol Hydrate (3 in 250,000) as directed under Ultraviolet-visible Spectrophotometry $< 2.24°$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave-
lengths.

(2) Determine the infrared absorption spectrum of Acrinol Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Acrinol Hydrate (1 in 100) add 5 mL of dilute sulfuric acid, shake well, allow to stand for about 10 minutes at room temperature, and filter: the filtrate responds to the Qualitative Tests <1.09> for lactate.

**Purity** (1) Chloride <1.03>—Dissolve 1.0 g of Acrinol Hydrate in 80 mL of water by warming on a water bath, cool, and add 10 mL of sodium hydroxide TS and water to make 100 mL. Shake well, allow to stand for 30 minutes, filter, to 40 mL of the filtrate add 7 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare 50 mL of the control solution with 4 mL of sodium hydroxide TS, 7 mL of dilute nitric acid, 0.30 mL of 0.01 mol/L hydrochloric acid VS and sufficient water (not more than 0.026%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Acrinol Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Volatile fatty acids—Dissolve 0.5 g of Acrinol Hydrate in a mixture of 20 mL of water and 5 mL of dilute sulfuric acid, shake well, filter, and heat the filtrate: no odor of volatile fatty acids is perceptible.

(4) Related substances—Dissolve 10 mg of Acrinol Hydrate in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 10 µL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than acrinol is not larger than 3 times the peak area of acrinol obtained with the standard solution (2), and the total area of the peaks other than acrinol is not larger than the peak area of acrinol with the standard solution (1).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate in 900 mL of water, adjust to pH 2.8 with phosphoric acid, and add water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile for liquid chromatography, and add 1.0 g of sodium 1-octanesulfonate to dissolve. Flow rate: Adjust the flow rate so that the retention time of acrinol is about 15 minutes.

Time span of measurement: About 3 times as long as the retention time of acrinol beginning after the solvent peak.

**System suitability**—

Test for required detectability: Confirm that the peak area of acrinol obtained with 10 µL of the standard solution (2) is equivalent to 7 to 13% of that with 10 µL of the standard solution (1).

System performance: When the procedure is run with 10 µL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acrinol are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of acrinol is not more than 1.5%.

**Water** <2.48> 4.5 – 5.5% (0.2 g, volumetric titration, direct titration)

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.27 g of Acrinol Hydrate, dissolve in 5 mL of formic acid, add 60 mL of a mixture of acetic anhydride and acetic acid (100):1:1, and titrate <2.50> immediately with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 34.34 mg of acrinol (C₁₅H₁₅N₃O₆C₃H₆O₃)

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Acrinol and Zinc Oxide Oil**

**Method of preparation**

Acrinol Hydrate, very finely powdered 10 g
Zinc Oxide Oil 990 g

To make 1000 g

Prepare by mixing the above ingredients.

**Description** Acrinol and Zinc Oxide Oil is a yellowish white, slimy substance. Separation of a part of its ingredients occurs on prolonged standing.

**Identification** (1) Shake well 1 g of Acrinol and Zinc Oxide Oil with 10 mL of diethyl ether, 2 mL of acetic acid (100) and 10 mL of water, and separate the water layer. Shake the layer with 5 mL of hydrochloric acid and 2 to 3 drops of sodium nitrite TS, and allow to stand: a dark red color is produced (acrinol).

(2) Place 1 g of Acrinol and Zinc Oxide Oil in a crucible, melt by warming, heat, gradually raising the temperature until the mass is thoroughly charred, and then ignite strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, filter after thorough shaking, and to the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

(3) Shake well 0.2 g of Acrinol and Zinc Oxide Oil with 20 mL of ethanol (95) and 1 mL of acetic acid (100), centri-
Compound Acrinol and Zinc Oxide Oil

複方アクリノール・チンク油

Method of preparation

- Acrinol Hydrate, very finely powdered: 10 g
- Zinc Oxide Oil: 650 g
- Ethyl Aminobenzoate, finely powdered: 50 g
- White Beeswax: 20 g
- Hydrophilic Petrolatum: 270 g

To make 1000 g

Prepare by mixing the above ingredients.

Description

Compound Acrinol and Zinc Oxide Oil is light yellow to yellow in color.

Identification (1)

Shake well 1 g of Compound Acrinol and Zinc Oxide Oil with 10 mL of diethyl ether, 2 mL of acetic acid (100) and 10 mL of water, and separate the water layer. Shake the layer with 5 mL of hydrochloric acid and 2 to 3 drops of sodium nitrite TS, and allow to stand: a dark red color is produced (acrinol).

(2) Place 1 g of Compound Acrinol and Zinc Oxide Oil in a crucible, melt by warming, heat, gradually raising the temperature until the mass is thoroughly charred, and then ignite strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is produced (zinc oxide).

(3) Shake well 0.2 g of Compound Acrinol and Zinc Oxide Oil with 20 mL of ethanol (95) and 1 mL of acetic acid (100), centrifuge, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of acrinol and 25 mg of ethylaminobenzoate in 50 mL of ethanol (95) and in 2.5 mL of acetic acid (100), respectively, and use both solutions as the standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, ethanol (95) and acetic acid (100) (40:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots from the sample solution and standard solution exhibit a blue fluorescence and show the same Rf value.

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.

Acrinol and Zinc Oxide Ointment

アクリノール・亜鉛華軟膏

Method of preparation

- Acrinol Hydrate, very finely powdered: 10 g
- Zinc Oxide Ointment: 990 g

To make 1000 g

Prepare as directed under Ointments, with the above ingredients.

Description

Acrinol and zinc oxide ointment is yellow in color.

Identification (1)

Shake 0.5 g of Acrinol and Zinc Oxide Ointment with 5 mL of diethyl ether, 5 mL of dilute hydrochloric acid and 2 to 3 drops of sodium nitrite TS, and allow to stand: a dark red color develops in the water layer (acrinol).

(2) Ignite 0.5 g of Acrinol and Zinc Oxide Ointment to char, and dissolve the residue in 5 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for zinc salt.

(3) Shake 0.5 g of Acrinol and Zinc Oxide Ointment with 5 mL of diethyl ether, 1 mL of acetic acid (100) and 5 mL of water, separate the water layer, and use the water layer as the sample solution. Dissolve 5 mg of acrinol in 1 mL of acetic acid (100) and 5 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, ethanol (95) and acetic acid (100) (40:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots from the sample solution and the standard solution exhibit a blue fluorescence and show the same Rf value.

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.
Actinomycin D

アクチノマイシン D

Actinomycin D is a peptide substance having anti-tumor activity produced by the growth of Streptomyces parvulus.

It, when dried, contains not less than 950 μg (potency) and not more than 1030 μg (potency) per mg. The potency of Actinomycin D is expressed as mass (potency) of actinomycin D (C_{62}H_{86}N_{12}O_{16}).

**Description**

Actinomycin D occurs as an orange-red to red crystalline powder.

It is freely soluble in acetone, sparingly soluble in acetonitrile and in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

**Identification (1)**

Determine the absorption spectrum of a solution of Actinomycin D in methanol (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2,24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Actinomycin D RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g each of Actinomycin D and Actinomycin D RS in 10 mL of acetone, and use these solutions as the sample solution and standard solution. Perform the test with exactly 25 μL of the standard solution under the above operating conditions, and determine the peak area of actinomycin D, A_t, and A_s, of both solutions.

System suitability—

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of actinomycin D are not less than 2000 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 5 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of actinomycin D is not more than 2.0%.

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant.

Adrenaline

アドレナリン

Adrenaline occurs as a white to grayish white, crystalline powder.

It is freely soluble in formic acid and in acetic acid (100), very slightly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It gradually changes to brown in color by air and by light.

**Identification (1)**

Determine the absorption spectrum of a solution of Adrenaline in 0.01 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2,24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of
Adrenaline as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{25} - 50.0 - 53.5 ^\circ$ (after drying, 1 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Adrenaline in 10 mL of dilute hydrochloric acid: the solution is clear, and is not more colored than Matching Fluid A.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Adrenaline according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Adrenalone—Dissolve 50 mg of Adrenaline in 0.05 mol/L hydrochloric acid TS to make exactly 25 mL, and determine the absorbance of this solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.2.

(4) Noradrenaline—Dissolve 0.20 g of Adrenaline in 1 mL of formic acid, add methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 8.0 mg of Noradrenaline Bitartrate RS in methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and formic acid (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Folin’s TS on the plate: the spot obtained from the sample solution, corresponding to the spot from the standard solution, is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, silica gel, 18 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Adrenaline, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS $= 18.32$ mg of $C_9H_{13}NO_3$.

Containers and storage—Tight containers.

Storage—Light-resistant, and under Nitrogen atmosphere.

Method of preparation—Dissolve Adrenaline in diluted Hydrochloric Acid (9 in 10,000), and prepare as directed under Injections.

Identification (1) To 1 mL of Adrenaline Injection add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to red.

(2) Place 1 mL each of Adrenaline Injection in test tubes A and B, and proceed as directed in the Identification (2) under Adrenaline.

Extractable volume <6.05> It meets the requirement.

Assay Pipet 30 mL of Adrenaline Injection into a separator, add 25 mL of carbon tetrachloride, shake vigorously for 1 minute, allow the liquids to separate, and discard the carbon tetrachloride. Repeat this procedure three times. Rinse the stopper and mouth of the separator with a small amount of water. Add 0.2 mL of starch TS, then while swirling the separator add iodine TS dropwise until a persistent blue color develops, and immediately add sodium thiosulfate TS to discharge the blue color. Add 2.1 g of sodium hydrogen carbonate to the liquid in the separator, preventing it from coming in contact with the mouth of the separator, and shake until most of the sodium hydrogen carbonate dissolves. Rapidly inject 1.0 mL of acetic anhydride into the contents of the separator. Immediately stopper the separator loosely, and allow to stand until the evolution of gas ceases. Shake vigorously, allow to stand for 5 minutes, extract with six 25-mL portions of chloroform, and filter each chloroform extract through a pledget of absorbent cotton. Evaporate the combined chloroform extracts on a water bath in a current of air to 3 mL, completely transfer this residue by means of small portions of chloroform to a tared beaker, and heat again to evaporate to dryness. Dry the residue at 105°C for 30 minutes, cool in a desiccator (silica gel), and accurately measure the mass $M$ (mg) of the dried residue. Dissolve in chloroform to make exactly 5 mL, and determine the optical rotation <2.49> $[\alpha]_D^{20}$ using a 100-mm cell.

Amount (mg) of adrenaline ($C_9H_{13}NO_3$) $= M \times (0.5 + (0.5 \times [\alpha]_D^{20} / 93)) \times 0.592$

Containers and storage—Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Adrenaline Injection

Epinephrine Injection

Adrenaline Injection is aqueous solution for injection.

It contains not less than 0.085 w/v% and not more than 0.115 w/v% of adrenaline ($C_9H_{13}NO_3$: 183.20).

Adrenaline Solution

Epinephrine Solution

Adrenaline Solution contains not less than 0.085 w/v% and not more than 0.115 w/v% of adrenaline ($C_9H_{13}NO_3$: 183.20).
Method of preparation

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline</td>
<td>1 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>8.5 g</td>
</tr>
<tr>
<td>Diluted Hydrochloric Acid (9 in 100)</td>
<td>10 mL</td>
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<tr>
<td>Stabilizer</td>
<td>a suitable quantity</td>
</tr>
<tr>
<td>Preservative</td>
<td>a suitable quantity</td>
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<tr>
<td>Purified Water or Purified Water</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td>Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

Prepare by mixing the above ingredients.

Description Adrenaline Solution is clear, colorless or slightly reddish liquid.
It changes gradually to pale red and then to brown on exposure to air and light.

pH: 2.3 – 5.0

Identification Proceed as directed in the Identification under Adrenaline Injection.

Assay Proceed as directed in the Assay under Adrenaline Injection.

Amount (mg) of adrenaline (C₉H₁₅NO₃)

\[ M = \frac{0.5 + (0.5 \times [\alpha]^{20})/93}{0.592} \]

Containers and storage  Containers—Tight containers.
Storage—Light-resistant.

Afloqualone

アフロクアロン

![Afloqualone molecule](image)

C₁₆H₁₄FN₃O: 283.30
6-Amino-2-fluoromethyl-3-(2-tolyl)-3H-quinazolin-4-one [56287-74-2]

Afloqualone, when dried, contains not less than 98.5% of C₁₆H₁₄FN₃O.

Description Afloqualone occurs as white to light yellow crystals or crystalline powder.
It is soluble in acetonitrile, sparingly soluble in ethanol (99.5), and practically insoluble in water.
It is gradually colored by light.
Melting point: about 197°C (with decomposition).

Identification (1) Conduct this procedure without exposure to light, using light-resistant containers. Determine the absorption spectrum of a solution of Afloqualone in ethanol (99.5) (1 in 150,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Afloqualone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Acidity or alkalinity—Take 1.0 g of Afloqualone in a light-resistant vessel, add 20 mL of freshly boiled and cooled water, shake well, and filter. To 10 mL of the filtrate add 2 drops of bromothymol blue TS: a yellow color develops. The color changes to blue by adding 0.20 mL of 0.01 mol/L sodium hydroxide TS.

(2) Heavy metals: <1.07>—Proceed with 2.0 g of Afloqualone in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 10 mg of Afloqualone in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the total of the peak areas other than the peak area of afloqualone from the sample solution is not larger than the peak area of afloqualone from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 7.2 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, adjust to pH 5.5 with diluted phosphoric acid (1 in 10). To 600 mL of this solution add 400 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of afloqualone is about 5.5 minutes.
Time span of measurement: About 4 times as long as the retention time of afloqualone beginning after the solvent peak.
System suitability—
Test for required detection: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 25 mL, and confirm that the peak area of afloqualone obtained from 20 μL of this solution is equivalent to 15 to 25% of that of afloqualone obtained from 20 μL of the standard solution.
System performance: Dissolve 0.01 g of Afloqualone in a suitable amount of the mobile phase, add 5 mL of a solution of propyl parahydroxybenzoate in the mobile phase (1 in 2000) and the mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, afloqualone and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of afloqualone is not more than 5%.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacu-
 Ajmaline Tablets

Ajmaline Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of ajmaline (C20H26N2O2: 326.43).

**Method of preparation** Prepare as directed under Tablets, with Ajmaline.

**Identification (1)** Shake a quantity of powdered Ajmaline Tablets, equivalent to 0.1 g of Ajmaline according to the labeled amount, with 30 mL of chloroform, and filter. Evaporate the filtrate on a water bath to dryness. With the residue, proceed as directed in the Identification under Ajmaline.

(2) Dissolve 0.01 g of the residue of (1) in 100 mL of methanol, and use this solution as the sample solution. Add 3 mL of nitric acid to 1 mL of the sample solution: a deep red color develops.

**Optical rotation** \( \left[ \alpha \right] _{D}^{20} +136^\circ - +151^\circ \) (after drying, 0.5 g, chloroform, 50 mL, 100 mm).

**Uniformity of dosage units** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ajmaline Tablets add 150 mL of 2nd fluid for dissolution test, shake to disintegrate the tablet, then add 2nd fluid for dissolution test to make exactly 200 mL, and filter this solution through a membrane filter with a pore size not exceeding 0.8 \( \mu \)m. Discard the first 10 mL of the filtrate, pipet \( V \) mL of the subsequent filtrate equivalent to about 0.5 mg of ajmaline (C20H26N2O2), add 2nd fluid for dissolution test to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of
ajimaline for assay, previously dried in vacuum at 80°C for 3 hours, dissolve in 2nd fluid for dissolution test to make exactly 500 mL, and use this solution as the standard solution. Determine the absorbances at 288 nm, $A_T$ and $A_S$, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry. 

$$M_a = M_b \times A_T/A_S \times V/V \times 1/C \times 180$$

$M_a$: Amount (mg) of ajimaline for assay.

**Assay**

Weigh accurately and powder not less than 20 Ajimaline Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 g of ajimaline (C$_{20}$H$_{26}$N$_2$O$_2$), add 15 Ajimaline Tablets. Weigh accurately a portion of the powder, and use this solution as the sample solution. 

**Dissolution**

When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Ajimaline Tablets is not less than 75%.

Start the test with 1 tablet of Ajimaline Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add the dissolution medium to make exactly $V' \text{ mL}$ so that each mL contains about 56 μg of ajimaline (C$_{20}$H$_{26}$N$_2$O$_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of ajimaline for assay, previously dried in vacuum at 80°C for 3 hours, dissolve in the dissolution medium to make exactly 500 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 288 nm as directed under Ultraviolet-visible Spectrophotometry. 

**Optical rotation**

$\alpha_{D}^{20} = -81°\text{ to } -85°$ (after drying, 0.25 g, ethanol (95), 25 mL, 100 mm).

**Melting point**

153° to 157°C

**Purity**

(1) Chloride $<1.03$—Dissolve 0.5 g of Alacepril in 30 mL of methanol, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate $<1.14$—Dissolve 0.5 g of Alacepril in 30 mL of methanol, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 30 mL of methanol, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals $<1.07$—Proceed with 1.0 g of Alacepril according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 50 mg of Alacepril in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to
make exactly 200 mL and use this solution as the standard solution. Perform the test with exactly 10 \( \mu L \) of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than alacepril from the sample solution is not larger than 1/5 times the peak area of alacepril from the standard solution, and the total area of the peaks other than the peak of alacepril from the sample solution is not larger than the peak area of alacepril from the standard solution. For this calculation, use the areas of the peaks, having the relative retention time of about 2.3 and about 2.6 with respect to alacepril, after multiplying by their sensitivity factors, 1.5 and 1.9, respectively.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylosilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100), acetonitrile, methanol and tetrahydrofuran (6:2:1:1).

Flow rate: Adjust the flow rate so that the retention time of alacepril is about 5 minutes.

Time span of measurement: About 3 times as long as the retention time of alacepril beginning after the solvent peak.

**System suitability**

Test for required detectability: To exactly 4 mL of the standard solution add ethanol (95) to make exactly 10 mL. Confirm that the peak area of alacepril obtained with 10 \( \mu L \) of this solution is equivalent to 30 to 50% of that with 10 \( \mu L \) of the standard solution.

System performance: Dissolve 20 mg of Alacepril in 50 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 80,000). When the procedure is run with 10 \( \mu L \) of this solution under the above operating conditions, alacepril and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alacepril is not more than 2.0%.

**Loss on drying** <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.6 g of Alacepril, previously dried, dissolve in 75 mL of a mixture of methanol and water (2:1), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 40.65 mg of C\(_{20}H_{26}N_2O_5S\)

**Containers and storage** Containers—Tight containers.

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### Alacepril Tablets

Alacepril Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of alacepril (C\(_{20}H_{26}N_2O_5S\): 406.50).

**Method of preparation** Prepare as directed under Tablets, with Alacepril.

**Identification** Shake well a quantity of powdered Alacepril Tablets, equivalent to 0.1 g of Alacepril according to the labeled amount, with 10 mL of ethanol (95), filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of alacepril in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.02>. Spot 5 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of ethanol (99.5) and hexane (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same color tone and \( R_f \) value.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Alacepril Tablets add 2 mL of water, disperse the particle with the aid of ultrasonic wave, and add exactly 2 mL of the internal standard solution every 10 mg of alacepril (C\(_{20}H_{26}N_2O_5S\)) according to the labeled amount. To this solution add a suitable amount of methanol, extract for 15 minutes with the aid of ultrasonic wave while occasional shaking, and shake more 15 minutes. Add methanol to make \( V \) mL so that each mL of the solution contains about 0.5 mg of alacepril (C\(_{20}H_{26}N_2O_5S\)), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of alacepril for assay, previously dried at 105°C for 3 hours, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of alacepril to that of the internal standard.

Amount (mg) of alacepril (C\(_{20}H_{26}N_2O_5S\)) = \( M_5 \times Q_1 / Q_2 \times V / 50 \)

\( M_5 \): Amount (mg) of alacepril for assay

**Internal standard solution**—A solution of propyl parahydroxybenzoate in methanol (3 in 20,000).

**Operating conditions**

Proceed as directed in the operating conditions in the Assay.

**System suitability**

Proceed as directed in the system suitability in the Assay.

**Dissolution** <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate...
of a 12.5-mg tablet and a 25-mg tablet in 30 minutes is not less than 75%, and that of a 50-mg tablet in 30 minutes is not less than 70%.

Start the test with 1 tablet of Alacepril Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 14 μg of alacepril (C₂₀H₂₆N₂O₅S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 14 mg of alacepril for assay, previously dried at 105°C for 3 hours, dissolve in 2 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances at A₁₁, A₁₂, A₁₃, and A₁₄ at 300 nm, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.2₄₅>, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of alacepril (C₂₀H₂₆N₂O₅S) = M₅ × (A₁₁ - A₁₃)/(A₁₁ - A₁₃) × V/V × 1/C × 90

M₅: Amount (mg) of alacepril for assay
C: Labeled amount (mg) of alacepril (C₂₀H₂₆N₂O₅S) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Alacepril Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of alacepril (C₂₀H₂₆N₂O₅S), moisten with 2 mL of water, add exactly 3 mL of the internal standard solution and 40 mL of methanol, extract for 15 minutes with the aid of ultrasonic wave, cool, and add methanol to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of alacepril for assay, previously dried at 105°C for 3 hours, dissolve in 2 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A₁₁ and A₁₃, at 230 nm, and A₁₂ and A₁₄, at 300 nm, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.2₄₅>, using water as the blank.

System suitability—


dissolution rate (%) with respect to the labeled amount of alacepril (C₂₀H₂₆N₂O₅S) = M₅ × (A₁₁ - A₁₃)/(A₁₁ - A₁₃) × V/V × 1/C × 90

M₅: Amount (mg) of alacepril for assay
C: Labeled amount (mg) of alacepril (C₂₀H₂₆N₂O₅S) in 1 tablet

Containers and storage—

L-Alanine, when dried, contains not less than 98.5% and not more than 101.0% of C₃H₇NO₂.

Description L-Alanine occurs as white crystals or crystalline powder. It has a slightly sweet taste. It is freely soluble in water and in formic acid, and practically insoluble in ethanol (99.5). It dissolves in 6 mol/L hydrochloric acid TS.

Identification—

Determine the infrared absorption spectrum of L-Alanine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.2₅₉>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.₄₉₉> [α]₀°: +13.5 – +15.5° (after drying, 2.5 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.₅₄₉>—Dissolve 1.0 g of L-Alanine in 20 mL of water: the pH of the solution is between 5.7 and 6.7.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Alanine in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.₀₇₉>—Perform the test with 0.5 g of L-Alanine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%)

(3) Sulfate <1.₁₄₉>—Perform the test with 0.6 g of L-Alanine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%)

(4) Ammonium <1.₀₂₉>—Perform the test with 0.25 g of L-Alanine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.₀₇₉>—Proceed with 1.0 g of L-Alanine according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <1.₁₇₉>—Prepare the test solution with 1.0 g of L-Alanine according to Method 1, and perform the test according to Method A. Prepare the control solution with
1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Weigh accurately about 0.5 g of L-Alanine, dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, accurately measure 2.5 mmol amounts of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, glycine, L-alanine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine and L-arginine, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Based on the peak heights obtained from the sample solution and standard solution, determine the mass of the amino acids other than alanine contained in 1 mL of the sample solution, and calculate the mass percent: the amount of each amino acid other than alanine is not more than 0.1%.

Operating conditions—
Detector: A visible spectrophotometer (wavelength: 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (Na type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57°C. Chemical reaction bath temperature: A constant temperature of about 130°C.

Reaction time: About 1 minute.
Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table, and add 0.1 mL of capric acid to each mobile phase.

<table>
<thead>
<tr>
<th></th>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
<th>Mobile phase C</th>
<th>Mobile phase D</th>
<th>Mobile phase E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid monohydrate</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>12.80 g</td>
<td>6.10 g</td>
<td>—</td>
</tr>
<tr>
<td>Trisodium citrate dihydrate</td>
<td>6.19 g</td>
<td>7.74 g</td>
<td>13.31 g</td>
<td>26.67 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>3.74 g</td>
<td>54.35 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>8.00 g</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol (99.5%)</td>
<td>130 mL</td>
<td>20 mL</td>
<td>4 mL</td>
<td>100 mL</td>
<td>—</td>
</tr>
<tr>
<td>Thiodiglycol</td>
<td>5 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>—</td>
<td>—</td>
<td>5 mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lauromacrogol solution (1 in 4)</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
</tr>
<tr>
<td>Water</td>
<td>Appropriate amount</td>
<td>Appropriate amount</td>
<td>Appropriate amount</td>
<td>Appropriate amount</td>
<td>Appropriate amount</td>
</tr>
</tbody>
</table>

Total volume 1000 mL 1000 mL 1000 mL 1000 mL 1000 mL

Changing mobile phases: When the procedure is run with 20 μL of the standard solution under the above operating conditions, switchover in sequence to mobile phases A, B, C, D and E so that aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine, and arginine are eluted in this order with the resolution between the peaks of isoleucine and leucine being not less than 1.2.

Reaction reagents: Dissolve 204 g of lithium acetate dihydrate in water, add 123 mL of acetic acid (100) and 401 mL of 1-methoxy-2-propanol, and water to make 1000 mL, introduce nitrogen for 10 minutes, and use this solution as solution (I). Separately, add 39 g of ninhydrin to 979 mL of 1-methoxy-2-propanol, introduce nitrogen for 5 minutes, add 81 mg of sodium borohydride, introduce nitrogen for 30 minutes, and use this solution as solution (II). To 1 volume of solution (I) add 1 volume of solution (II). Prepare before use.

Flow rate of mobile phase: 0.20 mL per minute.
Flow rate of reaction reagent: 0.24 mL per minute.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and alanine is not less than 1.2.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak height and retention time of each amino acid obtained from the standard solution are not more than 5.0% and not more than 1.0%, respectively.

Loss on ignition <2.44> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 90 mg of L-Alanine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 8.909 mg of C,H$_2$NO$_2$

Containers and storage Containers—Tight containers.

**Albumin Tannate**

タンニン酸アルブミン

Albumin Tannate is a compound of tannic acid and a protein. The label states the origin of the protein of Albumin Tannate.

**Description** Albumin Tannate occurs as a light brown powder. It is odorless, or has a faint, characteristic odor. It is practically insoluble in water and in ethanol (95). It dissolves in sodium hydroxide TS with turbidity.

**Identification** (1) To 0.1 g of Albumin Tannate add 10 mL of ethanol (95), and heat in a water bath for 3 minutes with shaking. After cooling, filter, and to 5 mL of the fil-
trate add 1 drop of iron (III) chloride TS: a blue-purple to bluish black color is produced. On standing, a bluish black precipitate is produced.

(2) To 0.1 g of Albumin Tannate add 5 mL of nitric acid: an orange-yellow color develops.

**Purity (1)** Acidity—Shake 1.0 g of Albumin Tannate with 50 mL of water for 5 minutes, and filter. To 25 mL of the filtrate add 1.0 mL of 0.1 mol/L sodium hydroxide VS 2 drops of phenolphthalein TS: a red color develops.

(2) Fats—To 2.0 g of Albumin Tannate add 20 mL of petroleum benzine, shake vigorously for 15 minutes, and filter. Evaporate 10 mL of the filtrate on a water bath: the mass of the residue is not more than 50 mg.

**Residue on ignition**<sup>2.44</sup> Not more than 1.0% (0.5 g).

**Digestion test** To 1.00 g of Albumin Tannate add 0.25 g of saccharated pepsin and 100 mL of water, shake well, and allow to stand for 20 minutes at 40±1°C in a water bath. Add 1.0 mL of dilute hydrochloric acid, shake, and allow to stand for 3 hours at 40±1°C. Cool rapidly to ordinary temperature, and filter. Wash the residue with 3-10 mL portions of water, dry in a desiccator (silica gel) for 18 hours, and dry at 105°C for 3 hours: the mass of the residue is 0.50 to 0.58 g.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Aldioxa**

アルジオキサ

![Chemical Structure](image)

C₃H₁₉AlN₅O₇: 218.10
Dihydroxo(5-oxo-4-ureido-4,5-dihydro-1H-imidazol-2-yl)oxyaluminium [5579-81-7]

Aldioxa is a condensation product of allantoin and aluminum hydroxide. When dried, it contains not less than 65.3% and not more than 74.3% of allantoin (C₆H₃N₃O₇: 158.12), and not less than 11.1% and not more than 13.0% of aluminum (Al: 26.98).

**Description** Aldioxa occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Melting point: about 230°C (with decomposition).

**Identification (1)** To 0.2 g of Aldioxa add 10 mL of dilute hydrochloric acid, boil for 5 minutes, and add 10 mL of a solution of phenylhydrazinium chloride (1 in 100). After cooling, mix well with 0.5 mL of potassium hexacyanoferrate (III) TS, and shake with 1 mL of hydrochloric acid: a red color develops.

(2) To 0.2 g of Aldioxa add 10 mL of dilute hydrochloric acid, dissolve by warming, and cool: the solution responds to the Qualitative Tests.<sup>1.09</sup> for aluminum salt.

**Purity (1)** Chloride <1.03>—To 0.10 g of Aldioxa add 6 mL of dilute nitric acid, boil with shaking for 5 minutes, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142%).

(2) Sulfate <1.14>—To 0.20 g of Aldioxa add 6 mL of dilute hydrochloric acid, boil with shaking for 5 minutes, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

(3) Nitrate—To 0.10 g of Aldioxa add carefully 5 mL of water and 5 mL of sulfuric acid, dissolve by shaking, cool, and superimpose 2 mL of iron (II) sulfate TS: no brown ring is produced at the zone of contact.

(4) Heavy metals <1.07>—To 1.0 g of Aldioxa add 3 mL of hydrochloric acid and 3 mL of water, heat gently to boil with shaking, and evaporate on a water bath to dryness. To the residue add 30 mL of water, shake under warming, cool, filter, and to the filtrate add 2 mL of dilute acetic acid (31) and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 3 mL of hydrochloric acid add 3 mL of water, evaporate on a water bath to dryness, and add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid (31) and water to make 50 mL (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Aldioxa according to Method 2, and perform the test (not more than 2 ppm).

**Loss on drying**<sup>2.44</sup> Not more than 4.0% (1 g, 105°C, 2 hours).

**Assay (1)** Allantoin—Weigh accurately about 0.1 g of Aldioxa, previously dried, dissolve in 50 mL of dilute sulfuric acid by heating, cool, and add water to make exactly 100 mL. Pipet 10 mL of this solution, and perform the test as directed under Nitrogen Determination<sup>1.08</sup>. Each mL of 0.005 mol/L sulfuric acid VS = 0.3953 mg of C₃H₉N₅O₇

(2) Aluminum—Weigh accurately about 0.2 g of Aldioxa, previously dried, dissolve carefully in 50 mL of dilute hydrochloric acid by heating, cool, and add dilute hydrochloric acid to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Aluminum Solution, dilute with water so that each mL of the solution contains not less than 16.0 μg and not more than 64.0 μg of aluminum (Al: 26.98), and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry<sup>2.23</sup> according to the following conditions, and calculate the aluminum content of the sample solution from the calibration curve obtained from the absorbance of the standard solu-
Alendronate Sodium Hydrate

アルレンドロン酸ナトリウム水和物

\[
\text{C}_4\text{H}_{12}\text{NNaO}_7\text{P}_2\cdot3\text{H}_2\text{O}: 325.12
\]

Monosodium trihydrogen 4-amino-1-hydroxybutane-1,1-diylidiphosphonate trihydrate

\[\text{H}_2\text{N} \quad \text{PO}_3\text{HNa} \quad \text{PO}_3\text{H}_2 \quad \cdot 3\text{H}_2\text{O}\]

Alendronate Sodium Hydrate contains not less than 99.0% and not more than 101.0% of alendronate sodium (\(\text{C}_4\text{H}_{12}\text{NNaO}_7\text{P}_2: 271.08\)), calculated on the dried basis.

**Description**

Alendronate Sodium Hydrate occurs as a white crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 0.1 mol/L trisodium citrate TS.

Melting point: about 252°C (with decomposition, after drying).

**Identification** (1)

To 5 mL of a solution of Alendronate Sodium Hydrate (1 in 50) add 1 mL of ninhydrin TS, and heat: a blue-purple color develops.

(2) Determine the infrared absorption spectrum of Alendronate Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum or the spectrum of Alendronate Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Alendronate Sodium Hydrate add 10 mL of a mixture of nitric acid and perchloric acid (1:1). Heat to concentrate to about 1 mL, add about 10 mL of water while hot, and neutralize with a solution of sodium hydroxide (2 in 5): the solution responds to the Qualitative Tests <1.09> for phosphate.

(4) A solution of Alendronate Sodium Hydrate (1 in 100) responds to the Qualitative Tests <1.09> for sodium salt.

**pH** <2.54>

The pH of a solution of 1.0 g of Alendronate Sodium Hydrate in 100 mL of freshly boiled and cooled water is between 4.0 and 5.0.

**Purity** (1) Heavy metals <1.07>—Put 1.0 g of Alendronate Sodium Hydrate in a Kjeldahl flask, add 9 mL of a mixture of nitric acid and sulfuric acid (5:4), and heat until the solution becomes brown. After cooling, add 9 mL of a mixture of nitric acid and sulfuric acid (5:4), and heat again until the color changes from colorless to brown. After cooling, add 2 mL of nitric acid, strongly heat until brown fumes are no longer evolved, and heat until large amounts of white fumes are evolved. After cooling, add carefully 5 mL of water and 1 mL of hydrogen peroxide (30), heat until white fumes are no longer evolved, and continue heating for more 5 minutes. After cooling, if any yellow color remains, add 2 mL of nitric acid, and repeat the same procedure. After cooling, transfer the solution in the Kjeldahl flask to a beaker, wash out the inside of the flask with 5 mL of water, and add the washing to the beaker. Adjust to pH 3 – 5 with ammonia solution (28), transfer to a Nessler tube, add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution in the same procedure using the same amount of the reagents used for the preparation of the sample solution, add 1.0 mL of Standard Lead Solution and add water to make 50 mL (not more than 10 ppm).

(2) Related substances—Dissolve 15 mg of Alendronate Sodium Hydrate in 25 mL of 0.1 mol/L trisodium citrate TS, and use this solution as the sample stock solution. Pipet 5 mL of the sample stock solution, and add 0.1 mol/L trisodium citrate TS to make exactly 50 mL. Pipet 1 mL of this solution, add 0.1 mol/L trisodium citrate TS to make exactly 100 mL, and use this solution as the standard stock solution. To exactly 5 mL each of the sample stock solution and standard stock solution, add exactly 5 mL each of a solution of sodium tetraborate decahydrate (19 in 1000), acetonitrile and a solution of 9-fluorenylmethyl chloroformate in acetonitrile (1 in 250), shake for 45 seconds, and allow to stand for 30 minutes at room temperature. Then, add 20 mL of dichloromethane to them, shake for 60 seconds, centrifuge, and use the supernatant liquids so obtained as the sample solution and the standard solution, respectively. Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography (2.21) according to the following conditions. Determine each peak area of these solutions by the automatic integration method: each peak area other than alendronic acid obtained from the sample solution is not larger than the peak area of alendronic acid from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 266 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase A: Dissolve 2.94 g of trisodium citrate dihydrate and 1.42 g of anhydrous disodium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 850 mL of this solution add 150 mL of acetonitrile, and use this solution as the sample stock solution.

Mobile phase B: Dissolve 2.94 g of trisodium citrate dihydrate and 1.42 g of anhydrous disodium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 700 mL of acetonitrile, and use this solution as the standard stock solution.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Mobile Phase A</th>
<th>Mobile Phase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>0.9</td>
<td>0.1</td>
</tr>
<tr>
<td>1.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%)
---|---|---
0 – 15 | 100 → 50 | 0 → 50
15 – 25 | 50 → 0 | 50 → 100

Flow rate: About 1.8 mL per minute.
Time span of measurement: About 5 times as long as the retention time of alendronic acid, beginning after the solvent peak.

System suitability—
System performance: Dissolve 15 mg of Alendronate Sodium Hydrate and 2 mg of 4-aminobutylic acid in 100 mL of 0.1 mol/L trisodium citrate TS. To 5 mL of this solution add 5 mL each of a solution of sodium tetraborate decahydrate (19 in 1000), acetonitrile and a solution of 9-fluorenylmethyl chloroformate in acetonitrile (1 in 250), then, proceed in the same manner as the sample solution. When the procedure is run with 20 μL of this solution under the above operating conditions, alendronic acid and 4-aminobutylic acid are eluted in this order with the resolution between these peaks being not less than 6.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alendronic acid is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying 2.41 | 16.1 - 17.1% (1 g, 140°C, 3 hours).

Assay Weigh accurately about 10 mg each of Alendronate Sodium Hydrate and Alendronate Sodium RS (separately determine the loss on drying in the same conditions as Alendronate Sodium Hydrate), dissolve in 0.1 mol/L trisodium citrate TS to make exactly 100 mL, and use these solutions as the sample stock solution and the standard stock solution, respectively. To exactly 5 mL each of these solutions add exactly 5 mL each of a solution of sodium tetraborate decahydrate (19 in 1000) and a solution of 9-fluorenylmethyl chloroformate in acetonitrile (1 in 2000), shake for 30 seconds, and allow to stand for 25 minutes. Then, add 25 mL of dichloromethane, shake for 60 seconds, centrifuge, and use the supernatant liquids so obtained as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A1 and A5, of alendronic acid.

Amount (mg) of alendronate sodium (C4H7N2NaO7P2)

\[ M_S = M_S \times \frac{A_1}{A_5} \]

M_S: Amount (mg) of Alendronate Sodium RS, calculated on the dried basis.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 266 nm).
Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 14.7 g of trisodium citrate dihydrate and 7.1 g of anhydrous disodium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 700 mL of this solution add 250 mL of acetonitrile for liquid chromatography and 50 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of alendronic acid is about 3 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of alendronic acid are not less than 1500 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alendronic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Alendronate Sodium Injection
アレンドロン酸ナトリウム注射液

Alendronate Sodium Injection is an aqueous solution for injection.
It contains not less than 95.0% and not more than 101.0% of the labeled amount of alendronic acid (C4H7N2NaO7P2: 249.10).

Method of preparation Prepare as directed under Injections, with Alendronate Sodium Hydrate.

Description Alendronate Sodium Injection is a clear, colorless liquid.

Identification Use Alendronate Sodium Injection as the sample solution. Separate, dissolve 33 mg of alendronate sodium hydrate in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.01) according to the following conditions, and determine the peak areas, A1 and A5, of alendronic acid.

Amount (mg) of alendronate sodium (C4H7N2NaO7P2)

\[ M_S = M_S \times \frac{A_1}{A_5} \]

M_S: Amount (mg) of Alendronate Sodium RS, calculated on the dried basis.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 266 nm).
Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 14.7 g of trisodium citrate dihydrate and 7.1 g of anhydrous disodium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 700 mL of this solution add 250 mL of acetonitrile for liquid chromatography and 50 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of alendronic acid is about 3 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of alendronic acid are not less than 1500 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alendronic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Alendronate Sodium Injection
アレンドロン酸ナトリウム注射液

Alendronate Sodium Injection is an aqueous solution for injection.
It contains not less than 95.0% and not more than 101.0% of the labeled amount of alendronic acid (C4H7N2NaO7P2: 249.10).

Method of preparation Prepare as directed under Injections, with Alendronate Sodium Hydrate.

Description Alendronate Sodium Injection is a clear, colorless liquid.

Identification Use Alendronate Sodium Injection as the sample solution. Separate, dissolve 33 mg of alendronate sodium hydrate in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.01) according to the following conditions, and determine the peak areas, A1 and A5, of alendronic acid.

Amount (mg) of alendronate sodium (C4H7N2NaO7P2)

\[ M_S = M_S \times \frac{A_1}{A_5} \]

M_S: Amount (mg) of Alendronate Sodium RS, calculated on the dried basis.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 266 nm).
Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 14.7 g of trisodium citrate dihydrate and 7.1 g of anhydrous disodium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 700 mL of this solution add 250 mL of acetonitrile for liquid chromatography and 50 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of alendronic acid is about 3 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of alendronic acid are not less than 1500 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alendronic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Alendronate Sodium Injection
アレンドロン酸ナトリウム注射液

Alendronate Sodium Injection is an aqueous solution for injection.
It contains not less than 95.0% and not more than 101.0% of the labeled amount of alendronic acid (C4H7N2NaO7P2: 249.10).

Method of preparation Prepare as directed under Injections, with Alendronate Sodium Hydrate.

Description Alendronate Sodium Injection is a clear, colorless liquid.

Identification Use Alendronate Sodium Injection as the sample solution. Separate, dissolve 33 mg of alendronate sodium hydrate in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.01) according to the following conditions, and determine the peak areas, A1 and A5, of alendronic acid.

Amount (mg) of alendronate sodium (C4H7N2NaO7P2)

\[ M_S = M_S \times \frac{A_1}{A_5} \]

M_S: Amount (mg) of Alendronate Sodium RS, calculated on the dried basis.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 266 nm).
Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Alendronate Sodium Tablets

アレンドロン酸ナトリウム錠

Alendronate Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of alendronic acid (C$_{4}$H$_{13}$NO$_{7}$P$_{2}$: 249.10).

**Method of preparation** Prepare as directed under Tablets, with Alendronate Sodium Hydrate.

**Identification** To a quantity of powdered Alendronate Sodium Tablets, equivalent to 25 mg of alendronic acid (C$_{4}$H$_{13}$NO$_{7}$P$_{2}$) according to the labeled amount, add 25 mL of water, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh 33 mg of alendronic acid hydrate, and dissolve in 25 mL of water as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.0D). Spot 5 µL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of water, pyrigin, acetic acid (100) and ethyl acetate (1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 100°C for 10 minutes: the principal spots from the sample solution and standard solution show a blue-purple color and the same $R_f$ value.

**Uniformity of dosage units** (6.0D) Perform the test according to the following method: it meets the requirement of the content uniformity test.

To 1 tablet of Alendronate Sodium Tablets add 0.1 mol/L trisodium citrate TS to make exactly 100 mL, and stir until the tablet is completely disintegrated. Centrifuge this solution, pipet $V$ mL of the supernatant liquid, and add 0.1 mol/L trisodium citrate TS to make exactly $V$ mL so that each mL contains about 25 µg of alendronic acid (C$_{4}$H$_{13}$NO$_{7}$P$_{2}$), and use this solution as the sample stock solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of alendronic acid (C}_4\text{H}_{13}\text{NO}_7\text{P}_2) = M_5 \times A_1 / A_5 \times 1/5 \times 0.919$$

$M_5$: Amount (mg) of Alendronate Sodium RS, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 14.7 g of trisodium citrate dihydrate and 8.7 g of dipotassium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 750 mL of this solution add 200 mL of acetonitrile for liquid chromatography and 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of alendronic acid is about 7 minutes.

System suitability—

System performance: When the procedure is run with 50 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of alendronic acid are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak of alendronic acid is not more than 1.0%.

Containers and storage Container—Hermetic containers.

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**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 14.7 g of trisodium citrate dihydrate and 8.7 g of dipotassium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 750 mL of this solution add 200 mL of acetonitrile for liquid chromatography and 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of alendronic acid is about 7 minutes.

System suitability—

System performance: When the procedure is run with 50 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of alendronic acid are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak of alendronic acid is not more than 1.0%.

Containers and storage Container—Hermetic containers.
the standard stock solution. Pipet 5 mL each of the sample stock solution and standard stock solution, add exactly 1 mL of trisodium citrate dihydrate solution (22 in 125), exactly 5 mL of a solution obtained by dissolving 6.2 g of boric acid in 950 mL of water, adjusting to pH 9.0 with sodium hydroxide TS, and adding water to make 1000 mL, and add exactly 4 mL of a solution of 9-fluorenylmethyl chloroformate in acetonitrile (1 in 2000), shake for 30 seconds, and allow to stand at room temperature for 25 minutes. Then, add 25 mL of dichloromethane, shake for 45 seconds, then centrifuge, and use the supernatant liquid as the sample solution and the standard solution, respectively. Then, proceed as directed in the Assay.

Dissolution rate (%) with respect to the labeled amount of alendronic acid (C\(_{4}H_{13}NO_{7}P_{2}\))

\[ M \times A_{T}/A_{S} \times V/V \times 1/C \times 108/5 \times 0.919 \]

\( M \): Amount (mg) of Alendronate Sodium RS, calculated on the dried basis
\( C \): Labeled amount (mg) of alendronic acid (C\(_{4}H_{13}NO_{7}P_{2}\)) in 1 tablet

**Assay**

Weigh accurately and powder not less than 20 Alendronate Sodium Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of alendronic acid (C\(_{4}H_{13}NO_{7}P_{2}\)), add 0.1 mol/L trisodium citrate TS to make exactly 1000 mL, stir for 30 minutes, and centrifuge. Pipet 5 mL of the supernatant liquid, add 0.1 mL/L trisodium citrate TS to make exactly 50 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 39 mg of Alendronate Sodium RS (separately determine the loss on drying <2.41> in the same conditions as Alendronate Sodium Hydrate), dissolve in 0.1 mol/L trisodium citrate TS to make exactly 50 mL. Pipet 2 mL of this solution, add 0.1 mol/L trisodium citrate TS to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 5 mL each of the sample stock solution and standard stock solution, add exactly 5 mL of a solution of sodium tetraborate decahydrate (19 in 500) and exactly 4 mL of a solution of 9-fluorenylmethyl chloroformate in acetonitrile (1 in 2000), shake for 30 seconds, and allow to stand at room temperature for 25 minutes. Then, add 25 mL of dichloromethane to them, shake for 45 seconds, centrifuge, and use the supernatant liquid as the sample solution and the standard solution, respectively. Perform the test with exactly 50 mL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \( A_{T} \) and \( A_{S} \), of alendronic acid.

\[ M \times A_{T}/A_{S} \times 8/5 \times 0.919 \]

\( M \): Amount (mg) of Alendronate Sodium RS, calculated on the dried basis

**Operating conditions**

**Detector:** An ultraviolet absorption photometer (wavelength: 266 nm).

**Column:** A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 \( \mu m \) in particle diameter).

**Column temperature:** A constant temperature of about 35°C.

Mobile phase: Dissolve 14.7 g of trisodium citrate dihydrate and 7.1 g of anhydrous disodium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 750 mL of this solution add 200 mL of acetonitrile for liquid chromatography and 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of alendronic acid is about 7 minutes.

**System suitability**

System performance: When the procedure is run with 50 \( \mu L \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of alendronic acid are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 \( \mu L \) of the standard solution under the above operations conditions, the relative standard deviation of the peak of alendronic acid is not more than 1.0%.

**Containers and storage**

Container—Well-closed containers.

### Alimemazine Tartrate

アリメマジン酒石酸塩

![Chemical Structure](image)

(C\(_{18}H_{22}N_{2}S\))\(_{2}\).C\(_{4}H\(_{6}\)O\(_{6}\): 746.98

\( N/N,2\)-Trimethyl-3-(10H-phenothiazin-10-yl)propylamine hemitartrate

[41375-66-0]

Alimemazine Tartrate, when dried, contains not less than 98.0% of \( (C_{18}H_{22}N_{2}S)_{2}C_{4}H_{6}O_{6} \).

**Description**

Alimemazine Tartrate occurs as a white powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Alimemazine Tartrate (1 in 50) is between 5.0 and 6.5.

It is gradually colored by light.

**Identification**

(1) To 2 mL of a solution of Alimemazine Tartrate (1 in 100) add 1 drop of iron (III) chloride TS: A red-brown color is produced, and immediately a yellow precipitate is formed.

(2) Dissolve 1 g of Alimemazine Tartrate in 5 mL of water, add 3 mL of sodium hydroxide TS, extract with two 10-mL portions of diethyl ether [use the aqueous layer obtained in the Identification (4)]. Shake the combined diethyl ether extracts with 3 g of anhydrous sodium sulfate, filter, and evaporate the diethyl ether with the aid of a current of air. Dry the residue in a desiccator (in vacuum, phosphorus oxide) for 16 hours: it melts <2.60> between 66°C and 70°C.

(3) Determine the absorption spectrum of a solution of
Alimemazine Tartrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) The aqueous layer, obtained in the identification (2), when neutralized with dilute acetic acid, responds to the Qualitative Tests <1.69> (1) and (2) for tartrate.

**Melting point** <2.60> 159 – 163°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Alimemazine Tartrate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Alimemazine Tartrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 2 ppm).

(3) Arsenic <1.17>—Prepare the test solution with 1.0 g of Alimemazine Tartrate according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 2 ppm).

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.8 g of Alimemazine Tartrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from red through brown to green-brown (indicator: 2 mL of p-naphtholbenzene TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 37.35 mg of (C₄H₄N₄O₂).₆C₂H₆O₄

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

### Allopurinol

アルポリノール

C₉H₈N₄O: 136.11
1H-Pyrazolo[3,4-d]pyrimidin-4-ol
[315-30-0]

Allopurinol, when dried, contains not less than 98.0% and not more than 101.0% of C₉H₈N₄O.

**Description** Allopurinol occurs as white to pale yellowish white crystals or crystalline powder.

It is slightly soluble in N,N-dimethylformamide, and very slightly soluble in water and in ethanol (99.5).

It dissolves in ammonia TS.

**Identification** (1) Determine the absorption spectrum of a solution of Allopurinol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Allopurinol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Allopurinol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 1.0 g of Allopurinol according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Allopurinol in 10 mL of ammonia TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ammonia TS to make exactly 500 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of cellulose with fluorescent indicator for thin-layer chromatography. Develop the plate with ammonia TS-saturated 1-butanol to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.16 g of Allopurinol, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from red through brown to green-brown (indicator: 2 mL of p-naphtholbenzene TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 13.61 mg of C₉H₈N₄O

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

### Allopurinol Tablets

アルポリノール錠

Allopurinol Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of allopurinol (C₉H₈N₄O: 136.11).

**Method of preparation** Prepare as directed under Tablets, with Allopurinol.

**Identification** (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 248 nm and 252 nm.

(2) To a quantity of powdered Allopurinol Tablets,
equivalent to 0.1 g of Allopurinol according to the labeled amount, add 5 mL of a solution of diethylamine (1 in 10), shake well, add 5 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.1 g of allopurinol in 5 mL of a solution of diethylamine (1 in 10), add 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.2.4. Spot 2.5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-butanol, ammonia solution (28) and 2-methoxyethanol (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spots obtained from the sample solution and standard solution show the same Rf value.

Uniformity of dosage units 6.02 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Allopurinol Tablets add V/10 mL of a 0.05 mol/L sodium hydroxide TS, shake well, and treat with ultrasonic waves for 10 minutes. After cooling, add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.5 mg of allopurinol (C₆H₄N₄O), and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L of hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of allopurinol for assay, previously dried at 105°C for 4 hours, dissolve in 20 mL of 0.05 mol/L sodium hydroxide TS, and add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₅, of the sample solution and standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry 2.2.4.

\[
\text{Amount (mg) of allopurinol (C₆H₄N₄O)} = M₅ \times A₁ / A₅ \times V / 100
\]

\[ M₅: \text{Amount (mg) of allopurinol for assay} \]

Dissolution 6.10 When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Allopurinol Tablets is not less than 80%.

Start the test with 1 tablet of Allopurinol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 11 μg of allopurinol (C₆H₄N₄O) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 11 mg of allopurinol for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₅, of the sample solution and standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry 2.2.4.

\[
\text{Dissolution rate (％) with respect to the labeled amount of allopurinol (C₆H₄N₄O)} = M₅ \times A₁ / A₅ \times V / V \times 1 / C \times 90
\]

\[ M₅: \text{Amount (mg) of allopurinol for assay} \]

C: Labeled amount (mg) of allopurinol (C₆H₄N₄O) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Allopurinol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of allopurinol (C₆H₄N₄O), add 20 mL of 0.05 mol/L sodium hydroxide TS, shake well, and treat with ultrasonic waves for 10 minutes. After cooling, add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of allopurinol for assay, previously dried at 105°C for 4 hours, dissolve in 20 mL of 0.05 mol/L sodium hydroxide TS, and add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₅, of the sample solution and standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry 2.2.4.

\[
\text{Amount (mg) of allopurinol (C₆H₄N₄O)} = M₅ \times A₁ / A₅
\]

\[ M₅: \text{Amount (mg) of allopurinol for assay} \]

Containers and storage Containers—Well-closed containers.

Alminoprofen

アルミノプロフェン

C₁₃H₁₇NO₂: 219.28
(2RS)-2-[[4-(2-Methylprop-2-en-1-yl)amino]phenyl]propanoic acid [39718-89-3]

Alminoprofen, when dried, contains not less than 99.0% and not more than 101.0% of C₁₃H₁₇NO₂.

Description Alminoprofen occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in ethanol (99.5) and in acetic acid (100), and very slightly soluble in water.

It gradually turns brown on exposure to light.

A solution of Alminoprofen in ethanol (99.5) (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Alminoprofen in ethanol (99.5) (3 in 500,000) as directed under Ultraviolet-visible Spectrophotometry 2.2.4, and compare the spectrum with the Reference Spectrum:
both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the infrared absorption spectrum of Alminoprofen as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** \(<2.60>\) 106 – 108°C

**Purity** (1) Heavy metals \(<1.07>\)—Proceed with 2.0 g of Alminoprofen according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.11>\)—Prepare the test solution with 1.0 g of Alminoprofen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Alminoprofen in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of the peak other than alminoprofen obtained from the sample solution is not larger than 1/5 times the peak area of alminoprofen from the standard solution. Furthermore, the total area of the peaks other than alminoprofen from the sample solution is not larger than the peak area of alminoprofen from the standard solution.

**Operating conditions**—
- **Detector:** An ultraviolet absorption photometer (wavelength: 254 nm).
- **Column:** A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
- **Column temperature:** A constant temperature of about 25°C.
- **Mobile phase:** A mixture of methanol and diluted acetic acid (100) (1 in 1000) (4:1).
- **Flow rate:** Adjust the flow rate so that the retention time of alminoprofen is about 5 minutes.
- **Time span of measurement:** About 5 times as long as the retention time of alminoprofen, beginning after the solvent peak.

**System suitability**—
- **Test for required detectability:** Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of alminoprofen obtained from 5 \(\mu\)L of this solution is equivalent to 7 to 13% of that from 5 \(\mu\)L of the standard solution.
- **System performance:** Dissolve 10 mg each of Alminoprofen and butyl parahydroxybenzoate in 100 mL of methanol. Pipet 10 mL of this solution, and add methanol to make exactly 50 mL. When the procedure is run with 5 \(\mu\)L of this solution under the above operating conditions, alminoprofen and butyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.
- **System repeatability:** When the test is repeated 6 times with 5 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alminoprofen is not more than 2.0%.

**Loss on drying** \(<2.41>\) Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 1 hour).

**Residue on ignition** \(<2.44>\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Alminoprofen, previously dried, dissolve in 50 mL of acetic acid (100), and titrate \(<2.50>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 21.93 \text{ mg of C}_{13}H_{17}NO_2
\]

**Containers and storage** Containers—Well-closed containers. Storage—Light-resistant.

### Alminoprofen Tablets

アルミノプロフェン錠

Alminoprofen Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of alminoprofen (\(\text{C}_{13}H_{17}NO_2\): 219.28).

**Method of preparation** Prepare as directed under Tablets, with Alminoprofen.

**Identification** Take an amount of powdered Alminoprofen Tablets, equivalent to 30 mg of Alminoprofen according to the labeled amount, add ethanol (99.5) to make 100 mL, shake thoroughly, and centrifuge. To 2 mL of the supernatant liquid add ethanol (99.5) to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\): it exhibits maxima between 253 nm and 257 nm, and between 298 nm and 302 nm.

**Purity** Related substances—Conduct this procedure using light-resistant vessels. Powder 10 tablets of Alminoprofen Tablets, weigh a portion of the powder equivalent to 50 mg of Alminoprofen according to the labeled amount, add 50 mL of the mobile phase, shake for 15 minutes, add the mobile phase to make exactly 100 mL, centrifuge, and use the supernatant liquid as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the conditions described in the Purity (3) under Alminoprofen. Determine each peak area of each solution by the automatic integration method: the area of the peak other than alminoprofen obtained from the sample solution is not larger than 1/2 times the peak area of alminoprofen from the standard solution. Furthermore, the total area of the peaks other than alminoprofen from the sample solution is not larger than 2 times the peak area of alminoprofen from the standard solution.

**Uniformity of dosage units** \(<6.02>\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.
To 1 tablet of Alminoprofen Tablets add 5 mL of water, shake until the tablet is disintegrated, add 50 mL of ethanol (99.5), shake for 20 minutes, then add ethanol (99.5) to make exactly 100 mL, and centrifuge. Pipet 3 mL of the supernatant liquid, add ethanol (99.5) to make exactly 50 mL. Pipet V mL of this solution, add ethanol (99.5) to make exactly V mL so that each mL contains about 6 μg of alminoprofen (C₁₃H₁₇NO₂), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of alminoprofen (C₁₃H₁₇NO₂) = Mₛ × A₁/Aₙ × V/V × 1/3

Mₛ: Amount (mg) of alminoprofen for assay

Dissolution [6.10] When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Alminoprofen Tablets is not less than 80%.

Start the test with 1 tablet of Alminoprofen Tablets, withdraw not less than 20 mL of the medium at specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.05 mol/L sodium hydroxide TS to make exactly V mL so that each mL contains about 8.9 μg of alminoprofen (C₁₃H₁₇NO₂) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of alminoprofen for assay, previously dried in vacuum for 1 hour using phosphorus (V) oxide as the desiccant, and dissolve in 0.05 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 3 mL of this solution, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and Aₙ, at 245 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of alminoprofen (C₁₃H₁₇NO₂) = Mₛ × A₁/Aₙ × V/V × 1/C × 27

Mₛ: Amount (mg) of alminoprofen for assay

C: Labeled amount (mg) of alminoprofen (C₁₃H₁₇NO₂) in 1 tablet

Assay Weigh accurately the mass of not less than 20 tablets of Alminoprofen Tablets, and powder. Weigh accurately an amount equivalent to about 60 mg of alminoprofen (C₁₃H₁₇NO₂), add ethanol (99.5) and shake well, add ethanol (99.5) to make exactly 200 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add ethanol (99.5) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of alminoprofen for assay, previously dried in vacuum for 1 hour using phosphorus (V) oxide as the desiccant, dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2 mL of this solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and Aₙ, at the wavelength of maximum absorption at about 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of alminoprofen (C₁₃H₁₇NO₂) = Mₛ × A₁/Aₙ × 2

Mₛ: Amount (mg) of alminoprofen for assay

Containers and storage Containers—Well-closed containers.

Alprazolam

アルプラゾラム

C₁₇H₁₃ClN₄: 308.76
8-Chloro-1-methyl-6-phenyl-4-
[1,2,4]triazolo[4,3-]
H: 4H-
[1,2,4]triazolo[4,3-a]1,4benzdiazepine
[28981-97-7]

Alprazolam, when dried, contains not less than 98.5% of C₁₇H₁₃ClN₄.

Description Alprazolam occurs as white crystals or crystaline powder.

It is freely soluble in chloroform, soluble in methanol and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in water.

It dissolves in dilute nitric acid.

Identification (1) Determine the absorption spectrum of a solution of Alprazolam in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.05 g of Alprazolam in 0.7 mL of deuterchloroform for nuclear magnetic resonance spectroscopy, and determine the spectrum of this solution using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (H): it exhibits a single signal A at around δ 2.6 ppm, doublet signals B and C at around δ 4.0 ppm and δ 5.4 ppm, and a broad signal D between δ 7.1 ppm and 7.9 ppm. The ratio of integrated intensity of each signal, A:B:C:D, is about 3:1:1:8.

(3) Perform the test with Alprazolam as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 228 – 232°C

Purity (1) Chloride <1.02>—Dissolve 0.5 g of Alprazolam in 10 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Alprazolam according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Alprazolam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to
make exactly 100 mL, then pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of acetone, hexane, ethyl acetate and ethanol (95) (4:2:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.25 g of Alprazolam, previously dried, dissolve in 100 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 15.44 mg of C\(_{17}\)H\(_{13}\)ClN\(_4\).

**Containers and storage** Containers—Well-closed containers.

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**Alprenolol Hydrochloride**

アルプレノール塩酸塩

\[
\text{C}_{15}\text{H}_{23}\text{NO}_{2}\cdot\text{HCl}: 285.81
\]

(2R,5S)-1-(2-Allylphenox)-3-[1-(methylamino)propan-2-ol monohydrochloride [13707-88-5]

Alprenolol Hydrochloride, when dried, contains not less than 99.0% of C\(_{15}\)H\(_{23}\)NO\(_2\)\cdot\text{HCl}.

**Description** Alprenolol Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, in ethanol (95) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

**Identification (1)** To 2 mL of a solution of Alprenolol Hydrochloride (1 in 100) add 0.05 mL of copper (II) sulfate TS and 2 mL of sodium hydrosulfite: a blue-purple color develops. To this solution add 1 mL of diethyl ether, shake well, and allow to stand: a red-purple color develops in the diethyl ether layer.

(2) Dissolve 0.05 g of Alprenolol Hydrochloride in 5 mL of water, add 1 to 2 drops of bromine TS, and shake: the color of the test solution disappears.

(3) Determine the absorption spectrum of a solution of Alprenolol Hydrochloride in ethanol (95) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Alprenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Alprenolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.99> for chloride.

**pH** <2.54> Dissolve 1.0 g of Alprenolol Hydrochloride in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

**Melting point** <2.60> 108 – 112°C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Alprenolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Alprenolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Alprenolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Alprenolol Hydrochloride in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 100 mL. Pipet 2.5 mL of this solution, add ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, acetone, acetic acid (100) and water (60:42:5:3) to a distance of about 10 cm, air-dry the plate, and then dry at 80°C for 30 minutes. After cooling, allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot and the spot on the starting point from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Alprenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 28.58 mg of C\(_{15}\)H\(_{23}\)NO\(_2\)\cdot\text{HCl}

**Containers and storage** Containers—Well-closed containers.
Alprostadil

Prostaglandin E₁

アルプロスタジル

\[
\begin{align*}
\text{C}_{20}\text{H}_{34}\text{O}_5: 354.48 \\
7-[1(R,2R,3R)-3-Hydroxy-2-[(1E,3S)-3-hydroxyoct-1-en-1-yl]-5-oxocyclopentyl]heptanoic acid [745-65-3]
\end{align*}
\]

Alprostadil, when dried, contains not less than 97.0% and not more than 103.0% of \( \text{C}_{20}\text{H}_{34}\text{O}_5 \).

**Description**

Alprostadil occurs as white crystals or crystaline powder.

It is freely soluble in ethanol (99.5) and in tetrahydrofuran, slightly soluble in acetonitrile, and practically insoluble in water.

**Identification (1)**

The absorption spectrum of a solution of Alprostadil in ethanol (99.5) (1 in 100,000) determined as directed under Ultraviolet-visible Spectrophotometry shows no absorption between 210 nm and 350 nm. Separately, to 10 mL of this solution add 1 mL of potassium hydroxide-ethanol TS, allow to stand for 15 minutes, and determine the absorption spectrum in the same way. Compare the spectrum so obtained with the Reference Spectrum or the spectrum of a solution of Alprostadil RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)**

Determine the infrared absorption spectrum of Alprostadil, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Alprostadil RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation**

\[ [\alpha]_{D}^{20}: -53 \text{ to } -61^\circ \text{ (after drying, 25 mg, tetrahydrofuran, 5 mL, 100 mm).} \]

**Melting point**

\[ \text{114} \text{ – 118}^\circ \text{C} \]

**Purity**

Related substances—Dissolve 4 mg of Alprostadil in 2 mL of a mixture of acetonitrile for liquid chromatography and water (9:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, and add the mixture of acetonitrile for liquid chromatography and water (9:1) to make exactly 10 mL. Pipet 2 mL of this solution, add the mixture of acetonitrile for liquid chromatography and water (9:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 5 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks, having the relative retention time of about 0.70 and 1.26 with respect to alprostadil, is not larger than 1/2 times the peak area of alprostadil with the standard solution, the area of the peaks, having the relative retention time of about 0.88 and 1.18 with respect to alprostadil, is not larger than the peak area of alprostadil with the standard solution, the area of the peaks other than alprostadil and the peaks mentioned above is not larger than 1/10 times the peak area of alprostadil with the standard solution and the total area of the peaks other than alprostadil is not larger than 2 times the peak area of alprostadil with the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of alprostadil beginning after the solvent peak.

**System suitability**

Test for required detectability: Measure exactly 2 mL of the standard solution, add the mixture of acetonitrile for liquid chromatography and water (9:1) to make exactly 20 mL. Confirm that the peak area of alprostadil obtained with 5 \( \mu L \) of this solution is equivalent to 7 to 13% of that with 5 \( \mu L \) of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

**System repeatability:** When the test is repeated 6 times with 5 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alprostadil is not more than 1.5%.

**Loss on drying**

\[ \text{Not more than 1.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).} \]

**Assay**

Weigh accurately about 5 mg each of Alprostadil and Alprostadil RS, previously dried, dissolve in exactly 5 mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (9:1) to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 5 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, \( Q_{al} \) and \( Q_{RS} \), of the peak area of alprostadil to that of the internal standard.

Amount (mg) of \( \text{C}_{20}\text{H}_{34}\text{O}_5 \) = \( M_{S} \times Q_{al}/Q_{RS} \)

\( M_{S}: \) Amount (mg) of Alprostadil RS

**Internal standard solution**

A solution of dimethyl phthalate in the mixture of acetonitrile for liquid chromatography and water (9:1) (1 in 10,000).

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 196 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 9.07 g of potassium dihydrogen phosphate in water to make 1000 mL. Adjust the pH to 6.3 with a solution prepared by dissolving 9.46 g of disodium hydrogen phosphate in water to make 1000 mL, and dilute to 10 times its volume with water. To 360 mL of this solution add 110 mL of acetonitrile for liquid chromatography and
30 mL of methanol for liquid chromatography.
Flow rate: Adjust the flow rate so that the retention time of alprostadil is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 µL of the standard solution under the above operating conditions, alprostadil and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.
System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of alprostadil to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.
Storage—Light-resistant, and at a temperature not exceeding 5°C.

Alprostadil Injection
アルプロスタジル注射液

Alprostadil Injection is an emulsion-type injection. It contains not less than 80.0% and not more than 125.0% of the labeled amount of alprostadil (C_{20}H_{34}O_{5}: 354.48).

Method of preparation—Prepare as directed under Injections, with Alprostadil.

Description—Alprostadil Injection occurs as a white emulsion and is slightly viscous. It has a distinctive odor.

Identification—To a quantity of Alprostadil Injection, corresponding to 10 µg of Alprostadil according to the labeled amount, add 2 mL of acetonitrile, shake well, and centrifuge. To 3.5 mL of the supernatant liquid add 7 mL of diluted phosphoric acid (1 in 1000), and then run this solution on a column (prepared by filling a 10 mm inside diameter, 9 mm long chromatography tube with 0.4 g of 70 µm octadecylsilanized silica gel for pretreatment) previously having undergone elution with 2.5 mL of a mixture of methanol and then 10 mL of water. Wash the octadecylsilanized silica gel for pretreatment) prewashed layer Chromatography

Separately, dissolve 1 mg of Alprostadil RS in 10 mL of ethyl acetate, and use this solution as the sample solution.

Purity—(1) Heavy metals (≤1.07)—Proceed with 4.0 mL of Alprostadil Injection according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).
(2) Prostaglandin A₁—Use the sample solution obtained in the Assay as the sample solution. Separately, weigh accurately about 10 mg of prostaglandin A₁, previously dried for 4 hours in a desiccator (in vacuum, phosphorus (V) oxide), and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2.5 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 40 µL each of the sample solution and standard solution as directed under Liquid Chromatography (≤2.01>) according to the following conditions, calculate the ratios, Q₁ and Q₂, of the peak area of prostaglandin A₁ to that of the internal standard, and calculate the amount of prostaglandin A₁ converted to alprostadil using the following equation: not more than 3.0 µg per a volume, equivalent to 5 µg of alprostadil (C_{20}H_{32}O_{5}).

Amount (µg) of prostaglandin A₁ (C_{20}H_{32}O_{5}) converted to alprostadil
\[ M_{\text{A₁}} \times Q_{1}/Q_{2} \times 1/2 \times 1.054 \]

M_{\text{A₁}}: Amount (mg) of prostaglandin A₁

Internal standard solution—Dissolve 50 mg of 1-naphthol in 20 mL of ethanol (99.5). To 3 mL of this solution add the mobile phase to make 100 mL.

Operating conditions—Proceed as directed in the operating conditions in the Assay.

System suitability—
Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 5 mL. Confirm that the peak area of prostaglandin A₁ obtained with 40 µL of this solution is equivalent to 14 to 26% of that with 40 µL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

(3) Peroxide—Pipet 4 mL of Alprostadil Injection, place in a glass-stoppered flask, add 15 mL of a mixture of acetic acid (100) and isooctane (3:2), previously having undergone a 30 minute nitrogen substitution, and dissolve with gentle shaking. To this solution add 0.5 mL of saturated potassium iodide TS, replace the inside of the vessel with nitrogen, and shake for exactly 5 minutes. Then, add 0.5 mL of starch TS, shake vigorously, add 15 mL of water, and shake vigorously. Under a stream of nitrogen, titrate (≤2.50>) with 0.01 mol/L sodium thiosulfate VS until the color of the solution disappears. Separately, perform a blank determination using 4 mL of water, and make any necessary correction. Calculate the amount of peroxides using the following equation: not more than 0.5 meq/L.

Amount (meq/L) of peroxides = V × 2.5

V: Amount (mL) of 0.01 mol/L sodium thiosulfate VS consumed

(4) Free fatty acids—Pipet 3 mL of Alprostadil Injection, add exactly 15 mL of a mixture of 2-propanol, heptane and 0.5 mol/L sulfuric acid TS (40:10:1), and shake for 1 minute. After leaving for 10 minutes, add exactly 9 mL of heptane and exactly 9 mL of water, shake the test tube by in-
Amount (meq/L) of free fatty acids = \( V_f / V_s \times f \times 15 \)

**Bacterial endotoxins** &lt;4.00 &gt; Less than 10 EU/mL.

**Extractable volume** &lt;6.00 &gt; It meets the requirement.

**Foreign insoluble matter** &lt;6.00 &gt; Perform the test according to Method 1: no easily detectable foreign matter is observed.

**Sterility** &lt;4.06 &gt; Perform the test according to the Membrane filter method: it meets the requirement. However, use the sample solution consisting of equal volume of Alprostadil Injection and a solution prepared by adding water to 0.1 g of polysorbate 80 to make 100 mL.

**Particle diameter** Being specified separately.

**Assay** Measure exactly a volume of Alprostadil Injection corresponding to 5 mg of alprostadil (C20H34O5), add exactly 1 mL of the internal standard solution, shake, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of Alprostadil RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, dissolve in ethanol (99.5) to make exactly 50 mL, and use this solution as standard stock solution. Pipet 2.5 mL of the standard stock solution, add the mobile phase to make exactly 50 mL, pipet 1 mL, add exactly 1 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 40 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography &lt;2.01 &gt; according to the following conditions using an apparatus equipped with an automatic pretreatment device (using a postcolumn reaction), and calculate the ratios, \( Q_f \) and \( Q_s \), of the peak area of alprostadil to that of the internal standard.

\[
\text{Amount (\( \mu g \)) of alprostadil (C20H34O5) = } M_s \times \frac{Q_f}{Q_s} 
\]

\[
M_C = \text{Amount (mg) of Alprostadil RS}
\]

**Internal standard solution**—Dissolve 50 mg of 1-naphthol in 20 mL of ethanol (99.5). To 3 mL of this solution add 90 mL of the mobile phase to make 100 mL.

**Operating conditions**—

Equipment: Liquid chromatograph consisting of 2 pumps for pumping the mobile phase and the reaction reagent, an automatic pretreatment device, column, reaction coil, detector, and recording apparatus. Use a reaction coil that is maintained at a constant temperature.

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 60°C.

Reaction coil: Polytetrafluoroethylene tube 0.5 mm in inside diameter and 10 m in length.

Mobile phase: Dissolve 9.07 g of potassium dihydrogen phosphate in water to make 1000 mL and adjust the pH to 6.3 by adding a solution prepared by dissolving 9.46 g of disodium hydrogen phosphate in water to make 1000 mL. To 1 volume of this solution add 9 volumes of water. To 3 volumes of this solution add 1 volume of acetonitrile for liquid chromatography.

Reaction reagent: Potassium hydroxide TS.

Reaction temperature: A constant temperature of about 60°C.

Mobile phase flow rate: Adjust the flow rate so that the retention time of alprostadil is about 7 minutes.

Reaction reagent flow rate: 0.5 mL per minute.

Automatic pretreatment device: Composed of a pretreatment column, pump for pumping pretreatment column wash solution, and routing valve for 2 high pressure flow paths.

Pretreatment column: A stainless steel column 4 mm in inside diameter and 2.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Pretreatment column wash solution: Ethanol (99.5).

Flow rate of wash solution: A constant flow rate of about 2.0 mL per minute.

Flow path operating conditions: Change the flow path operating conditions at the times shown in the table below using the valves shown in the figure.

<table>
<thead>
<tr>
<th>Valve</th>
<th>Time of switchover (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVA</td>
<td>0    9.0  9.1  *1)  *2)</td>
</tr>
<tr>
<td>RVB</td>
<td>0    1    1    1    0</td>
</tr>
</tbody>
</table>

*1) After the internal standard has completely eluted

*2) 0.1 minutes after *1)
Alprostadil Alfadex / Official Monographs

**Prostaglandin E₁ α-Cyclodextrin Clathrate Compound**

アルプロスタジル アルファデクス

C₂₀H₃₄O₅·αC₃₆H₆₀O₃₀
7-[(1R,2R,3R)-3-Hydroxy-2-[(1E,3S)-3-hydroxyoct-1-en-1-yl]-5-oxocyclopentyl]heptanoic acid—α-cyclodextrin [55648-20-9]

Alprostadil Alfadex is a α-cyclodextrin clathrate compound of alprostadil.

It contains not less than 2.8% and not more than 3.2% of alprostadil (C₂₀H₃₄O₅: 354.48), calculated on the anhydrous basis.

**Description**

Alprostadil Alfadex occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (95), in ethyl acetate and in diethyl ether.

It is hygroscopic.

**Identification (1)**

Dissolve 0.02 g of Alprostadil Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, and centrifuge. Use the supernatant liquid as the sample solution (1). Separately, to 0.02 g of Alprostadil Alfadex add 5 mL of ethyl acetate, shake, and centrifuge. Use the supernatant liquid as the sample solution (2). Evaporate the solvent from these solutions under reduced pressure, add 2 mL of sulfuric acid to the residue, and shake for 5 minutes: the liquid obtained from the sample solution (1) shows an orange-yellow color, while the liquid obtained from the sample solution (2) does not show that color.

**Identification (2)**

Dissolve 0.02 g of Alprostadil Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, and centrifuge, and evaporate the solvent from the supernatant liquid under reduced pressure. Dissolve the residue in 2 mL of ethanol (95), add 5 mL of 1,3-dinitrobenzene TS, then add 5 mL of a solution of potassium hydroxide in ethanol (95) (17 in 100) under ice-cooling, and allow to stand for 20 minutes in a dark place under ice-cooling: a purple color develops.

**Identification (3)**

Dissolve 0.05 g of Alprostadil Alfadex in 1 mL of iodine TS, by heating on a water bath, and allow to stand: a dark blue precipitate is formed.

**Identification (4)**

Determine the absorption spectrum of a solution of Alprostadil Alfadex in dilute ethanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2,24>: it exhibits no absorption between 220 nm and 400 nm. Separately, to 10 mL of the solution add 1 mL of potassium hydroxide-ethanol TS, allow to stand for 15 minutes, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2,24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**System suitability**

System performance: Dissolve 10 mg of prostaglandin A₁, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, in ethanol (99.5) to make 100 mL. To 2.5 mL of this solution add 2.5 mL of the standard stock solution, and add the mobile phase to make 50 mL. To 1 mL of this solution add 1 mL of the internal standard solution, shake, and perform the test under the above conditions with 40 µL of the solution. Alprostadil, prostaglandin A₁ and the internal standard are eluted in this order, and the resolution between the peaks of alprostadil and prostaglandin A₁ is not less than 10, and that between prostaglandin A₁ and the internal standard is not less than 2.0.

System repeatability: When the test is repeated 6 times with 40 µL of the sample solution under the above conditions, the relative standard deviation of the ratio of the peak area of alprostadil to that of the internal standard is not more than 2.0%.

**Containers and storage**

Containers—Hermetic containers.

Storage—Light-resistant, not exceeding 5°C, avoiding freezing.

**Figure**

Components of automatic pretreatment system

A: RVA valve  
B: RVB valve  
C: Sample injector  
D: Mobile phase  
E: Column for pressure correction  
F: Column  
G: Pretreatment column  
H: Wash solution  
I: Drain  
J: Pump
Optical rotation $<2.49^\circ$ [α]$^D_{20}$; +126 - +138° (0.1 g calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

pH $<2.54^\circ$ Dissolve 0.10 g of Alprostadil Alfadex in 20 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Alprostadil Alfadex in 10 mL of water: the solution is colorless. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry within 30 minutes after preparation of the solution: the absorbance at 450 nm is not larger than 0.10.

(2) Prostaglandin $A_1$—Dissolve 0.10 g of Alprostadil Alfadex in 5 mL of water, add exactly 5 mL of the internal standard solution and ethanol (95) to make 15 mL, and use this solution as the sample solution. Separately, dissolve 1.5 mg of prostaglandin $A_1$ in ethanol (95) to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 5 mL of the internal standard solution, 2 mL of ethanol (95) and water to make 15 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01^\circ$ according to the operating conditions described in the Assay, and calculate the ratios, $Q_7$ and $Q_8$, of the peak area of prostaglandin $A_1$ to that of the internal standard: $Q_7$ is not larger than $Q_8$.

Internal standard solution—A solution of propyl parahydroxybenzoate in dilute ethanol (1 in 15,000).

(3) Related substances—Dissolve 0.10 g of Alprostadil Alfadex in 3 mL of water, add exactly 3 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid obtained as the sample solution. Separately, weigh accurately about 0.1 g of Alprostadil RS, dissolve in 5 mL of ethanol (95) (3 in 200,000) and 5 mL of the internal standard solution. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of alprostadil, the internal standard and prostaglandin $A_1$ in this order and complete separation of these peaks.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding 5°C.

Expiration date 24 months after preparation.

Dried Aluminum Hydroxide Gel

Dried Aluminum Hydroxide Gel contains not less than 50.0% of aluminum oxide ($\text{Al}_2\text{O}_3$: 101.96).

Description Dried Aluminum Hydroxide Gel occurs as a white, amorphous powder. It is odorless and tasteless.

Most of it dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

Identification To 0.2 g of Dried Aluminum Hydroxide Gel add 20 mL of dilute hydrochloric acid, warm, and centrifuge: the supernatant liquid responds to the Qualitative Tests $<1.09^\circ$ for aluminum salt.

Purity (1) Acidity or alkalinity—To 1.0 g of Dried Aluminum Hydroxide Gel add 25 mL of water, shake well, and centrifuge: the supernatant liquid is neutral.

(2) Chloride $<1.03^\circ$—To 1.0 g of Dried Aluminum Hydroxide Gel add 30 mL of dilute nitric acid, heat gently to boil while shaking, cool, add water to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.284%).

(3) Sulfate $<1.14^\circ$—To 1.0 g of Dried Aluminum Hydroxide Gel add 15 mL of dilute hydrochloric acid, heat

$$M_5: \text{Amount (mg) of Alprostadil}$$

Internal standard solution—A solution of propyl parahydroxybenzoate in dilute ethanol (1 in 15,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogenphosphate and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of alprostadil is about 6 minutes.

Selection of column: Dissolve about 0.1 g of Alprostadil Alfadex in 5 mL of water, add 5 mL of a solution of prostaglandin $A_1$ in ethanol (95) (3 in 200,000) and 5 mL of the internal standard solution. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of alprostadil, the internal standard and prostaglandin $A_1$ in this order and complete separation of these peaks.

Related substances—Dissolve 0.10 g of Alprostadil Alfadex in 20 mL of water, add exactly 5 mL of the internal standard solution and ethanol (95) to make 15 mL, and use this solution as the sample solution. Separately, dissolve 1.5 mg of prostaglandin $A_1$ in ethanol (95) to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 5 mL of the internal standard solution, 2 mL of ethanol (95) and water to make 15 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01^\circ$ according to the operating conditions described in the Assay, and calculate the ratios, $Q_7$ and $Q_8$, of the peak area of prostaglandin $A_1$ to that of the internal standard: $Q_7$ is not larger than $Q_8$.

Internal standard solution—A solution of propyl parahydroxybenzoate in dilute ethanol (1 in 15,000).

(3) Related substances—Dissolve 0.10 g of Alprostadil Alfadex in 3 mL of water, add exactly 3 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid obtained as the sample solution. Separately, dissolve 1.0 mg of prostaglandin $A_1$ in ethyl acetate to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 5 mL of the internal standard solution, 2 mL of ethyl acetate to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Thin-layer Chromatography $<2.03^\circ$. Spot 10 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of phosphomolybdic acid in ethanol (95) (1 in 4) on the plate, and heat at 100°C for 5 minutes: the spots other than the principal spot from the sample solution, and the spots other than the spot corresponding to the spot from the standard solution are all not more intense than the spot from the standard solution.

Water $<2.48^\circ$ Not more than 6.0% (0.2 g, direct titration).

Assay Weigh accurately about 0.1 g of Alprostadil Alfadex, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution and water to make 15 mL, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of Alprostadil RS, dissolve in 5 mL of ethanol (95), add exactly 5 mL of the internal standard solution and water to make 15 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01^\circ$ according to the following conditions, and calculate the ratios, $Q_7$ and $Q_8$, of the peak area of alprostadil to that of the internal standard.

Amount (mg) of alprostadil (C$_7$H$_8$O$_4$) = $M_5 \times Q_7 / Q_8$
Dried Aluminum Hydroxide Gel Fine Granules

乾燥水酸化アルミニウムゲル細粒

Dried Aluminum Hydroxide Gel Fine Granules contain not less than 47.0% of aluminum oxide (Al₂O₃: 101.96).

**Method of preparation** Prepare as directed under Granules, with Dried Aluminum Hydroxide Gel.

**Identification** To 0.2 g of Dried Aluminum Hydroxide Gel Fine Granules add 20 mL of dilute hydrochloric acid, warm and centrifuge: the supernatant liquid responds to the Qualitative Tests <1.09> for aluminum salt.

**Acid-consuming capacity** Proceed as directed for Acid-consuming capacity under Dried Aluminum Hydroxide Gel: the volume of 0.1 mol/L hydrochloric acid VS consumed is not less than 235 mL per g of Dried Aluminum Hydroxide Gel Fine Granules.

**Assay** Proceed as directed in the Assay under Dried Aluminum Hydroxide Gel.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.549 mg of Al₂O₃

**Containers and storage** Containers—Tight containers.

Natural Aluminum Silicate

天然ケイ酸アルミニウム

**Description** Natural Aluminum Silicate occurs as a white or slightly colored powder. It is odorless and tasteless. It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Natural Aluminum Silicate (1 g) dissolves when heated in 20 mL of a solution of sodium hydroxide (1 in 5), with some decomposition, leaving a large amount of insoluble substance.

**Identification** (1) To 0.5 g of Natural Aluminum Silicate add 3 mL of diluted sulfuric acid (1 in 3), heat until white fumes evolve, cool, add 20 mL of water, and filter. Render the filtrate slightly acid with ammonia TS: the solution responds to the Qualitative Tests <1.09> for aluminum salt.

(2) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Place the bead in contact with Natural Aluminum Silicate, and fuse again: an infusible material appears in the bead, producing, upon cooling, an opaque bead with a web-like structure.

**Purity** (1) Acidity or alkalinity—Shake 5.0 g of Natural Aluminum Silicate with 100 mL of water, and centrifuge: the supernatant liquid so obtained is neutral.
Chloride—To 5.0 g of Natural Aluminum Silicate add 100 mL of water, boil gently for 15 minutes while shaking, then cool, add water to restore the original volume, and centrifuge. To 10 mL of the supernatant liquid add 6 mL of dilute hydrochloric acid, dilute to 50 mL with water, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

Sulfate—To the residue obtained in (6) add 3 mL of dilute hydrochloric acid, heat on a water bath for 10 minutes, dilute to 50 mL with water, and filter. To 2.0 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

Heavy metals—To 1.5 g of Natural Aluminum Silicate add 50 mL of water and 5 mL of hydrochloric acid, boil gently for 20 minutes while shaking, then cool, centrifuge, remove the supernatant liquid, wash the residue with two 10-mL portions of water, centrifuging each time, combine these washings with the filtrate, and add ammonia solution (28) dropwise, until a precipitate just appears. Add dropwise dilute hydrochloric acid with vigorous shaking and redissolve the precipitate. Heat the mixture with 0.45 g of hydroxylammonium chloride, cool, and add 0.45 g of sodium acetate trihydrate, 6 mL of dilute acetic acid and water to make 150 mL. Perform the test, using 50 mL of this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, 2 mL of dilute acetic acid and water to make 50 mL (not more than 40 ppm).

Arsenic—To 1.0 g of Natural Aluminum Silicate, add 5 mL of dilute hydrochloric acid, heat gently to boil while shaking well, cool rapidly, and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue, and repeat the extraction in the same manner. Concentrate the combined extracts on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 2 ppm).

Soluble salts—Evaporate 50 mL of the supernatant liquid obtained in (1) on a water bath to dryness, and ignite the residue at 700°C for 2 hours: the mass of the ignited residue is not more than 40 mg.

Fluoride—(i) Apparatus: Use a hard glass apparatus as illustrated in the figure. Ground-glass joints may be used.

(ii) Procedure: Transfer 5.0 g of Natural Aluminum Silicate to the distilling flask A with the aid of 20 mL of water, add about 1 g of glass wool and 50 mL of diluted purified sulfuric acid (1 in 2), and connect A to the distillation apparatus, previously washed with steam and then connected to the steam generator B vigorously, and introduce the generated steam into F. Simultaneously, heat A gradually until the temperature of the solution in A reaches 130°C, then open the rubber tube F, close the rubber tube G, boil water in the steam generator B vigorously, and introduce the generated steam into F. Simultaneously, heat A, and maintain the temperature of the solution in A between 135°C and 145°C. Adjust the distilling rate to about 10 mL per minute. Collect about 170 mL of the distillate, then stop the distillation, wash C with a small quantity of water, combine the washings with the distillate, add water to make exactly 200 mL, and use this solution as the test solution. Perform the test with the test solution as directed in the procedure of determination for fluoride under Oxygen Flask Combustion Method 1.06. No corrective solution is used in this procedure. The content of fluoride (F) is not more than 0.01%.

Amount (mg) of fluoride (F: 19.00) in the test solution

\[
\text{Amount (mg) of fluoride in 5 mL of the standard solution} = \frac{V_1}{V_2} \times \frac{A_{f}}{A_{S}} \times \frac{200}{V}
\]

Loss on drying Not more than 20.0% (1 g, 105°C, 3 hours).

Adsorptive power To 0.10 g of Natural Aluminum Silicate add 20 mL of a solution of methylene blue trihydrate (3 in 2000), shake for 15 minutes, allow to stand for 5 hours at 37 ± 2°C, and centrifuge. Dilute 1.0 mL of the supernatant liquid with water to 200 mL. Place 50 mL of the solution in a Nessler tube and observe horizontally or vertically against a white background: the color of the solution is not deeper than that of the following control solution.

Control solution: Dilute 1.0 mL of a solution of methylene blue trihydrate (3 in 2000) with water to 400 mL, and use 50 mL of this solution.

Containers and storage Containers—Well-closed containers.
Synthetic Aluminum Silicate

**Description**  Synthetic Aluminum Silicate occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Synthetic Aluminum Silicate (1 g) dissolves when heated in 20 mL of a solution of sodium hydroxide (1 in 5), leaving a small amount of insoluble substance.

**Identification** (1) To 0.5 g of Synthetic Aluminum Silicate add 3 mL of diluted sulfuric acid (1 in 3), heat until white fumes evolve, cool, add 20 mL of water, and filter. Render the filtrate slightly acid with ammonia TS: the solution responds to the Qualitative Tests for aluminum salt.

(2) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Place the bead in contact with Synthetic Aluminum Silicate, and fuse again: an infusible material appears in the bead, producing, upon cooling, an opaque bead with a web-like structure.

**Purity** (1) Acidity or alkalinity—Shake 1.0 g of Synthetic Aluminum Silicate with 20 mL of water, and centrifuge: the supernatant liquid so obtained is neutral.

(2) Chloride  To 5.0 g of Synthetic Aluminum Silicate add 100 mL of water, boil gently for 15 minutes while shaking, then cool, add water to restore the original volume, and centrifuge. To 10 mL of the supernatant liquid add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate  To 2.0 mL of the supernatant liquid obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Heavy metals  To 3.0 g of Synthetic Aluminum Silicate add 50 mL of water and 5 mL of hydrochloric acid, boil gently for 20 minutes while shaking, then after cooling, centrifuge, remove the supernatant liquid, wash the precipitate with two 10-mL portions of water, centrifuging each time, combine these washings with the filtrate, and add ammonia solution (28) dropwise until a precipitate just appears. Add dropwise dilute hydrochloric acid with vigorous shaking to redissolve the precipitate. Heat the solution with 0.45 g of hydroxylammonium chloride, and after cooling, add 0.45 g of sodium acetate trihydrate, 6 mL of dilute acetic acid and water to make 150 mL. Perform the test with 50 mL of this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution, 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, 2 mL of dilute acetic acid and water to make 50 mL (not more than 30 ppm).

(5) Arsenic  To 1.0 g of Synthetic Aluminum Silicate add 10 mL of dilute hydrochloric acid, heat gently to boiling while shaking well, cool rapidly, and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue, and repeat the extraction in the same manner. Concentrate the combined extracts on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 2 ppm).

**Loss on drying**  Not more than 20.0% (1 g, 105°C, 3 hours).

**Acid-consuming capacity**  Weigh accurately about 1 g of Synthetic Aluminum Silicate, transfer to a glass-stoppered flask, add 200 mL of 0.1 mol/L hydrochloric acid VS, exactly measured, stopper the flask, and shake at 37 ± 2°C for 1 hour. Filter, pipet 50 mL of the filtrate, and titrate by stirring well the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS until the pH of the solution changes to 3.5. The volume of 0.1 mol/L hydrochloric acid VS consumed is not less than 50.0 mL per g of Synthetic Aluminum Silicate.

**Containers and storage**  Containers—Well-closed containers.

Aluminum Monostearate

モノステアリン酸アルミニウム

Aluminum Monostearate is mainly aluminum compounds of stearic acid (C_{18}H_{36}O_{2}: 284.48) and palmitic acid (C_{16}H_{32}O_{2}: 256.42).

Aluminum Monostearate, when dried, contains not less than 7.2% and not more than 8.9% of aluminum (AI: 26.98).

**Description**  Aluminum Monostearate occurs as a white to yellowish white powder. It is odorless or has a faint, characteristic odor.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

**Identification** (1) Heat 3 g of Aluminum Monostearate with 30 mL of hydrochloric acid in a water bath with occasional shaking for 10 minutes. After cooling, shake the mixture vigorously with 50 mL of water and 30 mL of diethyl ether for 3 minutes, and allow to stand. To the separated aqueous layer add sodium hydroxide TS until the solution becomes slightly turbid, and filter: the filtrate responds to the Qualitative Tests for aluminum salt.

(2) Wash the diethyl ether layer separated in (1) with two 20-mL portions of water, and evaporate the diethyl ether layer on a water bath: the residue melts at above 54°C.

**Acid value for fatty acid**  Weigh accurately about 1 g of fatty acid obtained in the Identification (2), transfer a 250-mL glass-stoppered flask, add 100 mL of a mixture of diethyl ether and ethanol (95) (2:1), warm to dissolve, add several drops of phenolphthalein TS, and proceed as directed under Acid Value.

**Purity** (1) Free fatty acid—Mix 1.0 g of Aluminum Monostearate with about 50 mL of a mixture of neutralized ethanol and diethyl ether
(1:1), combine the filtrate and the washings, and add 2.1 mL of 0.1 mol/L potassium hydroxide VS: a red color develops.

(2) Water-soluble salts—Heat 2.0 g of Aluminum Monostearate with 80 mL of water in a loosely stoppered conical flask on a water bath for 30 minutes with occasional shaking. After cooling, filter through dry filter paper, wash the residue with a small amount of water, combine the washings with the filtrate, add water to make 100 mL, evaporate 50 mL of this solution on a water bath, and heat strongly at 600°C: the mass of the residue is not more than 10.0 mg.

(3) Heavy metals <1.07>—Heat 1.0 g of Aluminum Monostearate over a small flame with caution at the beginning, and continue the heating, gradually raising the temperature, to ash. After cooling, add 10 mL of diluted hydrochloric acid (1 in 2), evaporate on a water bath, and boil the residue with 20 mL of water for 1 minute. Cool, filter, wash the residue with water, combine the filtrate and the washings, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Evaporate 10 mL of diluted hydrochloric acid (1 in 2) on a water bath to dryness, add 2 mL of dilute acetic acid and 50 mL of Standard Lead Solution, dilute with water to make 50 mL, and use this solution as the control solution (not more than 50 ppm).

(4) Arsenic <1.11>—Mix 1.0 g of Aluminum Monostearate with 2 g of magnesium nitrate hexahydrate, ignite over a small flame, moisten the residue after cooling, and ignite gently to ash, and continue the heating, gradually raising the temperature, to ash. After cooling, add 2.4 mL of water to make 10 mL, evaporate on a water bath, and boil the residue with 0.5 mL of nitric acid, and heat to light red. Perform a blank determination.

Loss on drying <2.41> Not more than 3.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 1 g of Aluminum Monostearate, previously dried, ignite gently to ash, and cool. Add dropwise 0.5 mL of nitric acid, evaporate on a water bath by heating, and then heat strongly between 900°C and 1100°C to a constant mass. After cooling, weigh rapidly the ignited residue, and designate the mass as aluminum oxide (Al₂O₃): 101.96.

Amount (mg) of aluminum (Al)

\[ \text{Amount (mg) of aluminum (Al)} = \text{amount (mg) of aluminum oxide (Al}_2\text{O}_3) \times 0.529 \]

Containers and storage Containers—Well-closed containers.

Aluminum Potassium Sulfate Hydrate

Alum

硫酸アルミニウムカリウム水和物

AlK(SO₄)₂·12H₂O: 474.39

Aluminum Potassium Sulfate Hydrate contains not less than 99.5% of AlK(SO₄)₂·12H₂O.

Description Aluminum Potassium Sulfate Hydrate occurs as colorless or white crystals or powder. It is odorless. It has a slightly sweet, strongly astringent taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Aluminum Potassium Sulfate Hydrate (1 in 20) is acid.

Identification A solution of Aluminum Potassium Sulfate Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for aluminum salt, to the Qualitative Tests <1.09> (1), (3) and (4) for potassium salt, and to the Qualitative Tests <1.09> (1) and (3) for sulfite.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Aluminum Potassium Sulfate Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Iron <1.10>—Prepare the test solution with 1.0 g of Aluminum Potassium Sulfate Hydrate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.6 g of Aluminum Potassium Sulfate Hydrate, according to Method 1, and perform the test (not more than 3.3 ppm).

Assay Weigh accurately about 4.5 g of Aluminum Potassium Sulfate Hydrate, and dissolve in water to make exactly 200 mL. Take exactly 20 mL of this solution, and add exactly 30 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.8, boil for 5 minutes, and cool. Add 55 mL of ethanol (95), and titrate <2.50> with 0.05 mol/L zinc acetate VS (indicator: 2 mL of dithizone TS), until the color of the solution changes from light dark green to light red. Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

\[ = 23.72 \text{ mg of AlK(SO}_4)_2·12\text{H}_2\text{O} \]

Containers and storage Containers—Tight containers.

Dried Aluminum Potassium Sulfate

Burnt Alum

乾燥硫酸アルミニウムカリウム

AlK(SO₄)₂: 258.21

Dried Aluminum Potassium Sulfate, when dried, contains not less than 98.0% of AlK(SO₄)₂.

Description Dried Aluminum Potassium Sulfate occurs as white masses or white powder. It is odorless. It has a slightly sweet, astringent taste.

It is freely soluble in hot water and practically insoluble in ethanol (95).

It dissolves slowly in water.

Identification A solution of Dried Aluminum Potassium Sulfate (1 in 20) responds to the Qualitative Tests <1.09> for aluminum salt, to the Qualitative Tests <1.09> (1), (3) and (4) for potassium salt, and to the Qualitative Tests <1.09> (1) and (3) for sulfite.
Purity  (1) Water-insoluble substances—To 2.0 g of Dried Aluminum Potassium Sulfate add 40 mL of water, shake frequently, and allow to stand for 48 hours. Collect the insoluble residue on a glass filter (G4), wash with 50 mL of water, and dry at 105°C for 2 hours: the mass of the residue is not more than 50 mg.

(2) Heavy metals 《1.07》—Dissolve 0.5 g of Dried Aluminum Potassium Sulfate in 45 mL of water, and filter, if necessary. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 40 ppm).

(3) Iron 《1.1D》—Prepare the test solution with 0.54 g of Dried Aluminum Potassium Sulfate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 37 ppm).

(4) Arsenic 《1.1D》—Prepare the test solution with 0.40 g of Dried Aluminum Potassium Sulfate, according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying 《2.41》 Not more than 15.0% (2 g, 200°C, 4 hours).

Assay Weigh accurately about 1.2 g of Dried Aluminum Potassium Sulfate, previously dried, add 80 mL of water, and heat on a water bath with occasional shaking for 20 minutes. Cool, add water to make exactly 100 mL, and filter, if necessary. Discard the first 30 mL of the filtrate, take exactly the subsequent 20 mL of the filtrate, and add exactly 30 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium chloride TS and 1 mL of ammonia TS: a white, gelatinous precipitate is produced, which changes to red upon the addition of 5 drops of alizarin red S TS (aluminum sulfate).

Place 100 mL of Alum Solution in an evaporating dish, evaporate on a water bath to dryness, and dissolve the residue in 5 mL of water: the solution responds to the Qualitative Tests 《1.09》 for potassium salt.

(3) Alum Solution responds to the Qualitative Tests 《1.09》 (1) and (2) for sulfate.

Assay Pipet 50 mL of Alum Solution, add exactly 30 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and further add 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.8. Boil for 5 minutes, cool, add 55 mL of ethanol (95), and titrate 《2.50》 with 0.02 mol/L zinc acetate VS (indicator: 2 mL of dithizone TS), until the color of the solution changes from light dark green to light red. Perform a blank determination.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

= 9.488 mg of AlK(SO4)2.12H2O

Containers and storage Containers—Tight containers.

**Alum Solution**

ミョウバン水

Alum Solution contains not less than 0.27 w/v% and not more than 0.33 w/v% of aluminum potassium sulfate Hydrate [AlK(SO4)2.12H2O: 474.39].

Method of preparation

| Aluminum Potassium Sulfate Hydrate  | 3 g |
| Mentha Water                      | 50 mL |
| Water, Purified Water or Purified | a sufficient quantity |

To make 1000 mL

Dissolve and mix the above ingredients.

Description Alum Solution is a clear, colorless liquid. It has the odor of the mentha oil and an astringent taste.

**Amantadine Hydrochloride**

Amantadine Hydrochloride, when dried, contains not less than 99.0% of C10H17N.HCl.

Description Amantadine Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in formic acid, freely soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) To 0.1 g of Amantadine Hydrochloride add 1 mL of pyridine and 0.1 mL of acetic anhydride, dissolve by boiling for 1 hour, add 10 mL of dilute hydrochloric acid, and cool in ice water. Filter the crystals separated, wash with water, and dry at 105°C for 1 hour: the residue melts 《2.6D》 between 147°C and 151°C.

(2) Determine the infrared absorption spectrum of Amantadine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry 《2.2S》, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Amantadine Hydrochloride (1 in 50) responds to the Qualitative Tests 《1.09》 for chloride.

pH 《2.54》 Dissolve 1.0 g of Amantadine Hydrochloride in
JP XVI

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5 mL of water: the pH of this solution is between 4.0 and 6.0.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Amantadine Hydrochloride in 10 mL of water: the solution is clear and colorless.

**Heavy metals <1.07**—Proceed with 2.0 g of Amantadine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Arsenic <1.17**—Prepare the test solution with 1.0 g of Amantadine Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

**Related substances**—Dissolve 0.50 g of Amantadine Hydrochloride in 10 mL of water, add 10 mL of sodium hydroxide TS and 10 mL of chloroform, and shake. Filter the chloroform layer through absorbent cotton with 3 g of anhydrous sodium sulfate on a funnel, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: each peak area other than that of amantadine from the sample solution is not larger than 1/3 times the peak area of amantadine from the standard solution, and the total area of each peak is not larger than the peak area of amantadine from the standard solution.

**Operating conditions**—

- Detector: A hydrogen flame-ionization detector.
- Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm in particle diameter) coated with a mixture (L) of branched hydrocarbon of petroleum hexamethyldodecanese group for gas chromatography and potassium hydroxide at the ratios of 2% and 1%, respectively.
- Column temperature: Inject at a constant temperature of about 125°C, maintain the temperature for 5 minutes, raise at the rate of 5°C per minute to 150°C, and maintain at a constant temperature of about 150°C for 15 minutes.
- Carrier gas: Nitrogen
- Flow rate: Adjust the flow rate so that the retention time of amantadine is about 11 minutes.
- Selection of column: Dissolve 0.15 g of naphthalene in 5 mL of the sample solution, and add chloroform to make 100 mL. Proceed with 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of naphthalene and amantadine in this order with the resolution between these peaks being not less than 2.5.
- Detection sensitivity: Adjust the detection sensitivity so that the peak height of amantadine obtained from 2 μL of the standard solution composes about 10% of the full scale.
- Time span of measurement: About twice as long as the retention time of amantadine beginning after the solvent peak.

**Loss on drying <2.41** Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition <2.44** Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.2 g of Amantadine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add acetic acid (100) to make 70 mL, and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 18.77 mg of C₃H₇N.HCl

**Containers and storage** Containers—Well-closed containers.

**Ambenonium Chloride**

アンペノニウム塩化物

![Chemical structure of Ambenonium Chloride](image)

C₂₈H₃₇Cl₃N₄O₂: 608.47

2,2’-[(1,2-Dioxoethane-1,2-diyl)diimino]bis[N-(2-chlorobenzyl)-N,N-diethylethylamaminium] dichloride [115-79-7]

Ambenonium Chloride contains not less than 98.5% of C₂₈H₃₇Cl₃N₄O₂, calculated on the dried basis.

**Description** Ambenonium Chloride occurs as a white powder.
- It is freely soluble in water, in methanol and in acetic acid (100), soluble in ethanol (95), and slightly soluble in acetic anhydride.
- It is hygroscopic.
- Melting point: about 205°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Ambenonium Chloride in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ambenonium Chloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ambenonium Chloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Ambenonium Chloride in 10 mL of water: the solution is clear and colorless.

**Heavy metals <1.07**—Proceed with 1.0 g of Ambenonium Chloride according to Method 4, and perform the test. Use a solution of magnesium nitrate in ethanol (95) (1 in 5). Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Related substances**—Dissolve 0.10 g of Ambenonium
Amidotrizoic Acid

アミドトリゾ酸

C₁₁H₉I₃N₂O₄: 613.91
3,5-Bis(acetylamino)-2,4,6-triiodobenzoic acid
[117-96-4]

Amidotrizoic Acid, calculated on the dried basis, contains not less than 98.0% of C₁₁H₉I₃N₂O₄.

Description Amidotrizoic Acid occurs as a white, crystalline powder. It is odorless.

It is slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) Heat 0.1 g of Amidotrizoic Acid over a flame: a purple gas is evolved.

(2) Determine the infrared absorption spectrum of Amidotrizoic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Amidotrizoic Acid in 10 mL of 0.2 mol/L sodium hydroxide TS: the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.20 g of Amidotrizoic Acid in 5 mL of water and 1 mL of sodium hydroxide TS, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake, and allow to stand for 2 minutes. Add 5 mL of ammonium amidosulfate TS, shake well, allow to stand for 1 minute, and add 0.4 mL of a solution of 1-naphthol in ethanol (95) (1 in 10), 15 mL of sodium hydroxide TS and water to make exactly 50 mL. Determine the absorbance of this solution at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution, prepared in the same manner, as the blank: the absorbance is not more than 0.15.

(3) Soluble halides—Dissolve 2.5 g of Amidotrizoic Acid in 20 mL of water and 2.5 mL of ammonia TS, add 20 mL of dilute nitric acid and water to make 100 mL, allow to stand for 15 minutes with occasional shaking, and filter. Discard the first 10 mL of the filtrate, transfer the subsequent 25 mL of the filtrate to a Nessler tube, and add ethanol (95) to make 50 mL. Proceed as directed under Chloride Limit Test <1.03> using this solution as the test solution. Prepare the control solution as follows: to 0.10 mL of 0.01 mol/L hydrochloric acid VS, add 6 mL of dilute nitric acid and water to make 25 mL, then ethanol (95) to make 50 mL.

(4) Iodine—Dissolve 0.20 g of Amidotrizoic Acid in 2.0 mL of sodium hydroxide TS, add 2.5 mL of 0.5 mol/L sulfuric acid TS, allow to stand for 10 minutes with occasional shaking, add 5 mL of chloroform, shake well, and allow to stand: the solution is colorless in the chloroform layer.

(5) Heavy metals <1.07>—Proceed with 2.0 g of Amidotrizoic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Arsenic <1.17>—Prepare the test solution with 0.6 g of Amidotrizoic Acid according to Method 3, and perform the test (not more than 3.3 ppm).

Loss on drying <2.41> Not more than 7.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Ambenonium Chloride, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 30.42 mg of C₂₈H₄₂Cl₄N₄O₂

Containers and storage Containers—Tight containers.
**Amikacin Sulfate**

アミカシン硫酸塩

C₂₂H₄₃N₅O₁₃·2H₂SO₄: 781.76
3-Amino-3-deoxy-α-D-glucoopyranosyl-(1→6)-
[6-amino-6-deoxy-α-D-glucoopyranosyl-(1→4)]-1-N-
[(25)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine disulfate

Amikacin Sulfate is the sulfate of a derivative of kanamycin.

It contains not less than 691 μg (potency) and not more than 791 μg (potency) per mg, calculated on the dried basis. The potency of Amikacin Sulfate is expressed as mass (potency) of amikacin (C₂₂H₄₃N₅O₁₃·2H₂SO₄: 781.60).

Description

Amikacin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (95).

**Identification** (1) Determine the infrared absorption spectrum of Amikacin Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Amikacin Sulfate RS previously dried: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.1 g each of Amikacin Sulfate and Amikacin Sulfate RS in 4 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <2.05>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ammonia water (28%), methanol and tetrahydrofuran (1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.47> Not more than 4.0% (1 g, in vacuum, 60°C, 3 hours).

**Assay** Weigh accurately an amount of Amikacin Sulfate and Amikacin Sulfate RS, equivalent to about 50 mg (potency), dissolve each in water to make exactly 50 mL. Pipet 200 μL each of these solutions in the test tube with glass stopper, add exactly 3 mL of pyridine and exactly 2 mL of a solution of 2,4,6-trinitrobenzenesulfonic acid (1 in 100), stopper tightly, and heat in a water bath at 70°C for 30 minutes. After cooling, add exactly 2 mL each of acetic acid (100), and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the heights, H₁ and H₅, of the peak of amikacin derivative.

Amount [μg (potency)] of amikacin (C₂₂H₄₃N₅O₁₃) = M₅ × H₅/H₁ × 1000

M₅: Amount [mg (potency)] of Amikacin Sulfate RS

**Operating conditions**


Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadeclisilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 800 mL of water, adjust to pH 6.5 with a solution of potassium hydroxide (1 in 40), and add water to make 1000 mL. To 280 mL of this solution add 720 mL of methanol, and mix.

Flow rate: Adjust the flow rate so that the retention time of amikacin derivative is about 9 minutes.

**System suitability**

System performance: Dissolve about 5 mg (potency) of Amikacin Sulfate and about 5 mg (potency) of Kanamycin Sulfate in 5 mL of water. Transfer 200 μL of this solution in a glass-stoppered test tube, add 3 mL of pyridine and 2 mL of a solution of 2,4,6-trinitrobenzenesulfonic acid (1 in 100),
stopper tightly, heat in a water bath at 70°C for 30 minutes. After cooling, add 2 mL of acetic acid (100). When the procedure is run with 20 μL of this solution under the above operating conditions, amikacin derivative and kanamycin derivative are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of amikacin derivative is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Amikacin Sulfate for Injection

Amikacin Sulfate for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled amount of amikacin (C_{22}H_{43}N_{7}O_{15}; 585.60).

Method of preparation Prepare as directed under Injections, with Amikacin Sulfate.

Description Amikacin Sulfate for Injection occurs as white to yellowish white masses or powder.

Identification Dissolve an amount of Amikacin Sulfate for Injection, equivalent to 25 mg (potency) of Amikacin Sulfate according to the labeled amount, in 1 mL of water, and use this solution as the sample solution. Separately, dissolve 25 mg (potency) of Amikacin Sulfate RS in 1 mL of water, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Amikacin Sulfate.

Osmotic pressure ratio Being specified separately.

pH <2.54> Dissolve an amount of Amikacin Sulfate for Injection, equivalent to 0.1 g (potency) of Amikacin Sulfate according to the labeled amount, in 10 mL of water: the pH of this solution is 6.0 to 7.5.

Purity Clarity and color of solution—Dissolve an amount of Amikacin Sulfate for Injection, equivalent to 0.5 g (potency) of Amikacin Sulfate according to the labeled amount, in 5 mL of water: the solution is clear, and the absorbance at 405 nm of the solution determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.15.

Loss on drying <2.41> Not more than 4.0% (1 g, in vacuum, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 0.50 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the content of not less than 10 Amikacin Sulfate for Injection. Weigh accurately a portion of the content, equivalent to about 50 mg (potency) of Amikacin Sulfate, dissolve in water to make exactly 50 mL. Separately, weigh accurately an amount of Amikacin Sulfate RS, equivalent to about 50 mg (potency), and dissolve in water to make exactly 50 mL. Transfer exactly 200 μL of each of these solutions to separate glass stopped tubes, then proceed as directed in the Assay under Amikacin Sulfate RS.

\[
M_S = \frac{H_1 \times H_5}{M_S}
\]

Where:
- \( M_S \): Amount [mg (potency)] of amikacin (C_{22}H_{43}N_{7}O_{15})
- \( H_1 \): Amount [mg (potency)] of Amikacin Sulfate RS
- \( H_5 \): Amount [mg (potency)] of Amikacin Sulfate RS

Containers and storage Containers—Hermetic containers.

Amikacin Sulfate Injection

Amikacin Sulfate Injection is an aqueous injection. It contains not less than 90.0% and not more than 115.0% of the labeled amount of amikacin (C_{22}H_{43}N_{7}O_{15}; 585.60).

Method of preparation Prepare as directed under Injections, with Amikacin Sulfate.

Description Amikacin Sulfate Injection occurs as a colorless or pale yellow clear liquid.

Identification To a volume of Amikacin Sulfate Injection, equivalent to 0.1 g (potency) of Amikacin Sulfate according to the labeled amount, add water to make 4 mL, and use this solution as the sample solution. Separately, dissolve 25 mg (potency) of Amikacin Sulfate RS in 1 mL of water, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Amikacin Sulfate RS.

Osmotic pressure ratio Being specified separately.

pH <2.54> 6.0 – 7.5

Bacterial endotoxins <4.01> Less than 0.50 EU/mg (potency).

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take exactly a volume of Amikacin Sulfate Injection, equivalent to about 0.1 g (potency) of Amikacin Sulfate, and add water to make exactly 100 mL. Separately, weigh accurately an amount of Amikacin Sulfate RS, equivalent to about 50 mg (potency), and add water to make exactly 50 mL. Take exactly 200 μL of each of these solutions into stopped test tubes, then proceed as directed in the Assay under Amikacin Sulfate RS.
Aminophylline Hydrate

Aminophylline Hydrate occurs as white to pale yellow granules or powder. It is odorless or slightly ammonia-like odor, and has a bitter taste.

Identification (1) Dissolve 0.75 g of Aminophylline Hydrate in 30 mL of water, and use this solution as the sample solution. To 20 mL of the sample solution add 1 mL of dilute hydrochloric acid: a precipitate is gradually formed. Solution. To 20 mL of the sample solution add 1 mL of dilute hydrochloric acid: a precipitate is gradually formed.

Dissolve 0.1 g of the crystals obtained in (1) in 50 mL of water, and shake: it dissolves almost completely. Separation of crystals begins in 2 to 3 minutes, and these crystals dissolve on the addition of a small amount of ethylenediamine.

It is gradually affected by light, and gradually loses ethylenediamine in air.

Identification (1) Dissolve 0.75 g of Aminophylline Hydrate in 30 mL of water, and use this solution as the sample solution. To 20 mL of the sample solution add 1 mL of dilute hydrochloric acid: a precipitate is gradually formed. Filter the precipitate, recrystallize from water, and dry at dilute hydrochloric acid: a precipitate is gradually formed.

To 1 g of Aminophylline Hydrate add 5 mL of water, and shake: it dissolves almost completely. Separation of crystals begins in 2 to 3 minutes, and these crystals dissolve on the addition of a small amount of ethylenediamine.

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To 1 g of Aminophylline Hydrate add 5 mL of water, and shake: it dissolves almost completely. Separation of crystals begins in 2 to 3 minutes, and these crystals dissolve on the addition of a small amount of ethylenediamine.

Aminophylline Hydrate contains not less than 84.0% and not more than 86.0% of theophylline (C7H8N4O2: 180.16), and not less than 14.0% and not more than 15.0% of ethylenediamine (C2H8N2: 60.10), calculated on the anhydrous basis.

Aminophylline Hydrate is practically insoluble in ethanol (95) and in diethyl ether.

To 1 g of Aminophylline Hydrate in 10 mL of hot water: the solution is clear and colorless to pale yellow.

To 0.01 g of the crystals obtained in (1) add 10 drops of hydroquinone TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue shows a yellow-red color. Invert the dish containing the residue over a vessel containing 2 to 3 drops of ammonia TS: the color changes to red-purple, which is destroyed on the addition of 2 to 3 drops of sodium hydroxide TS.

To 0.01 g of the crystals obtained in (1) add 10 drops of hydroquinone TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue shows a yellow-red color. Invert the dish containing the residue over a vessel containing 2 to 3 drops of ammonia TS: the color changes to red-purple, which is destroyed on the addition of 2 to 3 drops of sodium hydroxide TS.

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To 0.01 g of the crystals obtained in (1) add 10 drops of hydroquinone TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue shows a yellow-red color. Invert the dish containing the residue over a vessel containing 2 to 3 drops of ammonia TS: the color changes to red-purple, which is destroyed on the addition of 2 to 3 drops of sodium hydroxide TS.

Residue on ignition (2) Not more than 0.1% (1 g).

Assay (1) Theophylline—Weigh accurately about 0.25 g of Aminophylline Hydrate, and dissolve in 50 mL of water and 8 mL of ammonia TS by gentle warming on a water bath. Add exactly 20 mL of 0.1 mol/L silver nitrate VS, warm on a water bath for 15 minutes, allow to stand between 5°C and 10°C for 20 minutes, collect the precipitate by suction, and wash with three 10-mL portions of water. Combine the filtrate and washings, and add dilute nitric acid to make neutral. Add 3 mL of dilute nitric acid, and titrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS is 18.02 mg of theophylline (C7H8N4O2).

(2) Ethylenediamine—Weigh accurately about 0.5 g of Aminophylline Hydrate, and dissolve in 30 mL of water, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of bromphenol blue TS).

Each mL of 0.1 mol/L hydrochloric acid VS is 3.005 mg of ethylenediamine (C2H8N2).

Aminophylline Injection

Aminophylline Injection is an aqueous solution for injection.

It contains not less than 75.0% and not more than 86.0% of the labeled amount of theophylline (C7H8N4O2: 180.16), and not less than 13.0% and not more than 20.0% of ethylenediamine (C2H8N2: 60.10).

The concentration of Aminophylline Injection is expressed as the quantity of aminophylline dihydrate (C18H24N10O4·2H2O: 456.46).

Method of preparation Prepare as directed under Injections, with Aminophylline Hydrate. It may be prepared with Theophylline and its equivalent Ethylenediamine, instead of Aminophylline Hydrate.

It may contain not more than 60 mg of Ethylenediamine
Amiodarone Hydrochloride / Official Monographs

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as a stabilizer for each g of Aminophylline Hydrate.

Description Aminophylline Injection is a clear and colorless liquid. It has a slightly bitter taste. It gradually changes in color by light.

pH: 8.0 – 10.0

Identification

To a volume of Aminophylline Injection, equivalent to 0.75 g of Aminophylline Hydrate according to the labeled amount, add water to make 30 mL. Proceed with this solution as directed in the Identification under Aminophylline Hydrate.

Bacterial endotoxins

Less than 0.6 EU/mg.

Extractable volume

It meets the requirement.

Foreign insoluble matter

Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter

It meets the requirement.

Sterility

Perform the test according to the Membrane filtration method: it meets the requirement.

Assay

(1) Theophylline—Pipet a volume of Aminophylline Injection, equivalent to about 39.4 mg of theophylline (C7H8N4O2) (about 50 mg of Aminophylline Hydrate), add water to make exactly 50 mL, and use this solution as the standard solution. Separate, weigh accurately about 40 mg of theophylline for assay, previously dried at 105°C for 4 hours, dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A1 and A5, of theophylline in each solution.

Amount (mg) of theophylline (C7H8N4O2)

= Mₛ × A₁/₁₅₈

Mₛ: Amount (mg) of theophylline for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100) and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of theophylline is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of theophylline are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of theophylline is not more than 1.0%.

(2) Ethylenediamine—To an accurately measured volume of Aminophylline Injection, equivalent to about 30 mg of ethylenediamine (C2H8N2) (about 0.2 g of Aminophylline Hydrate), add water to make 30 mL, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 2 to 3 drops of bromophenol blue TS).

Each mL of 0.1 mol/L hydrochloric acid VS = 3.005 mg of ethylenediamine (C2H8N2)

Containers and storage

Containers—Hermetic containers.

Storage—Light-resistant.

Amiodarone Hydrochloride

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C₂₅H₂₉I₂NO₃·HCl: 681.77
[2-Butylbenzofuran-3-yl][4-[2-(diethylaminomethoxy)-3,5-diiodophenyl]methanone monohydrochloride [19774-82-4]

Amiodarone Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of C₂₅H₂₉I₂NO₃·HCl.

Description

Amiodarone Hydrochloride occurs as a white to pale yellowish white crystalline powder.

It is very soluble in water at 80°C, freely soluble in dichloromethane, soluble in methanol, sparingly soluble in ethanol (95), and very slightly soluble in water.

Melting point: about 161°C (with decomposition).

Identification

(1) Determine the absorption spectrum of a solution of Amiodarone Hydrochloride in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Amiodarone Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Amiodarone Hydrochloride add 10 mL of water, dissolve by warming at 80°C, and cool; the solution responds to the Qualitative Tests (1.09) (2) for chloride.

pH < 2.5 To 1.0 g of Amiodarone Hydrochloride add 20 mL of freshly boiled and cooled water, dissolve by warming at 80°C, and cool: the pH of this solution is between 3.2 and 3.8.

Purity

(1) Clarity and color of solution—Dissolve 0.5 g of Amiodarone Hydrochloride in 10 mL of methanol: the solution is clear, and is not more colored than the following control solutions (1) and (2).

Control solution (1): To a mixture of 1.0 mL of Cobalt (II) Chloride CS, 2.4 mL of Iron (III) Chloride CS and 0.4 mL of Copper (II) Sulfate CS, add diluted hydrochloric acid
(1 in 40) to make 10.0 mL. To 2.5 mL of this solution add diluted hydrochloric acid (1 in 40) to make 20 mL.

Control solution (2): To 3.0 mL of a mixture of 0.2 mL of Cobalt (II) Chloride CS, 9.6 mL of a mixture of Copper (II) Sulfate CS and 0.2 mL of Hydrochloric acid (1 in 40) to make 100 mL.

(2) Iodine—To 1.50 g of Amiodarone Hydrochloride add 40 mL of water, dissolve by warming at 80°C, cool, add water to make exactly 50 mL, and use this solution as the sample stock solution. Pipet 15 mL of this solution, add exactly 1 mL of 0.1 mol/L hydrochloric acid TS and exactly 1 mL of a solution of potassium iodate (107 in 10,000), add water to make exactly 20 mL, and use this solution as the sample solution. Separately, pipet 15 mL of the sample stock solution, add exactly 1 mL of 0.1 mol/L hydrochloric acid TS, exactly 1 mL of a solution of potassium iodide (441 in 5,000,000) and exactly 1 mL of a solution of potassium iodate (107 in 10,000), add water to make exactly 20 mL, and use this solution as the standard solution. Separately, pipet 15 mL of the sample stock solution, add exactly 1 mL of 0.1 mol/L hydrochloric acid TS, add water to make exactly 20 mL, and use this solution as the control solution. Allow the sample solution, standard solution and control solution to stand in a dark place for 4 hours. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.2>, using the control solution as the blank: the absorbance of the sample solution at 420 nm is not larger than 1/2 times the absorbance of the standard solution.

(3) Heavy metals—Proceed with 1.0 g of Amiodarone Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substance 1—Dissolve 0.5 g of Amiodarone Hydrochloride in 5 mL of dichloromethane, and use this solution as the sample solution. Separately, dissolve 10 mg of 2-chloroethyl diethylamine hydrochloride in 50 mL of dichloromethane, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and formic acid (17:2:1) to a distance of about 15 cm, and air-dry the plate. Splay evenly bismuth subnitrate TS and then hydrogen peroxide TS: the spot obtained from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

(5) Related substance 2—Dissolve 0.125 g of Amiodarone Hydrochloride in 25 mL of a mixture of water and acetonitrile for liquid chromatography (1:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of water and acetonitrile for liquid chromatography (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile for liquid chromatography (1:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.03> according to the following conditions. Determine each peak area by the automatic integration method: the area of any peak other than amiodarone obtained from the sample solution is not larger than the peak area of amiodarone from the standard solution, and the total area of the peaks other than the peak of amiodarone is not larger than 2.5 times the peak area of amiodarone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: To 800 mL of water add 3.0 mL of acetic acid (100), adjust the pH to 4.95 with ammonium solution (28), and add water to make 1000 mL. To 300 mL of this solution add 400 mL of acetonitrile for liquid chromatography and 300 mL of methanol for liquid chromatography. Flow rate: Adjust the flow rate so that the retention time of amiodarone is about 24 minutes.

Time span of measurement: About 2 times as long as the retention time of amiodarone.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and acetonitrile for liquid chromatography (1:1) to make exactly 25 mL. Confirm that the peak area of amiodarone obtained from 10 μL of this solution is equivalent to 14 to 26% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amiodarone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amiodarone is not more than 1.0%.

(6) Residual solvent—Being specified separately.

Loss on drying <2.4f> Not more than 0.5% (1 g, reduced pressure not exceeding 0.3 kPa, 50°C, 4 hours).

Residue on ignition <2.4g> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Amiodarone Hydrochloride, previously dried, dissolve in 40 mL of a mixture of acetic anhydride and acetic acid (100) (3:1), and titrate <2.5g> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 68.18 mg of C25H29I2NO3.HCl

Containers and storage Containers—Tight containers. Storage—Light-resistant.
Amiodarone Hydrochloride Tablets

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Amiodarone Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of amiodarone hydrochloride (C$_{25}$H$_{29}$I$_2$NO$_3$.HCl: 681.77).

Method of preparation Prepare as directed under Tablets, with Amiodarone Hydrochloride.

Identification To 1 mL of the sample stock solution obtained in the Assay add the mobile phase to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 239 nm and 243 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Amiodarone Hydrochloride Tablets add 160 mL of the mobile phase, treat with ultrasonic waves for 10 minutes, add the mobile phase to make exactly 200 mL, and centrifuge. Pipet $V$ mL of the supernatant liquid, equivalent to about 1 mg of amiodarone hydrochloride (C$_{25}$H$_{29}$I$_2$NO$_3$.HCl), add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of amiodarone hydrochloride for assay, previously dried at 50°C for 4 hours under reduced pressure not exceeding 0.3 kPa, and dissolve in methanol to make exactly 100 mL. Prepare the test solution by adding methanol to 2 mL of the dissolution medium, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_9$, of the sample solution and standard solution at 241 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared by adding methanol to 2 mL of the dissolution medium to make 20 mL, as the blank.

Dissolution rate (%) with respect to the labeled amount of amiodarone hydrochloride (C$_{25}$H$_{29}$I$_2$NO$_3$.HCl) $M_5 = M_5 \times A_T/A_9 \times V/V \times 1/C \times 36$

$M_5$: Amount (mg) of amiodarone for assay

C: Labeled amount (mg) of amiodarone hydrochloride (C$_{25}$H$_{29}$I$_2$NO$_3$.HCl) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Amiodarone Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of amiodarone hydrochloride (C$_{25}$H$_{29}$I$_2$NO$_3$.HCl), add 80 mL of the mobile phase, treat with ultrasonic waves for 10 minutes, and add the mobile phase to make exactly 50 mL. Cenrifuge this solution, and use the supernatant liquid as the sample stock solution. Pipet 2 mL of the stock solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately 25 mg of amiodarone hydrochloride for assay, previously dried at 50°C for 4 hours under reduced pressure not exceeding 0.3 kPa, and dissolve in the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_9$, of the peak area of amiodarone to that of the internal standard.

Amount (mg) of amiodarone hydrochloride (C$_{25}$H$_{29}$I$_2$NO$_3$.HCl) $M_5 = M_5 \times Q_T/Q_9 \times 2$

$M_5$: Amount (mg) of amiodarone hydrochloride for assay

Internal standard solution—A solution of chlorhexidine hydrochloride in the mobile phase (1 in 2500).

Operating conditions—Detector: An ultraviolet absorption photometer (wavelength: 242 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about
50°C.

Mobile phase: A mixture of acetonitrile for liquid chromatography, a solution of sodium laurylsulfate (1 in 50) and phosphoric acid (750:250:1).

Flow rate: Adjust the flow rate so that the retention time of amiodarone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and amiodarone are eluted in this order with the resolution between these peaks not being less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of amiodarone to that of the internal standard is not more than 1.0%.

Containers and storage—Tight containers.

Storage—Light-resistant.

### Amitriptyline Hydrochloride

#### Description

Amitriptyline Hydrochloride occurs as colorless crystals or a white to pale yellow crystalline powder. It has a bitter taste and a numbing effect. It is freely soluble in water, in ethanol (95) and in acetic acid (100), soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Amitriptyline Hydrochloride (1 in 20) is between 4.0 and 5.0.

**Identification (1)** Dissolve 5 mg of Amitriptyline Hydrochloride in 3 mL of sulfuric acid: a red color develops. Add 5 drops of potassium dichromate TS to this solution: it turns dark brown.

**Identification (2)** Acidify 1 mL of a solution of Amitriptyline Hydrochloride (1 in 500) with 0.5 mL of dilute nitric acid, and add 1 drop of silver nitrate TS: a white, opalescent precipitate is produced.

**Identification (3)** Determine the absorption spectrum of a solution of Amitriptyline Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Amitriptyline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point** <2.6D> 195 – 198°C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Amitriptyline Hydrochloride in 20 mL of water: the solution is clear and colorless.

**Identification (2)** Heavy metals <1.07>—Proceed with 2.0 g of Amitriptyline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Loss on drying** <2.4I> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.4G> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Amitriptyline Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.5D> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 31.39 mg of C20H23N.HCl

Containers and storage—Tight containers.

Storage—Light-resistant.

### Amitriptyline Hydrochloride Tablets

Amitriptyline Hydrochloride Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of amitriptyline hydrochloride (C20H23N.HCl: 313.86).

**Method of preparation** Prepare as directed under Tablets, with Amitriptyline Hydrochloride.

**Identification (1)** Weigh a quantity of powdered Amitriptyline Hydrochloride Tablets, equivalent to 0.1 g of Amitriptyline Hydrochloride according to the labeled amount. Add 10 mL of chloroform, shake thoroughly, and filter. Evaporate the filtrate on a water bath to about 2 mL, add diethyl ether until turbidity is produced, and allow to stand. Filter the crystals formed through a glass filter (G4), and proceed as directed in the Identification (1) and (2) under Amitriptyline Hydrochloride.

**Identification (2)** Determine the absorption spectrum of a solution of the crystals obtained in (1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 238 nm and 240 nm, and a minimum between 228 nm and 230 nm.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Amitriptyline Hydrochloride Tablets add 50 mL of diluted methanol (1 in 2), shake to disintegrate the tablet, then add diluted methanol (1 in 2) to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, add methanol to make exactly V mL so that each mL contains about 10 μg of...
amitriptyline hydrochloride (C₂₀H₂₃N.HCl), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of amitriptyline hydrochloride (C₂₀H₂₃N.HCl) = \( M_6 \times A_T/\overline{A}_S \times V/V \times 1/20 \)

where

- \( M_6 \): Amount (mg) of Amitriptyline Hydrochloride RS

**Dissolution** (6.10)

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Amitriptyline Hydrochloride Tablets is not less than 70%.

Start the test with 1 tablet of Amitriptyline Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 µm. Discard the first 10 mL of the filtrate, pipet the subsequent \( V \) mL of the filtrate, add the dissolution medium to make exactly \( V \) mL so that each mL contains about 11 µg of amitriptyline hydrochloride (C₂₀H₂₃N.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 55 mg of Amitriptyline Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in the dissolution medium to make exactly 250 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 239 nm as directed under Ultraviolet-visible Spectrophotometry (6.24).

Dissolution rate (％) with respect to the labeled amount of amitriptyline hydrochloride (C₂₀H₂₃N.HCl) = \( M_6 \times A_T/\overline{A}_S \times V/V \times 1/C \times 18 \)

where

- \( M_6 \): Amount (mg) of Amitriptyline Hydrochloride RS

**C: Labeled amount (mg) of amitriptyline hydrochloride (C₂₀H₂₃N.HCl) in 1 tablet**

**Assay**

Weigh accurately and powder not less than 20 Amitriptyline Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of amitriptyline hydrochloride (C₂₀H₂₃N.HCl), and add 75 mL of diluted methanol (1 in 2). After shaking for 30 minutes, add diluted methanol (1 in 2) to make exactly 100 mL, and filter. Discard the first 20-mL portion of the filtrate, measure exactly the subsequent 5-mL portion, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Amitriptyline Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Measure exactly 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the sample solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 239 nm as directed under Ultraviolet-visible Spectrophotometry (6.24), respectively.

Amount (mg) of amitriptyline hydrochloride (C₂₀H₂₃N.HCl) = \( M_6 \times A_T/\overline{A}_S \)

where

- \( M_6 \): Amount (mg) of Amitriptyline Hydrochloride RS

**Containers and storage**

Containers—Tight containers.

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**Amlexanox**

アンレキサノクス

C₁₆H₁₈N₂O₄: 298.29
2-Amino-7-(1-methylethyl)-5-oxo-5H-[1]benzopyran-2,3-b]pyridine-3-carboxylic acid [68302-37-8]

Amlexanox, when dried, contains not less than 98.0％ and not more than 102.0％ of C₁₆H₁₈N₂O₄.

**Description**

Amlexanox occurs as white to yellowish white crystals or crystalline powder. It is very slightly soluble in ethanol (99.5), and practically insoluble in water. It dissolves in diluted sodium hydroxide TS (1 in 3).

**Identification** (1)

Determine the absorption spectrum of a solution of Amlexanox in ethanol (99.5) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry (6.24), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Amlexanox RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Amlexanox as directed in the potassium bromide disk method under Infrared Spectrophotometry (6.25), and compare the spectrum with the Reference Spectrum or the spectrum of Amlexanox RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** (1)

Chloride (6.07) — Dissolve 1.0 g of Amlexanox in 20 mL of water and 10 mL of sodium hydroxide TS, add 15 mL of dilute nitric acid and water to make 50 mL, centrifuge, and then filter the supernatant liquid. To 25 mL of this filtrate add water to make 50 mL. Perform the test using this solution as the test solution. The control solution consists of 5 mL of sodium hydroxide TS, 7.5 mL of dilute nitric acid, 0.30 mL of 0.01 mol/L hydrochloric acid VS, and water added to make 50 mL (not more than 0.021％).

(2) Heavy metals (6.07) — Proceed with 1.0 g of Amlexanox according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances — (i) Dissolve 30 mg of Amlexanox in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography (6.01) according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of the peak other than amlexanox obtained from the sample solution is not larger than 2 times the peak area of amlexanox from the standard solution.

**Operating conditions** —
The detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: Until completion of the elution of amlexanox beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of amlexanox obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlexanox is not more than 2.0%.

(ii) Dissolve 30 mg of Amlexanox in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than amlexanox obtained from the sample solution is not larger than 2 times the peak area of amlexanox from the standard solution.

Operating conditions—
Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 7.2 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. Adjust the pH of this solution to 8.0 by adding a solution prepared by dissolving 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: To 15 mL of a solution of benzophenone in the mobile phase (3 in 1,000,000) add the mobile phase to make 20 mL. Adjust the flow rate so that the retention time of benzophenone is about 6.5 minutes when the test with 10 μL of this solution under the conditions described above.

Time span of measurement: About 3 times as long as the retention time of benzophenone, beginning after the peak of amlexanox.

System suitability—
Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of amlexanox obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Pipet 1 mL of the sample solution, and add the mobile phase to make 100 mL. To 5 mL of this solution add 15 mL of the solution of benzophenone in the mobile phase (3 in 1,000,000). When perform the test with 10 μL of this solution according to the above conditions, amlexanox and benzophenone are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlexanox is not more than 2.0%.

(iii) The total amount of related substances, when calculated according to the following formula, is not more than 0.5%.

\[
\text{Total amount} \times \frac{1}{10} = \frac{(A_{T1}/A_{S1}) + (A_{T2}/A_{S2})}{2/0.1} = T1 \times Q1 = A_{T3} \times \frac{Q2}{Q3}
\]

\[
M \times \text{Amount (mg) of Amlexanox RS}
\]

Internal standard solution—A solution of 3-nitroaniline in the mobile phase (1 in 4000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 17.9 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. Adjust the pH of this solution to 8.0 by adding a solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water. To 760 mL of this solution add 240 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of amlexanox is about 10 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution according to the above conditions, amlexanox and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of amlexanox to that of the internal standard is not
Containers and storage  Containers—Well-closed containers.

Amlexanox Tablets
アンレキサノクス錠

Amlexanox Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of amlexanox (C\(_{16}H_{14}N_2O_4\): 298.29).

Method of preparation  Prepare as directed under Tablets, with Amlexanox.

Identification  (1) Take an amount of powdered Amlexanox Tablets, equivalent to 10 mg of Amlexanox according to the labeled amount, add 100 mL of ethanol (99.5), shake vigorously, and filter. Pipet 1 mL of the filtrate, add 25 mL of ethanol (99.5), and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits absorption maxima between 240 nm and 244 nm, so there is about 167 \(\times 6.02\) \(\mu g\) of amlexanox (C\(_{16}H_{14}N_2O_4\)) per 1 mg of Amlexanox.

Uniformity of dosage units  <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Amlexanox Tablets, add exactly 0.6 mL of the internal standard solution per 1 mg of amlexanox (C\(_{16}H_{14}N_2O_4\)), add the mobile phase to make exactly \(V\) mL so there is about 167 \(\mu g\) of amlexanox (C\(_{16}H_{14}N_2O_4\)) per 1 mL, disintegrate the tablet, and then shake vigorously for 5 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 30 mg of Amlexanox RS, previously dried at 105°C for 2 hours, and dissolve in 2 mL of dilute sodium hydroxide TS, add the dissolution medium to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), at 350 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24.

Dissolution rate (%) with respect to the labeled amount of amlexanox (C\(_{16}H_{14}N_2O_4\))

\[
M_S = M_A \times \frac{V}{Q} \times \frac{1/2}{1/18} \\
M_S: \text{Amount (mg) of Amlexanox RS}
\]

Internal standard solution—A solution of 3-nitroaniline in the mobile phase (1 in 500).

Containers and storage  Containers—Tight containers.

Amlodipine Besilate
アムロジピンベシル酸塩

Amlodipine Besilate contains not less than 98.0% and not more than 102.0% of C\(_{20}H_{25}ClN_2O_5\), C\(_6H_6O_3\)S, and enantiomer C\(_{20}H_2ClN_2O_5\), C\(_6H_6O_3\)S: 567.05
calculated on the anhydrous basis.

**Description**
Amlodipine Besilate occurs as a white to yellowish white crystalline powder.

It is freely soluble in methanol, sparingly soluble in ethanol (99.5), and slightly soluble in water.

A solution of Amlodipine Besilate in methanol (1 in 100) shows no optical rotation.

Melting point: about 198°C (with decomposition).

**Identification (1)**
Determine the absorption spectrum of a solution of Amlodipine Besilate in 0.01 mol/L hydrochloric acid-methanol TS (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Amlodipine Besilate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Amlodipine Besilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Amlodipine Besilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 30 mg of Amlodipine Besilate add 0.1 g of sodium nitrate and 0.1 g of anhydrous sodium carbonate, mix, and gradually ignite. After cooling, dissolve the residue in 2 mL of dilute hydrochloric acid and 10 mL of water, filter if necessary, and add barium chloride TS: a white precipitate is formed.

**Purity (1)**
Heavy metals <1.07)—Proceed with 1.0 g of Amlodipine Besilate according to Method 4, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 25 ppm).

(2) Related substances—Dissolve 0.10 g of Amlodipine Besilate in 50 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mixture of water and acetonitrile (1:1) to make exactly 100 mL. Pipet 3 mL of this solution, add the mixture of water and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.47> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of 0.90 with respect to amlodipine, obtained from the sample solution is not larger than the peak area of amlodipine from the standard solution, and the area of the peak other than amlodipine, other than benzenesulfonic acid having the relative retention time of about 0.15 with respect to amlodipine, and other than the peak mentioned above is not larger than 1/3 times the peak area of amlodipine from the standard solution. Furthermore, total peak area for peaks other than amlodipine and benzenesulfonic acid of the sample solution is not larger than 2.7 times the peak area of amlodipine from the standard solution.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 µm in particle diameter).

Flow rate: 1.0 mL per minute.

Time span of measurement: About 3 times as long as the retention time of amlodipine, beginning after the solvent peak.

**System suitability—**
Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and acetonitrile (1:1) to make exactly 10 mL. Confirm that the peak area of amlodipine obtained with 10 µL of this solution is equivalent to 7 to 13% of that with 10 µL of the standard solution.

**System performance:**
When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amlodipine are not less than 70,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlodipine is not more than 2.0%.

(3) Residual solvent—Being specified separately.

**Water** <2.48> Not more than 0.5% (1 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Assay**
Weigh accurately about 35 mg each of Amlodipine Besilate and Amlodipine Besilate RS (separately determine the water <2.48> using the same manner as Amlodipine Besilate), dissolve them separately in the mobile phase to make exactly 250 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 µL of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q1 and Q5, of the peak area of amlodipine to that of the internal standard.

\[
\text{Amount (mg) of C}_{20}\text{H}_{25}\text{ClN}_{2}\text{O}_{5}.\text{C}_{4}\text{H}_{6}\text{O}_{3}\text{S} = M_S \times \frac{Q_1}{Q_5}
\]

\[
M_S: \text{Amount (mg) of Amlodipine Besilate RS, calculated on the anhydrous basis}
\]

**Internal standard solution—**A solution of isobutyl parahydroxybenzoate in the mobile phase (3 in 20,000).

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeccylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and a solution of potassium dihydrogen phosphate (41 in 10,000) (13:7).

Flow rate: Adjust the flow rate so that the retention time of amloidipine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, amloidipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of amloidipine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Well-closed containers.

Storage—Light-resistant.

Amloidipine Besilate Tablets

アムロジピンベシル酸塩錠

Amloidipine Besilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of amloidipine besilate (C20H25ClN2O5.C6H6O3S: 567.05).

Method of preparation—Prepare as directed under Tablets, with Amloidipine Besilate.

Identification—To a quantity of powdered Amloidipine Besilate Tablets, equivalent to 2.5 mg of Amloidipine Besilate according to the labeled amount, add 100 mL of 0.01 mol/L hydrochloric acid-methanol TS, shake vigorously, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 235 nm and 239 nm, and between 358 nm and 362 nm.

Uniformity of dosage units—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Amloidipine Besilate Tablets add 10 mL of water to disintegrate, disperse with the aid of ultrasonic waves with occasional shaking, add the mobile phase to make exactly V mL so that each mL contains about 69 μg of amloidipine besilate (C20H25ClN2O5.C6H6O3S), and shake for 60 minutes. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of amloidipine besilate

\[ M_5 = \frac{M_S \times Q_r}{Q_S \times V} \times 500 \]

M₅: Amount (mg) of Amloidipine Besilate RS, calculated on the anhydrous basis

Internal standard solution—A solution of isobutyl parahydroxybenzoate in the mobile phase (3 in 20,000).

Dissolution—Being specified separately.

Assay—To 20 Amloidipine Besilate Tablets add 100 mL of water to disintegrate, disperse with the aid of ultrasonic waves with occasional shaking, add the mobile phase to make exactly 1000 mL, and shake for 60 minutes. Centrifuge this solution, pipet a volume of the supernatant liquid, and use this solution as the sample solution. Separately, weigh accurately about 35 mg of Amloidipine Besilate RS (separately, determine the water <2.46> in the same manner as Amloidipine Besilate), and dissolve in the mobile phase to make exactly 250 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Qₑ and Qₘ, of the peak area of amloidipine to that of the internal standard.

Amount (mg) of amloidipine besilate

\[ M_5 = \frac{M_S \times Q_r}{Q_S} \times 1/50 \]

M₅: Amount (mg) of Amloidipine Besilate RS, calculated on the anhydrous basis

Internal standard solution—A solution of isobutyl parahydroxybenzoate in the mobile phase (3 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeccylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate (41 in 10,000) (13:7).

Flow rate: Adjust the flow rate so that the retention time of amloidipine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, amloidipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of amloidipine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Well-closed containers.
Ammonia Water

アンモニア水

Ammonia Water contains not less than 9.5 w/v% and not more than 10.5 w/v% of ammonia (NH₃: 17.03).

**Description** Ammonia Water occurs as a clear, colorless liquid, having a very pungent, characteristic odor. It is alkaline.

Specific gravity d₃₀: 0.95 – 0.96

**Identification** (1) Hold a glass rod moistened with hydrochloric acid near the surface of Ammonia Water: dense white fumes are produced.

(2) Hold moistened red litmus paper near the surface of Ammonia Water: it turns blue.

**Purity** (1) Residue on evaporation—Evaporate 10.0 mL of Ammonia Water to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 2.0 mg.

(2) Heavy metals <1.07>—Evaporate 5.0 mL of Ammonia Water to dryness on a water bath, add 1 mL of dilute hydrochloric acid to the residue, and evaporate to dryness. Dissolve the residue in 2 mL of dilute acetic acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.5 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 5 ppm).

(3) Potassium permanganate-reducing substances—To 10.0 mL of Ammonia Water add 40 mL of dilute sulfuric acid while cooling, and add 0.10 mL of 0.02 mol/L potassium permanganate VS: the red color of the potassium permanganate does not disappear within 10 minutes.

**Assay** Measure exactly 5 mL of Ammonia Water, add 25 mL of water, and titrate <2.30> with 0.5 mol/L sulfuric acid VS (indicator: 2 drops of methyl red TS).

Each mL of 0.5 mol/L sulfuric acid VS = 17.03 mg of NH₃

**Containers and storage** Containers—Tight containers.

Storage—Not exceeding 30°C.

Amobarbital

アモバルビタール

C₁₁H₁₈N₂O₃: 226.27
5-Ethyl-5-(3-methylbutyl)pyrimidine-2,4,6(1H,3H,5H)-trione
[57-43-2]

Amobarbital, when dried, contains not less than 99.0% of C₁₁H₁₈N₂O₃.

**Description** Amobarbital occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste. It is freely soluble in ethanol (95), in acetone and in diethyl ether, sparingly soluble in chloroform, and practically insoluble in water.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

The pH of a saturated solution of Amobarbital is between 5.0 and 5.6.

**Identification** (1) Boil 0.2 g of Amobarbital with 10 mL of sodium hydroxide TS: the gas evolved changes moistened litmus paper to blue.

(2) Dissolve 0.05 g of Amobarbital in 2 to 3 drops of ammonia-ammonium chloride buffer solution, pH 10.7, and 5 mL of diluted pyridine (1 in 10). Add 5 mL of chloroform and 0.3 mL of copper (II) sulfate TS to the solution: a red-purple precipitate is produced in the aqueous layer. Shake the mixture: a red-purple color is produced in the chloroform layer.

(3) To 0.4 g of Amobarbital add 0.1 g of anhydrous sodium carbonate and 4 mL of water, shake, and add a solution of 0.3 g of 4-nitrobenzyl chloride in 7 mL of ethanol (95). Heat the mixture on a water bath for 30 minutes under a reflux condenser, and allow to stand for 1 hour. Filter the crystals produced, wash with 7 mL of sodium hydroxide TS and a small portion of water, recrystallize from ethanol, and dry at 105°C for 30 minutes: the crystals so obtained melt <2.60> between 168°C and 173°C or between 150°C and 154°C.

**Melting point** <2.60> 157 – 160°C

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Amobarbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.30 g of Amobarbital in 20 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.035%).

(3) Sulfate <1.14>—Dissolve 0.40 g of Amobarbital in 20 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.40 mL of 0.005 mol/L hydrochloric acid VS, 20 mL of acetone and 1 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Amobarbital according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Amobarbital. The solution is not more colored than Matching Fluid A.

**Loss on drying** <2.4> Not more than 1.0% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Amobarbital, previously dried, and dissolve in 5 mL of ethanol (95) and 50 mL of chloroform. Titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes
from yellow through light blue to purple (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 22.63 mg of C_{11}H_{17}N_{2}NaO_{3}

Containers and storage Containers—Well-closed containers.

**Amobarbital Sodium for Injection**

注射用アモバルビタールナトリウム

![Chemical Structure](image)

C_{11}H_{17}N_{2}NaO_{3}: 248.25
Monosodium 5-ethyl-5-(3-methylbutyl)-4,6-dioxo-1,4,5,6-tetrahydropyrimidin-2-olate [64-43-7]

Amobarbital Sodium for Injection is a preparation for injection which is dissolved before use.

When dried, it contains not less than 98.5% of amobarbital sodium (C_{11}H_{17}N_{2}NaO_{3}), and not less than 92.5% and not more than 107.5% of the labeled amount of amobarbital sodium (C_{11}H_{17}N_{2}NaO_{3}).

**Method of preparation** Prepare as directed under Injections.

**Description** Amobarbital Sodium for Injection occurs as white crystals or a crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether and in chloroform.

The pH of its solution (1 in 10) is between 10.0 and 11.0. It is hygroscopic.

**Identification** (1) Dissolve 1.5 g of Amobarbital Sodium for Injection in 20 mL of water, and add 10 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Collect the precipitate, wash with four 10-mL portions of water, and dry at 105°C for 3 hours: it melts <2.60> between 157°C and 160°C. With this precipitate, proceed as directed in the Identification under Amobarbital.

(2) Ignite 0.5 g of Amobarbital Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution responds to the Qualitative Tests <1.09> (1) for sodium salt.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Amobarbital Sodium for Injection in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

(2) Chloride <1.09>—Dissolve 1.0 g of Amobarbital Sodium for Injection in 49 mL of water, add 1 mL of acetic acid (100), shake, and filter. Discard the first 10 mL of the filtrate, and to the subsequent 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 0.5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.018%).

(3) Sulfate <1.14>—Dissolve 2.0 g of Amobarbital Sodium for Injection in 49 mL of water, add 1 mL of acetic acid (100), shake, and filter. Discard the first 10 mL of the filtrate, and to the subsequent 25 mL of the filtrate add 2.5 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 0.5 mL of acetic acid (100), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.019%).

(4) Heavy metals <1.07>—Dissolve 2.0 g of Amobarbital Sodium for Injection in 45 mL of water, add 5 mL of dilute hydrochloric acid, shake vigorously, and warm on a water bath for 2 minutes with occasional shaking. Cool, add 30 mL of water, shake, and filter. Discard the first 10 mL of the filtrate, add 1 drop of phenolphthalein TS to the subsequent 40 mL of the filtrate, add ammonia TS until a slight red color develops, and add 2.5 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.5 mL of dilute hydrochloric acid add 1 drop of phenolphthalein TS, add ammonia TS until a pale red color develops, and add 2.5 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(5) Neutral or basic substances—Dissolve about 1 g of Amobarbital Sodium for Injection, accurately weighed, in 10 mL of water and 5 mL of sodium hydroxide TS, then add 40 mL of chloroform, and shake well. Separate the chloroform layer, wash with two 5-mL portions of water, and filter. Evaporate the filtrate on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 0.30%.

(6) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Amobarbital Sodium for Injection: the solution is not more colored than Matching Fluid A.

**Loss on drying** <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

**Assay** Weigh accurately the contents of not less than 10 samples of Amobarbital Sodium for Injection. Weigh accurately about 0.5 g of the contents, previously dried, transfer to a separator, dissolve in 20 mL of water, add 5 mL of ethanol (95) and 10 mL of dilute hydrochloric acid, and extract with 50 mL of chloroform, then with three 25-mL portions of chloroform. Combine the chloroform extracts, wash with two 5-mL portions of water, and extract the washings with two 10-mL portions of chloroform. Filter the combined chloroform extracts into a conical flask, and wash the filter paper with three 5-mL portions of chloroform. Combine the filtrate and the washings, and add 10 mL of ethanol (95). Titrate <2.30> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through light blue to purple (indicator: 2 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination with a mixture of 160 mL of chloroform and 30 mL of ethanol (95), and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 24.83 mg of C_{11}H_{17}N_{2}NaO_{3}

Containers and storage Containers—Hermetic containers.
Amosulalol Hydrochloride

**Description**
Amosulalol Hydrochloride occurs as white crystals or a white crystalline powder. It has a bitter taste. It is very soluble in formic acid, freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5). It is hygroscopic.

A solution of Amosulalol Hydrochloride in methanol (1 in 100) shows no optical rotation.

**Identification**
(1) Determine the absorption spectrum of a solution of Amosulalol Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Amosulalol Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Amosulalol Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloro.

**Melting point**
<2.60> 158 – 162°C

**Purity**
(1) Heavy metals <1.07>—Place 1.0 g of Amosulalol Hydrochloride in a porcelain crucible, add 1.5 mL of sulfuric acid, cover loosely, and heat gently to carbonize. After cooling, add 2 mL of nitric acid, heat carefully until white fumes no longer are evolved, and then heat intensely to 500 – 600°C to incinerate. After cooling, add 2 mL of hydrochloric acid, proceed according to Method 2, and perform the test. The control solution, processed in the same manner as the test solution using the same amounts of reagents, is prepared by combining 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Amosulalol Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than amosulalol obtained from the sample solution is not larger than 2/5 times the peak area of amosulalol from the standard solution.

**Operating conditions**

- Detector: An ultraviolet absorption photometer (wavelength: 272 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 30°C.
- Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 5.7 by adding a solution prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To 670 mL of this solution add 330 mL of acetonitrile.
- Flow rate: Adjust the flow rate so that the retention time of amosulalol is about 7 minutes.
- Time span of measurement: About 2 times as long as the retention time of amosulalol, beginning after the solvent peak.

**System suitability**
- Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of amosulalol obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.
- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amosulalol are not less than 4000 and not more than 1.7, respectively.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amosulalol is not more than 1.0%.

**Water** <2.48> Not more than 4.0% (1 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay**
Weigh accurately about 0.6 g of Amosulalol Hydrochloride, dissolve in 3 mL of formic acid, add 80 mL of a mixture of acetic acid (100) and acetic anhydride (3:2), and titrate <2.50> within 5 minutes with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination using the same procedure, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 41.69 mg of C_{18}H_{24}N_{2}O_{5}S.HCl

**Containers and storage**
Containers—Tight containers.
Amosulalol Hydrochloride Tablets

アモスラロール塩酸塩錠

Amosulalol Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of amosulalol hydrochloride \((\text{C}_{18}\text{H}_{24}\text{N}_{2}\text{O}_{5}\text{S.HCl})\) (416.92).

**Method of preparation** Prepare as directed under Tablets, with Amosulalol Hydrochloride.

**Identification** To a quantity of powdered Amosulalol Hydrochloride Tablets, equivalent to 50 mg of Amosulalol Hydrochloride according to the labeled amount, add 25 mL of 0.1 mol/L hydrochloric acid TS, shake well, and then centrifuge. To 2.5 mL of the supernatant liquid add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\text{›}\) it exhibits a maximum between 270 nm and 274 nm, and a shoulder between 275 nm and 281 nm.

**Uniformity of dosage units** \(<6.02\text{›}\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Amosulalol Hydrochloride Tablets, disintegrate by adding 2 mL of 0.1 mol/L hydrochloric acid TS, add 15 mL of methanol, and shake well. Add methanol to make exactly \(V\) mL so that each mL contains about 0.4 mg of amosulalol hydrochloride \((\text{C}_{18}\text{H}_{24}\text{N}_{2}\text{O}_{5}\text{S.HCl})\), and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of amosulalol hydrochloride for assay (separately determine the water \(<2.49\text{›}\) in the same manner as Amosulalol Hydrochloride), and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay. 

\[
\text{Amount (mg) of amosulalol hydrochloride (C}_{18}\text{H}_{24}\text{N}_{2}\text{O}_{5}\text{S.HCl}) \quad M_5 = \frac{M_i}{Q_i/Q_s} \times \frac{V}{50}
\]

\(M_5\): Amount (mg) of amosulalol hydrochloride for assay, calculated on the anhydrous basis

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in methanol (1 in 6250).

**Dissolution** \(<6.10\text{›}\) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Amosulalol Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Amosulalol Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains about 5.5 \(\mu\)g of amosulalol hydrochloride \((\text{C}_{18}\text{H}_{24}\text{N}_{2}\text{O}_{5}\text{S.HCl})\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of amosulalol hydrochloride for assay (separately determine the water \(<2.49\text{›}\) in the same manner as Amosulalol Hydrochloride), and dissolve in water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\text{›}\) according to the following conditions, and determine the amosulalol peak areas, \(A_T\) and \(A_s\), of both solutions.

\[
\text{Dissolution rate (\%)} = \left( \frac{A_T}{A_s} \right) \times \frac{A_i/\sqrt{A_s} \times V/V \times 1/C \times 45/2}{M_i}
\]

\(M_i\): Amount (mg) of amosulalol hydrochloride for assay, calculated on the anhydrous basis

\(C\): Labeled amount (mg) of amosulalol hydrochloride \((\text{C}_{18}\text{H}_{24}\text{N}_{2}\text{O}_{5}\text{S.HCl})\) in 1 tablet

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 5.7 by adding a solution prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To 670 mL of this solution add 330 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of amosulalol is about 5 minutes.

**System suitability**—

System performance: When the procedure is run with 50 \(\mu\)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amosulalol are not less than 4000 and not more than 1.7, respectively.

System repeatability: When the test is repeated 6 times with 50 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amosulalol is not more than 1.8%.

**Assay** Take 10 Amosulalol Hydrochloride Tablets, add 20 mL of 0.1 mol/L hydrochloric acid TS, and shake well to disintegrate. Add 120 mL of methanol, again shake well, add methanol to make exactly 200 mL, and then centrifuge. Pipet a volume of supernatant liquid corresponding to about 5 mg of amosulalol hydrochloride \((\text{C}_{18}\text{H}_{24}\text{N}_{2}\text{O}_{5}\text{S.HCl})\), add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of amosulalol hydrochloride for assay (separately determine the water \(<2.49\text{›}\) in the same manner as Amosulalol Hydrochloride), and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\text{›}\) according to
the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of amosulalol to that of the internal standard.

Amount (mg) of amosulalol hydrochloride
\[
(C_{18}H_{24}N_{2}O_{5}S.HCl) = M_5 \times Q_2/Q_3 \times 1/5
\]

\( M_5 \): Amount (mg) of amosulalol hydrochloride for assay, calculated on the anhydrous basis

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in methanol (1 in 6250).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 272 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of diluted acetic acid (100) (1 in 25), acetonitrile and a solution of ammonium acetate (1 in 250) (5:3:2).
Flow rate: Adjust the flow rate so that the retention time of amosulalol is about 4 minutes.

**System suitability**—
- System performance: When the procedure is run with 10 \( \mu \)L of the standard solution under the above operating conditions, amosulalol and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.
- System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of amosulalol to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

### Amoxapine

アンモキサピン

\( C_{17}H_{16}ClN_{3}O: 313.78 \)
2-Chloro-11-(piperazin-1-yl)dibenzo[f][1,4]oxazepine [14028-44-3]

Amoxapine, when dried, contains not less than 98.5% of \( C_{17}H_{16}ClN_{3}O \).

**Description** Amoxapine occurs as white to light yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Amoxapine in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption as the same wavelengths.

(2) Determine the infrared absorption spectrum of Amoxapine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Amoxapine as directed under Flame Coloration Test <1.04> (2): a green color appears.

**Melting point** <2.60> 178 – 182°C

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 g of Amoxapine according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 15 ppm).

(2) Related substances—Dissolve 0.5 g of Amoxapine in 10 mL of a mixture of ethanol (95) and acetic acid (100) (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of ethanol (95) and acetic acid (100) (9:1) to make exactly 10 mL. Pipet 1 mL of this solution, add a mixture of ethanol (95) and acetic acid (100) (9:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 \( \mu \)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (95) and acetic acid (100) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.4% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Amoxapine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 15.69 mg of \( C_{17}H_{16}ClN_{3}O \)

**Containers and storage** Containers—Tight containers.
Amoxicillin Hydrate

アモキシシリン水和物

\[
\text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{5}\text{S}.3\text{H}_{2}\text{O}: 419.45 \\
(2S,5R,6R)-6-[(2R)-2-Amino-2-(4-hydroxyphenyl)acetamino]-3,3-dimethyl-7-oxo-4-thia-1-
azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate [61336-70-7]
\]

Amoxicillin Hydrate contains not less than 950 \( \mu g \) (potency) and not more than 1010 \( \mu g \) (potency) per mg, calculated on the anhydrous basis. The potency of Amoxicillin Hydrate is expressed as mass (potency) of amoxicillin \( (\text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{5}\text{S}) \): 365.40.

**Description** Amoxicillin Hydrate occurs as white to light yellowish white, crystals or crystalline powder.

It is slightly soluble in water and in methanol, and very slightly soluble in ethanol (95).

**Identification** Determine the infrared absorption spectrum of Amoxicillin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum or the spectrum of Amoxicillin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \(<2.49\rangle \ [\alpha]_{D}^{20} + 290 \pm 315^\circ \ (0.1 \ g \ calculated \ on \ the \ anhydrous \ basis, \ water, \ 100 \ mL, \ 100 \ mm).

**Purity** (1) Heavy metals \(<1.07\rangle\)—To 1.0 g of Amoxicillin Hydrate add 2 mL of a solution of magnesium sulfate heptahydrate (1 in 4), mix, and heat on a water bath to dryness. Carbonize the residue by gently heating. After cooling, add 1 mL of sulfuric acid, heat carefully, then heat at 500 – 600°C to incinerate. After cooling, add 1 mL of hydrochloric acid to the residue, and heat on a water bath to dryness. Then add 10 mL of water to the residue, and heat on a water bath to dissolve. After cooling, add ammonia TS to adjust the \( \text{pH} \) to 3 – 4, and add 2 mL of dilute acetic acid. If necessary, filter, wash the residue on the filter with 10 mL of water, transfer the filtrate and washings into a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: To 2.0 mL of Standard Lead Solution add 2 mL of a solution of magnesium sulfate heptahydrate (1 in 4), then proceed in the same manner as for preparation of the test solution (not more than 20 ppm).

(2) Arsenic \(<1.11\rangle\)—Prepare the test solution with 1.0 g of Amoxicillin Hydrate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Amoxicillin Hydrate in 50 mL of a solution of boric acid (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of boric acid (1 in 200) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\rangle\) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than amoxicillin obtained from the sample solution is not larger than the peak area of amoxicillin from the standard solution.

**Operating conditions**—

**Detector**—An ultraviolet absorption photometer (wavelength: 254 nm).

**Column**—A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (10 \( \mu \)m in particle diameter).

**Column temperature**—A constant temperature of about 25°C.

**Mobile phase**—Dissolve 1.36 g of sodium acetate trihydrate in 750 mL of water, adjust the \( \text{pH} \) to 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

**Flow rate**—Adjust the flow rate so that the retention time of amoxicillin is about 8 minutes.

**Time span of measurement**—About 4 times as long as the retention time of amoxicillin.

**System suitability**—

Test for required detection: To exactly 1 mL of the standard solution add a solution of boric acid (1 in 200) to make exactly 10 mL. Confirm that the peak area of amoxicillin obtained from 10 \( \mu L \) of this solution is equivalent to 7 to 13% of that from 10 \( \mu L \) of the standard solution.

**System performance**: When the procedure is run with 10 \( \mu L \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amoxicillin are not less than 2500 and not more than 1.5, respectively.

**System repeatability**: When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amoxicillin is not more than 1.0%.

**Water** \(<2.48\rangle\) Not less than 11.0% and not more than 15.0% (0.1 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Amoxicillin Hydrate and Amoxicillin RS, equivalent to about 30 mg (potency), dissolve each in a solution of boric acid (1 in 200) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\rangle\) according to the following conditions, and calculate the peak areas, \( A_T \) and \( A_S \), of amoxicillin of each solution.

**Amount** (\( \mu g \) (potency)) of amoxicillin (\( \text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{5}\text{S} \))

\[
M_S = M_5 \times A_T/A_S \times 1000 \\
M_5: \text{Amount [mg (potency)] of Amoxicillin RS}
\]

**Operating conditions**—

**Detector**—An ultraviolet absorption photometer (wavelength: 230 nm).

**Column**—A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

**Column temperature**—A constant temperature of about 25°C.
Mobile phase: Dissolve 1.361 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of amoxicillin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of amoxicillin is not less than 2500.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amoxicillin is not more than 1.0%.

Containers and storage—Containers—Tight containers.

### Amoxicillin Capsules

アモキシリンカプセル

Amoxicillin Capsules contain not less than 92.0% and not more than 105.0% of the labeled potency of Amoxicillin (C₁₆H₁₉N₃O₅S: 365.40).

Method of preparation—Prepare as directed under Capsules, with Amoxicillin Hydrate.

Identification—Take out the contents of Amoxicillin Capsules, to a quantity of the contents, equivalent to 8 mg (potency) of Amoxicillin Hydrate according to the labeled amount, add 2 mL of 0.01 mol/L hydrochloric acid TS, shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve an amount equivalent to 8 mg (potency) of Amoxicillin RS in 2 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of tetrahydrofuran, water and formic acid (50:5:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (95) (1 in 20) on the plate, and heat the plate at 110°C for 15 minutes: the principal spot obtained from the sample solution and the standard solution show a red-purple color and the same Rf value.

Purity—Related substances—Take out the contents of Amoxicillin Capsules, to a quantity of the contents, equivalent to 0.1 g (potency) of Amoxicillin Hydrate according to the labeled amount, add 30 mL of a solution of boric acid (1 in 200), shake for 15 minutes, and add a solution of boric acid (1 in 200) to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add a solution of boric acid (1 in 200) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than amoxicillin obtained from the sample solution is not larger than the peak area of amoxicillin from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Amoxicillin Hydrate.

System suitability—

Test for required detectability and system repeatability: Proceed as directed in the system suitability in the Purity (3) under Amoxicillin Hydrate.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amoxicillin is not less than 2500 and not more than 1.5, respectively.

Water <2.48> Not more than 15.0% (0.1 g, volumetric titration, direct titration).

Uniformity of dosage units <6.02> It meets the requirements of the Mass variation test.

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Amoxicillin Capsules is not less than 75%.

Start the test with 1 capsule of Amoxicillin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 56 μg (potency) of Amoxicillin Hydrate according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount equivalent to about 28 mg (potency) of Amoxicillin RS, dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, A₁ and A₅, of amoxicillin in each solution.

Dissolution rate (% with respect to the labeled amount of amoxicillin (C₁₆H₁₉N₃O₅S) = Mₛ × A₁/₅ × V/V × 1/C × 180

Mₛ: Amount [mg (potency)] of Amoxicillin RS C: Labeled amount [mg (potency)] of amoxicillin (C₁₆H₁₉N₃O₅S) in 1 capsule

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Amoxicillin Hydrate.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amoxicillin are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amoxicillin is not more than 1.5%.
Amphotericin B

Assay Weigh accurately the mass of not less than 10 Amoxicillin Capsules, take out the contents, and weigh accurately the mass of the emptied shells. Weigh accurately an amount equivalent to about 0.1 g (potency) of Amoxicillin Hydrate, add 70 mL of water, shake for 15 minutes, and add water to make exactly 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately an amount equivalent to about 20 mg (potency) of Amoxicillin RS, dissolve in water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of amoxicillin in each solution.

Amount [mg (potency)] of amoxicillin (C₁₆H₁₉N₃O₅S)  
= $M_S \times \frac{A_T}{A_S} \times 5$

$M_S$: Amount [mg (potency)] of Amoxicillin RS

Operating conditions—

Column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Amoxicillin Hydrate.

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amoxicillin are not less than 2500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of peak areas of amoxicillin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Amphotericin B is a polyene macrolide substance having antifungal activity produced by the growth of Streptomyces nodosus.

It contains not less than 840 μg (potency) per mg, calculated on the dried basis. The potency of Amphotericin B is expressed as mass (potency) of amphotericin B (C₄₇H₇₃NO₁₇).

Description Amphotericin B occurs as a yellow to orange powder.

It is freely soluble in dimethylsulfoxide and practically insoluble in water and in ethanol (95).

Identification (1) Dissolve 5 mg of Amphotericin B in 10 mL of dimethylsulfoxide. To 1 mL of this solution add 5 mL of phosphoric acid: a blue color develops between the two layers, and the solution becomes blue by shaking. After addition of 15 mL of water it becomes yellow to light yellow-brown by shaking.

(2) Dissolve 25 mg of Amphotericin B in 5 mL of dimethylsulfoxide, and add methanol to make 50 mL. To 1 mL of this solution add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of Amphotericin B RS: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity Amphotericin A—Weigh accurately about 50 mg each of Amphotericin B and Amphotericin B RS, add exactly 10 mL each of dimethylsulfoxide to dissolve, and add methanol to make exactly 50 mL. Pipet 4 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the sample solution and standard solution (1), respectively. Separately, weigh accurately about 20 mg of Nystatin RS, add exactly 40 mL of dimethylsulfoxide to dissolve, then add methanol to make exactly 200 mL. Pipet 4 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution (2). Perform
the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution obtained in the same manner as the sample solution as the blank, and determine the absorbances at 282 nm and at 304 nm. Calculate the amount of amphotericin A by the following equation: not more than 5% for Amphotericin B used for injections, and not more than 15% for Amphotericin B not used for injections.

\[
\text{Amount (\%)} = \frac{M_S \times ([A_{S1}] \times [A_{T1}] - [A_{S2}] \times [A_{T1}]) \times 25}{M_T \times ([A_{S1}] \times [A_{S2}] - [A_{S2}] \times [A_{S2}])}
\]

- \(M_S\): Amount (mg) of Nystatin RS
- \(M_T\): Amount (mg) of the sample
- \(A_{S1}\): Absorbance at 282 nm of the standard solution (1)
- \(A_{S2}\): Absorbance at 282 nm of the standard solution (2)
- \(A_{T1}\): Absorbance at 304 nm of the sample solution
- \(A_{T2}\): Absorbance at 304 nm of the sample solution

Loss on drying <2.41> Not more than 5.0% (0.1 g, in vacuum, 60°C, 3 hours).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—Saccharomyces cerevisiae ATCC 9763

(ii) Culture medium—Use the medium 2) under (1) Agar media for seed and base layer.

(iii) Preparation of cylinder-agar plate—Proceed as directed in 1.5 under the Cylinder plate method, using Petri dish plates not dispensing the agar medium for base layer and dispensing 8.0 mL of the seeded agar medium.

(iv) Standard solution—Use light-resistant vessels. Weigh accurately an amount of Amphotericin B RS equivalent to about 20 mg (potency), dissolve in dimethylsulfoxide to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, and add dimethylsulfoxide to make solutions so that each mL contains 200 \(\mu\)g (potency) and 50 \(\mu\)g (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(v) Sample solution—Use light-resistant vessels. Weigh accurately an amount of Amphotericin B equivalent to about 20 mg (potency), dissolve in dimethylsulfoxide to make exactly 20 mL, and use this solution as the sample solution. Take exactly a suitable amount of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains 200 \(\mu\)g (potency) and 50 \(\mu\)g (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

**Amphotericin B for Injection**

注射用アムホテリシン B

Amphotericin B for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 120.0% of the labeled amount of amphotericin B (C_{47}H_{73}NO_{17}: 924.08).

**Method of preparation** Prepare as directed under Injections, with Amphotericin B.

**Description** Amphotericin B for Injection occurs as yellow to orange, powder or masses.

**Identification** To an amount of Amphotericin B for Injection, equivalent to 25 mg (potency) of Amphotericin B according to the labeled amount, add 5 mL of dimethylsulfoxide and 45 mL of methanol, and shake. To 1 mL of this solution add methanol to make 50 mL, and filter if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits maxima between 361 nm and 365 nm, between 380 nm and 384 nm and between 403 nm and 407 nm.

**pH** <2.45> Dissolve an amount of Amphotericin B for Injection, equivalent to 50 mg (potency) of Amphotericin B according to the labeled amount, in 10 mL of water. To 1 mL of this solution add water to make 50 mL: 7.2 – 8.0.

**Purity** Clarity and color of solution—Dissolve an amount of Amphotericin B for Injection, equivalent to 50 mg (potency) of Amphotericin B according to the labeled amount, in 10 mL of water: the solution is clear and yellow to orange.

**Loss on drying** <2.41> Not more than 8.0% (0.3 g, in vacuum, 60°C, 3 hours).

**Bacterial endotoxins** <4.01> Less than 3.0 EU/mg (potency).

**Uniformity of dosage units** <6.02> It meets the requirements of the Mass variation test. However, use the average of the limits specified in the potency definition for \(T\).

**Foreign insoluble matter** <6.06> Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** <6.07> Perform the test according to Method 1: it meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, culture medium, preparation of cylinder-agar plate and standard solutions—Proceed as directed in the Assay under Amphotericin B.

(ii) Sample solutions—Prepare using light-resistant containers. Weigh accurately an amount of Amphotericin B for Injection, equivalent to about 50 mg (potency) according to the labeled amount, dissolve in dimethylsulfoxide to make exactly 50 mL, and use this solution as the sample stock solution. Measure exactly a suitable quantity of the sample
Amphotericin B Syrup

アムホテリシン B シロップ

Amphotericin B Syrup contain not less than 90.0% and not more than 115.0% of the labeled amount of amphotericin B (C_{47}H_{73}NO_{17}; 924.08).

Method of preparation  Prepare as directed under Syrup, with Amphotericin B.

Identification  To an amount of Amphotericin B Syrup, equivalent to 25 mg (potency) of Amphotericin B according to the labeled amount, add 5 mL of dimethylsulfoxide and 45 mL of methanol, and shake. To 1 mL of this solution add methanol to make 50 mL, and filter, if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 361 nm and 365 nm, between 380 nm and 384 nm and between 403 nm and 407 nm.

pH 〈2.54〉  5.0 – 7.0

Microbial limit 〈4.05〉  The acceptance criteria of TAMC and TYMC are 10^5 CFU/mL and 5 × 10^5 CFU/mL, respectively.

Assay  Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics (4.02) according to the following conditions.

(i)  Test organism, culture medium, preparation of cylinder-agar plate and standard solutions—Proceed as directed in the Assay under Amphotericin B.

(ii) Sample solutions—Prepare using light-resistant containers. Weigh accurately an amount of Amphotericin B Syrup, equivalent to about 0.1 g (potency) according to the labeled amount, add about 70 mL of dimethylsulfoxide, shake, then add dimethylsulfoxide to make exactly 100 mL, and use this solution as the sample stock solution. Measure exactly a suitable amount of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains about 200 μg (potency) and 50 μg (potency). Pipet 1 mL of each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage  Containers—Light-resistant, at a cold place.

Amphotericin B Tablets

アムホテリシン B 錠

Amphotericin B Tablets contain not less than 90.0% and not more than 120.0% of the labeled amount of amphotericin B (C_{47}H_{73}NO_{17}; 924.08).

Method of preparation  Prepare as directed under Tablets, with Amphotericin B.

Identification  To an amount of pulverized Amphotericin B Tablets, equivalent to 25 mg (potency) of Amphotericin B according to the labeled amount, add 5 mL of dimethylsulfoxide and 45 mL of methanol, and shake. To 1 mL of this solution add methanol to make 50 mL, and filter, if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 361 nm and 365 nm, between 380 nm and 384 nm and between 403 nm and 407 nm.

Uniformity of dosage units 〈6.02〉  It meets the requirement of the Mass variation test. However, use the average of the limits specified in the potency definition for T.

Loss on drying 〈2.41〉  Not more than 5.0% (0.3 g, in vacuum, 60°C, 3 hours).

Assay  Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics (4.02) according to the following conditions.

(i)  Test organism, culture medium, preparation of cylinder-agar plate and standard solutions—Proceed as directed in the Assay under Amphotericin B.

(ii) Sample solutions—Prepare using light-resistant containers. Weigh accurately and powder not less than 20 tablets of Amphotericin B Tablets. Weigh accurately a part of the powder, equivalent to about 0.1 g (potency) according to the labeled amount, add about 70 mL of dimethylsulfoxide, shake, then add dimethylsulfoxide to make exactly 100 mL, centrifuge, and use the supernatant liquid as the sample stock solution. Measure exactly a suitable amount of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage  Containers—Well-closed containers.
Anhydrous Ampicillin

Anhydrous Aminobenzylpenicillin

無水アンピシリン

\[
\begin{align*}
\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S} : 349.40 \\
(2S,5R,6R)-6-[(2R)-2\text{-Amino-2-phenylacetylamino}] &- \\
3,3\text{-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid} \\
[69-53-4]
\end{align*}
\]

Anhydrous Ampicillin contains not less than 960 µg (potency) and not more than 1005 µg (potency) per mg, calculated on the anhydrous basis. The potency of Anhydrous Ampicillin is expressed as mass (potency) of ampicillin (C\text{16}H\text{19}N\text{3}O\text{4}S).

**Description** Anhydrous Ampicillin occurs as white to light yellowish white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95%), and practically insoluble in acetonitrile.

**Identification** Determine the infrared absorption spectrum of Anhydrous Ampicillin as directed in the potassium bromide disk method under Infrared Spectrophotometry \(\text{\angle2.25}\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \(\text{\angle2.45}\) \([\alpha]_D^{\text{20}} + 280 - + 305^\circ\) (0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

**pH** \(\text{\angle2.45}\) The pH of a solution obtained by dissolving 1.0 g of Anhydrous Ampicillin in 100 mL of water is between 4.0 and 5.5.

**Purity (1)** Heavy metals \(\text{\angle1.07}\)—Proceed with 1.0 g of Anhydrous Ampicillin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \(\text{\angle1.11}\)—Prepare the test solution with 1.0 g of Anhydrous Ampicillin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Anhydrous Ampicillin in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography \(\text{\angle2.01}\) according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak other than ampicillin from the sample solution is not larger than the peak area of ampicillin from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: As long as about 10 times of the retention time of ampicillin.

**System suitability**—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 µL of this solution is equivalent to 7 to 13% of that from 10 µL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

**Water** \(\text{\angle2.45}\) Not more than 2.0% (2.5 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Anhydrous Ampicillin and Ampicillin RS, equivalent to about 50 mg (potency), add exactly 5 mL each of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography \(\text{\angle2.01}\) according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of ampicillin to that of the internal standard.

\[
\text{Amount \([\mu g \text{ (potency)}]\) of ampicillin (C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S})} = \frac{M_S \times Q_T}{Q_S} \times 1000
\]

**Containers and storage** Containers—Tight containers.
Ampicillin Hydrate
Aminobenzylpenicillin Hydrate
アンピシリン水和物

Ampicillin Hydrate contains not less than 960 μg (potency) and not more than 1005 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ampicillin Hydrate is expressed as mass (potency) of ampicillin (C₁₆H₁₉N₃O₄S: 349.40).

**Description** Ampicillin Hydrate occurs as a white to light yellowish white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

**Identification** Determine the infrared absorption spectrum of Ampicillin Hydrate as directed in the potassium bromide tablet, and compare the spectrum with the Reference Spectrum or the spectrum of Ampicillin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \[ \theta_d ^{25} + 280 - + 305^\circ \] (0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

**pH** \(< 2.5^\circ\) The pH of a solution obtained by dissolving 1.0 g of Ampicillin Hydrate in 400 mL of water is between 3.5 and 5.5.

**Purity**

(1) Heavy metals \(< l.0^\circ\)—Proceed with 1.0 g of Ampicillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \(< l.1^\circ\)—Prepare the test solution with 1.0 g of Ampicillin hydrate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Ampicillin hydrate in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(< 2.0^\circ\) according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than that of ampicillin obtained from the sample solution is not larger than the peak area of ampicillin from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 10 times as long as the retention time of ampicillin.

**System suitability**—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

(4) \( N,N \)-Dimethylaniline—Weigh accurately about 1 g of Ampicillin Hydrate, dissolve in 5 mL of sodium hydroxide TS, add exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand as the sample solution. Separately, weigh accurately about 50 mg of \( N,N \)-dimethylaniline, dissolve in 2 mL of hydrochloric acid and 20 mL of water, add water to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, and add water to make exactly 250 mL. Pipet 1 mL of this solution, add 5 mL of sodium hydroxide TS and exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography \(< 2.0^\circ\) according to the following conditions, calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of \( N,N \)-dimethylaniline to that of the internal standard, and calculate the amount of \( N,N \)-dimethylaniline by the following equation: not more than 20 ppm.

\[
\text{Amount (ppm) of } N,N\text{-dimethylaniline} = \frac{M_z}{M_t} \times \frac{Q_1}{Q_2} \times 400
\]

\( M_z \): Amount (g) of \( N,N \)-dimethylaniline

\( M_t \): Amount (g) of the sample

**Internal standard solution**—A solution of naphthalene in cyclohexane (1 in 20,000).

**Operating conditions**—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 2 m in length, packed with silicic earth for gas chromatography (180–250 μm in particle diameter) coated with 50% phenyl-50% methyl polysiloxane for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 120°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of \( N,N \)-dimethylaniline is about 5 minutes.

**System suitability**—

Test for required detectability: Measure exactly 1 mL of the standard stock solution, and add water to make exactly 250 mL. Pipet 1 mL of this solution, add 5 mL of sodium hydroxide TS and exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand for the test. Confirm that when the procedure is run with 1 μL of the upper layer liquid under the above operating conditions, the ratio of the peak area of \( N,N \)-dimethylaniline to that of the inter-
Ampicillin Sodium

Aminobenzylpenicillin Sodium

アンピシリンナトリウム

\[
\text{C}_{16}\text{H}_{18}\text{N}_{3}\text{NaO}_{4}\text{S} : 371.39 \\
\text{Monosodium (2S,5R,6R)-6-[(2R)-2-amino-2-phenylacetylaminol]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [69-52-3]}
\]

Ampicillin Sodium contains not less than 850 μg (potency) and not more than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ampicillin Sodium is expressed as mass (potency) of ampicillin (\(\text{C}_{16}\text{H}_{18}\text{N}_{3}\text{O}_{4}\text{S} : 349.40\)).

**Description**

Ampicillin Sodium occurs as white to light yellowish white, crystals or crystalline powder.

It is very soluble in water, and sparingly soluble in ethanol (99.5).

**Identification (1)**

Determine the infrared absorption spectrum of Ampicillin Sodium, previously dried in a desiccator (reduced pressure not exceeding 0.67 kPa, 60°C) for 3 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.01) according to the following conditions, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Ampicillin Sodium responds to the Qualitative Tests (1.09) (1) for sodium salt.

**Optical rotation**

\[ \alpha^\circ = +246 - +272^\circ \text{ (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).} \]

**pH**

The pH of a solution obtained by dissolving 1.0 g of Ampicillin Sodium in 10 mL of water is between 8.0 and 10.0.

**Purity (1)**

Clarity and color of solution—Dissolve 1.0 g of Ampicillin Sodium in 10 mL of water: the solution is clear and colorless or pale yellow.

(2) Heavy metals \(<1.07\) —Proceed with 1.0 g of Ampicillin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic \(<1.17\) —Prepare the test solution with 1.0 g of Ampicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Ampicillin Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine each peak area by the automatic integration method: the peak...
Ampicillin Sodium for Injection

Ampicillin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of ampicillin (C_{16}H_{19}N_{3}O_{4}S: 349.40).

Method of preparation
Prepare as directed under Injections, with Ampicillin Sodium.

Description
Ampicillin Sodium for Injection occurs as white to light yellowish white crystals or crystalline powder.

Identification
Proceed as directed in the Identification (1) under Ampicillin Sodium.

Osmotic pressure ratio
Being specified separately.

pH
The pH of a solution prepared by dissolving an amount of Ampicillin Sodium for Injection, equivalent to 1.0 g (potency) of Ampicillin Sodium according to the labeled amount, in 10 mL of water is 8.0 to 10.0.

Purity
Clarity and color of solution—Dissolve an amount of Ampicillin Sodium for Injection, equivalent to 0.25 g (potency) of Ampicillin Sodium according to the labeled amount, in 0.75 mL of water: the solution is clear. Perform the test with the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>:
the absorbance at 400 nm is not more than 0.40.

Water
Not more than 2.0% (0.2 g, volumetric titration, direct titration).

Bacterial endotoxins
Less than 0.075 EU/mg (potency).

Uniformity of dosage units
It meets the requirement of the Mass variation test.

Foreign insoluble matter
Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter
It meets the requirement.

Assay
Weigh accurately the mass of the contents of not less than 10 containers of Ampicillin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of Ampicillin Sodium, dissolve in 5 mL of the internal standard solution, and use this solution as the sample solution.

Sterility
Perform the test according to the Membrane filtration method: it meets the requirement.
Amyl Nitrite

C₅H₁₁NO₂: 117.15

Amyl Nitrite is the nitrous acid ester of 3-methylbutanol-1 and contains a small quantity of 2-methylbutanol-1 and the nitrous acid esters of other homologues.

It contains not less than 90.0% of C₅H₁₁NO₂.

Description Amyl Nitrite is a clear, light yellowish liquid, and has a characteristic, fruity odor.

It is miscible with ethanol (95), and with diethyl ether. It is practically insoluble in water. It is affected by light and by heat.

It is volatile at ordinary temperature and flammable even at a low temperature.

Boiling point: about 97°C

Identification Determine the infrared spectrum of Amyl Nitrite as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <2.56> d₅₀: 0.871 - 0.880

Purity (1) Acidity—To 5 mL of Amyl Nitrite add a mixture of 1.0 mL of 1 mol/L sodium hydroxide VS, 10 mL of water and 1 drop of phenolphthalein TS, shake, and allow to stand for 1 minute: the light red color of the water layer does not disappear.

(2) Water—Allow 2.0 mL of Amyl Nitrite to stand in ice water: no turbidity is produced.

(3) Aldehyde—To 3 mL of a mixture of equal volumes of silver nitrate TS and aldehyde free-ethanol add ammonia TS dropwise until the precipitate first formed is redissolved. Add 1.0 mL of Amyl Nitrite, and warm between 60°C and 70°C for 1 minute: a brown to black color is not produced.

(4) Residue on evaporation—Evaporate 10.0 mL of Amyl Nitrite on a water bath in a draft, carefully protecting from flame, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Assay Weigh accurately a volumetric flask containing 10 mL of ethanol (95), add about 0.5 g of Amyl Nitrite, and weigh accurately again. Add exactly 25 mL of 0.1 mol/L silver nitrate VS, then add 15 mL of potassium chlorate solution (1 in 20) and 10 mL of dilute nitric acid, stopper the flask immediately, and shake it vigorously for 5 minutes. Dilute with water to make exactly 100 mL, shake, and filter through dry filter paper. Discard the first 20 mL of the filtrate, measure exactly 50 mL of the subsequent filtrate, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS = 35.15 mg of C₅H₁₁NO₂

Containers and storage Containers—Hermetic containers. Not exceeding 10–ml capacity.

Storage—Light-resistant, in a cold place, and remote from fire.

Dental Antiformin

Dental Sodium Hypochlorite Solution

Dental Antiformin contains not less than 3.0 w/v% and not more than 6.0 w/v% of sodium hypochlorite (NaClO: 74.44).

Description Dental Antiformin is a slightly light yellow-green, clear liquid. It has a slight odor of chlorine.

It gradually changes by light.

Identification (1) Dental Antiformin changes red litmus paper to blue, and then decolorizes it.

(2) To Dental Antiformin add dilute hydrochloric acid: it evolves the odor of chlorine, and the gas changes potassium iodide starch paper moistened with water to blue.
Antipyrine / Official Monographs

(3) Dental Antiformin responds to the Qualitative Tests <1.09> (1) for sodium salt.

Assay Measure exactly 3 mL of Dental Antiformin in a glass-stoppered flask, add 50 mL of water, 2 g of potassium iodide and 10 mL of acetic acid (3L), and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS = 3.722 mg of NaClO

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 10°C.

Antipyrine

Phenazone

アンチピリン

C₁₁H₁₂N₂O: 188.23
1,5-Dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one [60-80-0]

Antipyrine, when dried, contains not less than 99.0% of C₁₁H₁₂N₂O.

Description Antipyrine occurs as colorless or white crystals, or a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in water, freely soluble in ethanol (95), and sparingly soluble in diethyl ether.

A solution of Antipyrine (1 in 10) is neutral.

Identification (1) To 5 mL of a solution of Antipyrine (1 in 100) add 2 drops of sodium nitrite TS and 1 mL of dilute sulfuric acid: a deep green color develops.

(2) To 2 mL of a solution of Antipyrine (1 in 100) add 4 drops of dilute iron (III) chloride TS: a yellow-red color develops. Then add 10 drops of dilute sulfuric acid: the color changes to light yellow.

(3) To 5 mL of a solution of Antipyrine (1 in 100) add 2 to 3 drops of tannic acid TS: a white precipitate is produced.

(4) To 0.1 g of Antipyrine add 0.1 g of vanillin, 5 mL of water and 2 mL of sulfuric acid, boil the mixture, and cool: a yellow-red precipitate is produced.

Melting point <2.60> 111 – 113°C

Purity (1) Chloride <1.07>—Perform the test with 1.0 g of Antipyrine. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Antipyrine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Readily carbonizable substances<1.15>—Perform the test with 0.5 g of Antipyrine: the solution remains colorless.

Loss on drying <2.41> Not more than 0.5% (1 g, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve about 0.2 g of Antipyrine, previously dried and accurately weighed, in 20 mL of sodium acetate TS, add exactly 30 mL of 0.05 mol/L iodine VS, and allow to stand for 20 minutes with occasional shaking. Dissolve the precipitate in 10 mL of chloroform, and titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 9.412 mg of C₁₁H₁₂N₂O

Containers and storage Containers—Well-closed containers.

Aprindine Hydrochloride

アプリンジン塩酸塩

C₂₂H₃₈N₂.HCl: 358.95
N-(2,3-Dihydro-1H-inden-2-yl)-N',N'-diethyl-N-phenylpropane-1,3-diamine monohydrochloride [33237-74-0]

Aprindine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of C₂₂H₃₈N₂.HCl.

Description Aprindine Hydrochloride occurs as a white to pale yellowish white crystalline powder. It has a bitter taste, numbing the tongue.

It is very soluble in water, in methanol and in acetic acid (100), and freely soluble in ethanol (99.5).

It gradually turns brown on exposure to light.

Identification (1) Dissolve 10 mg of Aprindine Hydrochloride in a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aprindine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Aprindine Hydrochloride (1 in 50) add 1 mL of dilute nitric acid: this solution responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Aprindine Hydrochloride in 50 mL of water: the pH of the solution is between 6.4 and 7.0.

Melting point <2.60> 127 – 131°C.
Purity (1) Clarity and color of solution—Dissolve 1.0 g of Aprindine Hydrochloride in 10 mL of methanol: the solution is clear, and its absorbance at 420 nm determined as directed under Ultraviolet-visible Spectrophotometry is not more than 0.10.

(2) Heavy metals. Proceed with 1.0 g of Aprindine Hydrochloride in the same manner, and make any necessary correction.

(3) Related substances. Dissolve 25 mg of Aprindine Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than aprindine obtained from the sample solution is not larger than 1/10 times the peak area of aprindine from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 3.40 g of potassium dihydrogen phosphate in 500 mL of water, and adjust the pH to 3.0 with hydrochloric acid. To 500 mL of this solution add 500 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of aprindine is about 6 minutes.
Time span of measurement: About 4 times as long as the retention time of aprindine.
System suitability—
Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of aprindine obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of aprindine are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aprindine is not more than 1.5%.

(4) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Aprindine Hydrochloride, previously dried, dissolve in 80 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 35.90 mg of C<sub>22</sub>H<sub>30</sub>N<sub>2</sub>HCl

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Aprindine Hydrochloride Capsules

Aprindine Hydrochloride Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of aprindine hydrochloride (C<sub>22</sub>H<sub>30</sub>N<sub>2</sub>HCl: 358.95).

Method of preparation Prepare as directed under Capsules, with Aprindine Hydrochloride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry, it exhibits maxima between 264 nm and 268 nm, and between 271 nm and 275 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Aprindine Hydrochloride Capsules, add 30 mL of a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125), shake vigorously for 20 minutes, add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly V mL so that each mL contains about 0.2 mg of aprindine hydrochloride (C<sub>22</sub>H<sub>30</sub>N<sub>2</sub>HCl), and filter. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Proceed as directed in the Assay.

Amount (mg) of aprindine hydrochloride (C<sub>22</sub>H<sub>30</sub>N<sub>2</sub>HCl) = M<sub>S</sub> × A<sub>S</sub>/A<sub>T</sub> × V/250

M<sub>S</sub>: Amount (mg) of aprindine hydrochloride for assay

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Aprindine Hydrochloride Capsules is not less than 80%.

Start the test with 1 capsule of Aprindine Hydrochloride Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 11 μg of aprindine hydrochloride (C<sub>22</sub>H<sub>30</sub>N<sub>2</sub>HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of aprindine hydrochloride for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A<sub>T</sub> and A<sub>S</sub>,
of aprindine in each solution.

Dissolution rate (%) with respect to the labeled amount of aprindine hydrochloride ($\text{C}_{22}\text{H}_{30}\text{N}_{2}\cdot\text{HCl}$)

$$M_S \times A_S / A_L \times V'/V \times 1/C \times 36$$

$M_S$: Amount (mg) of aprindine hydrochloride for assay

$C$: Labeled amount (mg) of aprindine hydrochloride ($\text{C}_{22}\text{H}_{30}\text{N}_{2}\cdot\text{HCl}$) in 1 capsule

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.40 g of potassium dihydrogen phosphate in 500 mL of water, and adjust the pH to 3.0 with hydrochloric acid. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of aprindine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of aprindine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aprindine is not more than 1.5%.

Assay

Take out the contents of not less than 20 Aprindine Hydrochloride Capsules, weigh accurately a portion of the powder, equivalent to about 0.1 g of aprindine hydrochloride ($\text{C}_{22}\text{H}_{30}\text{N}_{2}\cdot\text{HCl}$), add 60 mL of a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 100 mL. Pipet 10 mL of this solution, add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 50 mL, and filter, Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of aprindine hydrochloride for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 50 mL. Pipet 10 mL of this solution, add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 265 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of aprindine hydrochloride ($\text{C}_{22}\text{H}_{30}\text{N}_{2}\cdot\text{HCl}$)

$$M_S \times A_S / A_L \times 2$$

$M_S$: Amount (mg) of aprindine hydrochloride for assay

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.

Arbekacin Sulfate

アルベカシン硫酸塩

C$_{22}$H$_{44}$N$_6$O$_{10}$·xH$_2$SO$_4$ (x = 2 - 2½)

3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-[2,6-diamino-2,3,4,6-tetraoxo-α-D-erythro-hexopyranosyl-(1→4)]-1-N-[2(S)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine sulfate [51025-85-5, Arbekacin]

Arbekacin Sulfate is the sulfate of a derivative of dibekacin. It contains not less than 670 μg (potency) and not more than 750 μg (potency) per mg, calculated on the dried basis. The potency of Arbekacin Sulfate is expressed as mass (potency) of arbekacin ($\text{C}_{22}\text{H}_{44}\text{N}_{6}\text{O}_{10}$: 552.62).

Description

Arbekacin Sulfate occurs as a white powder. It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification

(1) Dissolve 10 mg each of Arbekacin Sulfate and Arbekacin Sulfate RS in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28), methanol, chloroform and ethanol (95) (7:6:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the principal spot obtained from the sample solution and the spot from the standard solution are purple-brown in color and their $R_f$ values are the same.

(2) A solution of Arbekacin Sulfate (1 in 50) responds to the Qualitative Tests <1.099> (1) for sulfate.

Optical rotation <2.49> $[\alpha]_D^2$: +69 - 79° (0.25 g after drying, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.75 g of Arbekacin Sulfate in 10 mL of water is between 6.0 and 8.0.

Purity

(1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Arbekacin Sulfate in 5 mL of water is clear and colorless.
(2) Heavy metals <0.07—Proceed with 2.0 g of Arbekacin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arbekacin—Weigh accurately about 20 mg of Arbekacin Sulfate, add exactly 10 mL of the internal standard solution to dissolve, add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Arbekacin Sulfate RS, equivalent to about 10 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the standard solution. Perform the test with 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of arbekacin to that of the internal standard. Calculate the amount of arbekacin by the following equation: not more than 2.0%.

\[
\text{Amount (mg) of arbekacin} = \frac{M_s}{M_f} \times \frac{Q_1}{Q_2} \times 1/10 \times 100
\]

\(M_s\): Amount (mg (potency)) of Arbekacin Sulfate RS
\(M_f\): Amount (mg) of the sample

Internal standard solution—A solution of becanamycin sulfate (1 in 2000).

Operating conditions—
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Reaction coil: A column about 0.3 mm in inside diameter and about 3 m in length.
Reaction coil temperature: A constant temperature of about 30°C.
Mobile phase: Dissolve 8.70 g of sodium 1-pentane sulfonate and 8.52 g of anhydrous sodium sulfate in 980 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 230 mL of this solution add 20 mL of methanol.
Reagent: Dissolve 12.36 g of boric acid in 960 mL of water, add 10 mL of a solution of o-phthalaldehyde in ethanol (99.5) (1 in 25), adjust the pH to 10.5 with 8 mol/L potassium hydroxide TS, and add water to make 1000 mL. To this solution add 1 mL of 2-mercaptoethanol.
Reaction temperature: A constant temperature of about 50°C.
Flow rate of the mobile phase: 0.5 mL per minute.
Flow rate of the reagent: 1 mL per minute.

System suitability—
System performance: Dissolve 20 mg each of Arbekacin Sulfate, becanamycin sulfate and dibekacin sulfate in 200 mL of water. When the procedure is run with 5 µL of this solution under the above operating conditions, becanamycin, arbekacin and dibekacin are eluted in this order, and the resolution between the peaks, becanamycin and arbekacin is not less than 5 and arbekacin and dibekacin is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dibekacin to that of the internal standard is not more than 2.0%.

(4) Related substances—Dissolve 20 mg of Arbekacin Sulfate in 200 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the area of each peak by the automatic integration method: the total area of the peaks other than arbekacin and dibekacin obtained from the sample solution is not larger than the peak area of arbekacin from the standard solution.

Operating conditions—
Detector, column, column temperature, reaction coil, reaction coil temperature, mobile phase, reagent, reaction temperature, flow rate of mobile phase, and flow rate of reagent: Proceed as directed in the operating conditions in the Purity (3).

Time span of measurement: About 1.5 times as long as the retention time of arbekacin.

System suitability—
System performance: Dissolve 10 mg each of Arbekacin Sulfate and dibekacin sulfate in 200 mL of water. When the procedure is run with 5 µL of this solution under the above operating conditions, arbekacin and dibekacin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of arbekacin is not more than 5.0%.

Loss on drying 2.41—Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay—Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics 4.02 according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633
(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer, having pH 7.8 – 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Arbekacin Sulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the stock solution in a refrigerator and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 µg (potency) and 5 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Arbekacin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 µg (potency) and 5 µg (potency), and use these
solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage  Containers—Tight containers.

### Arbekacin Sulfate Injection

アルベカシン硫酸塩注射液

Arbekacin Sulfate Injection is an aqueous injection. It contains not less than 90.0% and not more than 110.0% of the labeled amount of arbekacin sulfate (C_{22}H_{44}N_{6}O_{10}: 552.62).

**Method of preparation**  Prepare as directed under Injections, with Arbekacin Sulfate.

**Description**  Arbekacin Sulfate Injection occurs as a clear and colorless liquid.

**Identification**  To 0.2 mL of Arbekacin Sulfate Injection add 1 mL of water, and use this solution as the sample solution. Separately, dissolve 10 mg of Arbekacin Sulfate RS in 1 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 2 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia solution (28), methanol, chloroform and ethanol (95) (7:6:4:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 80°C for 10 minutes: the principal spot with the sample solution and the spot with the standard solution show a purple-brown color and the same spot with the sample solution and the spot with the standard solution show a violet-blue color. It is gradually decomposed on exposure to light.

**Osmotic pressure ratio**  <2.47> 0.8 – 1.2 (for the preparation intended for intramuscular use).

**pH**  <2.54> 6.0 – 8.0

**Extractable volume**  <6.05> It meets the requirement.

**Foreign insoluble matter**  <6.05> Perform the test according to the Method 1: it meets the requirement.

**Insoluble particulate matter**  <6.07> Perform the test according to the Method 1: it meets the requirement.

**Sterility**  <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay**  Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, Culture medium and Standard solutions: Proceed as directed in the Assay under Arbekacin Sulfate.

(ii) Sample solutions—Take exactly a volume of Arbekacin Sulfate Injection, equivalent to about 20 mg (potency), and add water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage  Containers—Hermetic containers.

### Argatroban Hydrate

アルガトロバン水和物

Argatroban Hydrate occurs as white crystals or crystalline powder. It has a bitter taste. It is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Argatroban Hydrate contains not less than 98.5% and not more than 101.0% of argatroban (C_{23}H_{36}N_{6}O_{5}S: 508.63), calculated on the anhydrous basis.

**Description**  Argatroban Hydrate occurs as white crystals or crystalline powder. It has a bitter taste.

**Extractable volume**  <2.47> 6.0 – 8.0

**Related substance 1—Dissolve 50 mg of Arbekacin Sulfate Injection, equivalent to about 20 mg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Assay**  Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, Culture medium and Standard solutions: Proceed as directed in the Assay under Arbekacin Sulfate.

(ii) Sample solutions—Take exactly a volume of Arbekacin Sulfate Injection, equivalent to about 20 mg (potency), and add water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage  Containers—Hermetic containers.

### Arbekacin Sulfate Injection

アルベカシン硫酸塩注射液

Arbekacin Sulfate Injection is an aqueous injection. It contains not less than 90.0% and not more than 110.0% of the labeled amount of arbekacin sulfate (C_{22}H_{44}N_{6}O_{10}: 552.62).

**Method of preparation**  Prepare as directed under Injections, with Arbekacin Sulfate.

**Description**  Arbekacin Sulfate Injection occurs as a clear and colorless liquid.

**Identification**  To 0.2 mL of Arbekacin Sulfate Injection add 1 mL of water, and use this solution as the sample solution. Separately, dissolve 10 mg of Arbekacin Sulfate RS in 1 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 2 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia solution (28), methanol, chloroform and ethanol (95) (7:6:4:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 80°C for 10 minutes: the principal spot with the sample solution and the spot with the standard solution show a purple-brown color and the same spot with the sample solution and the spot with the standard solution show a violet-blue color. It is gradually decomposed on exposure to light.

**Osmotic pressure ratio**  <2.47> 0.8 – 1.2 (for the preparation intended for intramuscular use).

**pH**  <2.54> 6.0 – 8.0

**Extractable volume**  <6.05> It meets the requirement.

**Foreign insoluble matter**  <6.05> Perform the test according to the Method 1: it meets the requirement.

**Insoluble particulate matter**  <6.07> Perform the test according to the Method 1: it meets the requirement.

**Sterility**  <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay**  Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, Culture medium and Standard solutions: Proceed as directed in the Assay under Arbekacin Sulfate.

(ii) Sample solutions—Take exactly a volume of Arbekacin Sulfate Injection, equivalent to about 20 mg (potency), and add water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage  Containers—Hermetic containers.

### Argatroban Hydrate

アルガトロバン水和物

Argatroban Hydrate occurs as white crystals or crystalline powder. It has a bitter taste. It is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Argatroban Hydrate contains not less than 98.5% and not more than 101.0% of argatroban (C_{23}H_{36}N_{6}O_{5}S: 508.63), calculated on the anhydrous basis.

**Description**  Argatroban Hydrate occurs as white crystals or crystalline powder. It has a bitter taste.

**Extractable volume**  <2.47> 6.0 – 8.0

**Related substance 1—Dissolve 50 mg of Arbekacin Sulfate Injection, equivalent to about 20 mg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Assay**  Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, Culture medium and Standard solutions: Proceed as directed in the Assay under Arbekacin Sulfate.

(ii) Sample solutions—Take exactly a volume of Arbekacin Sulfate Injection, equivalent to about 20 mg (potency), and add water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage  Containers—Hermetic containers.
Hydrate in 40 mL of methanol, add water to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography \(\text{<2.07>}\) according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak other than argatroban is not more than 0.1%.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase A: To 2.5 mL of acetic acid (100) add water to make 1000 mL, and adjust the pH to 5.0 with ammonia TS. To 500 mL of this solution add 500 mL of methanol.

Mobile phase B: To 2.5 mL of acetic acid (100) add water to make 1000 mL, and adjust the pH to 5.0 with ammonia TS. To 200 mL of this solution add 800 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5 - 35</td>
<td>100 → 5</td>
<td>0 → 95</td>
</tr>
</tbody>
</table>

Flow rate: About 1.0 mL per minute.

Time span of measurement: About 1.5 times as long as the retention time of argatroban beginning after the solvent peak.

**System suitability—**

Test for required detectability: To 1 mL of the sample solution add the mobile phase A to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of argatroban obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: Dissolve 5 mg of Argatroban Hydrate and 5 μL of methyl benzoate in 40 mL of methanol, and add water to make 100 mL. To 5 mL of this solution add 40 mL of methanol and water to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl benzoate and argatroban are eluted in this order with the resolution between these peaks other than argatroban is not more than 0.1.

**Residue on ignition** \(\text{<2.44>}\) No more than 0.1% (1 g).

**Isomer ratio** Dissolve 50 mg of Argatroban Hydrate in 50 mL of methanol, add water to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography \(\text{<2.07>}\) according to the following conditions. Determine the areas of two adjacent peaks, \(A_a\) and \(A_b\), having the retention times of about 40 minutes, where \(A_a\) is the peak area of shorter retention time and \(A_b\) is the peak area of longer retention time: \(A_b/(A_a + A_b)\) is between 0.30 and 0.40.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 500 mL of water add 500 mL of methanol, 13 mL of diluted 40% tetrabutylammonium hydroxide TS (1 in 4) and 0.68 mL of phosphoric acid, and adjust the pH to 6.8 with ammonia TS and diluted ammonia solution (28) (1 in 20).

Flow rate: Adjust the flow rate so that the retention time of the peak having the shorter retention time of the two peaks of argatroban is about 40 minutes.

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the resolution between the two peaks is not less than 1.2.

System repeatability: When the test is repeated 6 times with 10 μL of the sample solution under the above operating conditions, the relative standard deviation of the total area of the two separate peaks of argatroban is not more than 2.0%.

**Assay** Weigh accurately about 0.5 g of Argatroban Hydrate, dissolve in 20 mL of acetic acid for nonaqueous titration, add 40 mL of acetone for nonaqueous titration, and titrate \(\text{<2.50>}\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 50.86 mg of \(\text{C}_2\text{H}_4\text{N}_2\text{O}_7\).

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.
L-Arginine

L-アルギニン

\[
\begin{align*}
\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2 & : 174.20 \\
(2S)-2\text{-Amino-5-guanidinopentanoic acid} & [74-79-3]
\end{align*}
\]

L-Arginine, when dried, contains not less than 98.5% and not more than 101.0% of C₆H₁₄N₄O₂.

**Description** L-Arginine occurs as white crystals or crystalline powder. It has a characteristic odor.

- It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95%).
- It dissolves in dilute hydrochloric acid.
- It is hygroscopic.

**Identification** Determine the infrared absorption spectrum of previously dried L-Arginine as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** 2.49 \([\alpha]_D^0: +26.9\) – +27.9° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** 2.25 – 4.5. The pH of a solution prepared by dissolving 1 g of L-Arginine in 10 mL of water is between 10.5 and 12.0.

**Purity** (1) Clarity and color of solution—A solution obtained by dissolving 1 g of L-Arginine in 10 mL of water is clear and colorless.

- Chloride 1.0%—Perform the test with 0.5 g of L-Arginine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).
- Sulfate 1.10%—Perform the test with 0.6 g of L-Arginine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).
- Ammonium 1.02%—Perform the test with 0.25 g of L-Arginine. Use the distillation under reduced pressure.
- Heavy metals 1.07%—Dissolve 2.0 g of L-Arginine in 30 mL of water, add 1 drop of phenolphthalein TS, neutralize with dilute hydrochloric acid, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
- Iron 1.10%—Prepare the test solution with 1.0 g of L-Arginine according to Method 1, and perform the test using Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).
- Related substances—Dissolve 0.10 g of L-Arginine in 10 mL of water, and use this solution as the sample solution.

Purity (2) Dissolve 1.0 g of L-Arginine in 10 mL of water: the pH of this solution is between 4.7 and 6.2.

**Assay** Weigh accurately about 80 mg of L-Arginine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate 2.5° with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 8.710 mg of C₆H₁₄N₄O₂

**Containers and storage** Containers—Tight containers.

L-Arginine Hydrochloride

L-アルギニン塩酸塩

\[
\begin{align*}
\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2\text{·HCl} & : 210.66 \\
(2S)-2\text{-Amino-5-guanidinopentanoic acid monohydrochloride} & [1119-34-2]
\end{align*}
\]

L-Arginine Hydrochloride, when dried, contains not less than 98.5% of C₆H₁₄N₄O₂·HCl.

**Description** L-Arginine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a slight, characteristic taste.

- It is freely soluble in water and in formic acid, and very slightly soluble in ethanol (95%)

**Identification** (1) Determine the infrared absorption spectrum of L-Arginine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

- A solution of L-Arginine Hydrochloride (1 in 50) responds to the Qualitative Tests 1.0% for chloride.

**Optical rotation** 2.49 \([\alpha]_D^0: +21.5\) – +23.5° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** 2.54 – 4.5. Dissolve 1.0 g of L-Arginine Hydrochloride in 10 mL of water: the pH of this solution is between 4.7 and 6.2.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of L-Arginine Hydrochloride in 10 mL of water: the solution is clear and colorless.
L-Arginine Hydrochloride Injection

L-アルギニン塩酸塩注射液

L-Arginine Hydrochloride Injection is an aqueous solution for injection. It contains not less than 9.5 w/v% and not more than 10.5 w/v% of L-arginine hydrochloride (C₆H₁₄N₄O₂.HCl: 210.66).

Method of preparation

| L-Arginine Hydrochloride | 100 g |
| Water for Injection or Sterile Water | a sufficient quantity |

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

Description

L-Arginine Hydrochloride Injection is a clear, colorless liquid.

Identification (1)

To 5 mL of a solution of L-Arginine Hydrochloride Injection (1 in 100) add 1 mL of ninhydrin TS, and heat for 3 minutes: a blue-purple color develops.

(2) To 5 mL of a solution of L-Arginine Hydrochloride Injection (1 in 10) add 2 mL of sodium hydroxide TS and 1 to 2 drops of a solution of 1-naphthol in ethanol (95) (1 in 1000), allow to stand for 5 minutes, and add 1 to 2 drops of sodium hypochlorite TS: a red-orange color develops.

pH<2.54> 5.0–6.0

Bacterial endotoxins<4.01> Less than 0.50 EU/mL.

Extractable volume<6.05> It meets the requirement.

Foreign insoluble matter<6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter<6.07> It meets the requirement.

Sterility<4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay

Pipet 20 mL of L-Arginine Hydrochloride Injection, add 7.5 mol/L hydrochloric acid AS, and heat on a water bath for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate<2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 10.53 mg of C₆H₁₄N₄O₂·HCl

Amount (mg) of L-arginine hydrochloride (C₆H₁₄N₄O₂·HCl) = α₀ × 4444

Containers and storage Containers—Hermetic containers.
Arotinolol Hydrochloride

アロチノロール塩酸塩

C₁₅H₂₁N₃O₄S₃·HCl: 408.00
5-[2-[(2RS)-3-(1,1-Dimethylthyl)amino-2-hydroxypropylsulfanyl]-1,3-thiazol-4-yl]thiophene-2-carboxamide monohydrochloride

Arotinolol Hydrochloride, when dried, contains not less than 99.0% of C₁₅H₂₁N₃O₄S₃·HCl.

Description Arotinolol Hydrochloride occurs as a white to light yellow crystalline powder.

It is freely soluble in dimethylsulfoxide, slightly soluble in methanol and in water, very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

A solution of Arotinolol Hydrochloride in methanol (1 in 125) does not show optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Arotinolol Hydrochloride in methanol (1 in 75,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Arotinolol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectroscopy \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Arotinolol Hydrochloride (1 in 200) responds to the Qualitative Tests \(<1.09\) (2) for chloride.

Purity (1) Heavy metals \(<1.07\rangle—\)Proceed with 1.0 g of Arotinolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.05 g of Arotinolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.07\rangle\). Spot 40 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28:30:10:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying \(<2.44\rangle—\)Not more than 0.20% (1 g, in vacuum, 105°C, 4 hours).

Residue on ignition \(<2.44\rangle—\)Not more than 0.1% (1 g).

Assay Weigh accurately about 1.5 g of Arotinolol Hydrochloride, previously dried, dissolve in dimethylsulfoxide to make exactly 25 mL. Pipet 5 mL of this solution, add 100 mL of water and 5 mL of sodium hydroxide TS, and extract with three 50-mL portions of dichloromethane. Filter each dichloromethane extract through a pledget of absorbent cotton with anhydrous sodium sulfate on it. Evaporate combined filtrate to dryness in vacuum. Dissolve the residue in 70 mL of acetic acid (100), and titrate \(<2.50\rangle\) with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 20.40 mg of C₁₅H₂₁N₃O₄S₃·HCl

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Arsenical Paste

亜ヒ酸パスタ

Arsenical Paste contains not less than 36.0% and not more than 44.0% of arsenic (III) trioxide (As₂O₃: 197.84).

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic Trioxide, finely powdered</td>
<td>40 g</td>
</tr>
<tr>
<td>Procaine Hydrochloride, finely powdered</td>
<td>10 g</td>
</tr>
<tr>
<td>Hydrophilic Cream</td>
<td>30 g</td>
</tr>
<tr>
<td>Clove Oil</td>
<td>a suitable quantity</td>
</tr>
<tr>
<td>Medicinal Carbon</td>
<td>a suitable quantity</td>
</tr>
</tbody>
</table>

To make 100 g

Mix Arsenic Trioxide and Procaine Hydrochloride with Hydrophilic Cream, and add Clove Oil to make a suitably viscous liquid, followed by Medicinal Carbon for coloring.

Description Arsenical Paste is grayish black and has the odor of clove oil.

Identification (1) Place 0.1 g of Arsenical Paste in a small flask, add 5 mL of fuming nitric acid and 5 mL of sulfuric acid, and heat over a flame until the reacting liquid becomes colorless and white fumes begin to evolve. After cooling, add the reacting liquid to 20 mL of water cautiously, and add 10 mL of hydrogen sulfide TS while warming: a yellow precipitate is produced (arsenic (III) trioxide).

(2) Shake thoroughly 0.5 g of Arsenical Paste with 25 mL of diethyl ether, 5 mL of dilute hydrochloric acid and 20 mL of water, separate the water layer, and filter: 5 mL of the filtrate responds to the Qualitative Tests \(<1.09\) for primary aromatic amines (procaine hydrochloride).

(3) Shake thoroughly 0.5 g of Arsenical Paste with 25 mL of diethyl ether and 25 mL of water, separate the water layer, filter, and use the filtrate as the sample solution. Dissolve 0.01 g of procaine hydrochloride in 5 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.07\)\).

Spot 5 μL each of the sample solution and
standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 mm): the spots from the sample solution and standard solution exhibit the same Rf value.

**Assay**

Weigh accurately about 0.3 g of Arsenical Paste into a 150-mL Kjeldahl flask, add 5 mL of fuming nitric acid and 10 mL of sulfuric acid, and shake thoroughly. Heat cautiously the mixture, gently at first, and then continue strong heating, until red fumes of nitrogen oxide are sparingly evolved. After cooling, add 5 mL of fuming nitric acid, heat again until red fumes of nitrogen oxide are no longer evolved and the reacting liquid becomes clear, and cool. Add 30 mL of a saturated solution of ammonium oxalate monohydrate, heat again until white fumes of sulfuric acid are evolved, and continue the heating for 10 minutes. Decompose completely oxalic acid, cool, transfer cautiously the colorless reacting liquid to a glass-stoppered flask, containing 40 mL of water. Wash thoroughly the Kjeldahl flask with 60 mL of water, add the washings to the content of the glass-stoppered flask, and cool. Dissolve 3 g of potassium iodide in this solution, allow to stand in a dark place at room temperature for 45 minutes, and titrate $< 2.50$ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 5 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS = 4.946 mg of As$_2$O$_3$

**Containers and storage** Containers—Tight containers.

### Arsenic Trioxide

 arsenic trioxide

As$_2$O$_3$: 197.84

Arsenic Trioxide, when dried, contains not less than 99.5% of As$_2$O$_3$.

**Description**

Arsenic Trioxide occurs as a white powder. It is odorless. It is practically insoluble in water, in ethanol (95), and in diethyl ether.

It dissolves in sodium hydroxide TS.

**Identification**

Dissolve 0.2 g of Arsenic Trioxide in 40 mL of water by heating on a water bath: the solution responds to the Qualitative Tests $< 1.09$ for arsenite.

**Purity**

Clarity of solution—To 1.0 g of Arsenic Trioxide add 10 mL of ammonia TS, and heat gently: the solution is clear.

**Loss on drying** $< 2.41$ Not more than 0.5% (1 g, 105°C, 3 hours). 

**Assay**

Weigh accurately about 0.15 g of Arsenic Trioxide, previously dried, dissolve in 20 mL of a solution of sodium hydroxide (1 in 25), by warming, if necessary. Add 40 mL of water and 2 drops of methyl orange TS, then add dilute hydrochloric acid until the color of the solution becomes light red. Add 2 g of sodium hydrogen carbonate and 50 mL of water to this solution, and titrate $< 2.50$ with 0.05 mol/L iodine VS (indicator: 3 mL of starch TS).

Each mL of 0.05 mol/L iodine VS = 4.946 mg of As$_2$O$_3$

**Containers and storage** Containers—Tight containers.

### Ascorbic Acid

**Vitamin C**

アスコルビン酸

Ascorbic Acid, when dried, contains not less than 99.0% of L-ascorbic acid (C$_6$H$_8$O$_6$).

**Description**

Ascorbic Acid occurs as white crystals or a white, crystalline powder. It is odorless, and has an acid taste.

It is freely soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 190°C (with decomposition).

**Identification**

(1) To 5 mL each of a solution of Ascorbic Acid (1 in 50) add 1 drop of potassium permanganate TS or 1 drop of a solution of copper (II) sulfate pentahydrate (1 in 1000) and 1 drop of pyrrole, and warm the mixture at 50°C for 5 minutes: a blue color develops.

Optical rotation $< 2.49$ $[\alpha]_D^{20}: +20.5 – +21.5°$ (2.5 g, water, 25 mL, 100 mm).

pH $< 2.50$ Dissolve 1.0 g of Ascorbic Acid in 20 mL of water: the pH of this solution is between 2.2 and 2.5.

**Purity**

Clarity and color of solution—Dissolve 1.0 g of Ascorbic Acid in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals $< 1.07$—Perform the test with 1.0 g of Ascorbic Acid according to Method 1. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying $< 2.41$ Not more than 0.20% (1 g, silica gel, 24 hours).

Residue on ignition $< 2.44$ Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.2 g of Ascorbic Acid,
Ascorbic Acid Injection

Vitamin C Injection

Ascorbic Acid Injection is an aqueous solution for injection. It contains not less than 95.0% and not more than 115.0% of the labeled amount of L-ascorbic acid (C₆H₈O₆: 176.12).

Method of preparation  Prepare as directed under injections, with the sodium salt of Ascorbic Acid.

Description  Ascorbic Acid Injection occurs as a clear, colorless liquid.

Identification (1)  Measure a volume of Ascorbic Acid Injection, equivalent to 0.5 g of Ascorbic Acid according to the labeled amount, and add water to make 25 mL. Proceed with 5 mL each of the solution as directed in the Identification (1) under Ascorbic Acid.

(2) Measure a volume of Ascorbic Acid Injection, equivalent to 5 mg of Ascorbic Acid according to the labeled amount. Add a solution of metaphosphoric acid (1 in 50) to make 5 mL, and proceed with this solution as directed in the Identification (2) under Ascorbic Acid.

(3) Ascorbic Acid Injection responds to the Qualitative Tests (1) for sodium salt.

pH  2.54  5.6 – 7.4

Bacterial endotoxins  0.01  Less than 0.15 EU/mg.

Extractable volume  0.05  It meets requirement.

Foreign insoluble matter  0.06  Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter  0.07  It meets the requirement.

Sterility  0.06  Perform the test according to the Membrane filtration method: it meets the requirement.

Assay  Measure exactly a volume of Ascorbic Acid Injection, equivalent to about 0.1 g of L-ascorbic acid (C₆H₈O₆), previously diluted with metaphosphoric acid-acetic acid TS, if necessary, and add metaphosphoric acid-acetic acid TS to make exactly 200 mL. Measure exactly 2 mL of the solution, and shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS. Titrate 2.50 with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction.

Each mL of 2, 6-dichlorophenol-indophenol sodium TS for titration:

\[ A \text{ mg} \text{ of } \text{L-ascorbic acid (C}_6\text{H}_8\text{O}_6) \]

A is decided by the following standardization of 2,6-dichloroindophenol sodium TS for titration:

2,6-Dichlorophenol-indophenol sodium TS for titration:

Preparation  —Dissolve 42 mg of sodium hydrogen carbonate in 50 mL of water, add 0.5 g of 2,6-dichloroindophenol sodium dihydrate and water to make 200 mL, and filter. Prepare before use.

Standardization  —Weigh accurately about 50 mg of Ascorbic Acid RS, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in metaphosphoric acid-acetic acid TS to make exactly 100 mL. Pipet 2 mL of this solution, shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS, and titrate 2.50 with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction. Calculate the quantity (A mg) of L-ascorbic acid (C₆H₈O₆) equivalent to 1 mL of this test solution.

Containers and storage  Containers—Hermetic containers.

Storage—Under nitrogen atmosphere.

Ascorbic Acid Powder

Vitamin C Powder

Ascorbic Acid Powder contains not less than 95.0% and not more than 120.0% of the labeled amount of L-ascorbic acid (C₆H₈O₆: 176.12).

Method of preparation  Prepare as directed under Granules or Powders, with Ascorbic Acid.

Identification (1)  Weigh a portion of Ascorbic Acid Powder, equivalent to 0.5 g of Ascorbic Acid according to the labeled amount, add 30 mL of water, shake for 1 minute, and filter. Proceed with 5 mL each of the filtrate as directed in the Identification (1) under Ascorbic Acid.

(2) Weigh a portion of Ascorbic Acid Powder, equivalent to about 0.1 g of Ascorbic Acid according to the labeled amount, add 10 mL of a solution of metaphosphoric acid-acetic acid TS, shake for 1 minute, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (2) under Ascorbic Acid.

Purity  Rancidity—Ascorbic Acid Powder is free from any unpleasant or rancid odor and taste.

Assay  Weigh accurately a portion of Ascorbic Acid Powder, equivalent to about 0.1 g of L-ascorbic acid (C₆H₈O₆) according to the labeled amount, extract with several successive portions of metaphosphoric acid-acetic acid TS, combine the extracts, and filter. Wash the residue with metaphosphoric acid-acetic acid TS. Combine the filtrates and washings, and add metaphosphoric acid-acetic acid to make exactly 200 mL. Pipet 2 mL of the solution, and shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS. Titrate 2.50 with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction.

Each mL of 2, 6-dichlorophenol-indophenol sodium TS for titration:

\[ A \text{ mg} \text{ of } \text{L-ascorbic acid (C}_6\text{H}_8\text{O}_6) \]

A is decided by the following standardization of 2,6-dichloroindophenol sodium TS for titration:

2,6-Dichlorophenol-indophenol sodium TS for titration:

Preparation  —Dissolve 42 mg of sodium hydrogen carbonate in 50 mL of water, add 0.5 g of 2,6-dichloroindophenol sodium dihydrate and water to make 200 mL, and filter. Prepare before use.

Standardization  —Weigh accurately about 50 mg of Ascorbic Acid RS, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in metaphosphoric acid-acetic acid TS to make exactly 100 mL. Pipet 2 mL of this solution, shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS, and titrate 2.50 with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction. Calculate the quantity (A mg) of L-ascorbic acid (C₆H₈O₆) equivalent to 1 mL of this test solution.

Containers and storage  Containers—Hermetic containers.

Storage—Under nitrogen atmosphere.
dichlorophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction.

Each mL of 2,6-dichlorophenol-indophenol sodium TS for titration = A mg of C₆H₈O₆

A is decided by the following standardization of 2,6-dichlorophenoldisodium TS for titration:

Preparation—Dissolve 42 mg of sodium hydrogen carbonate in 50 mL of water, add 0.05 g of 2,6-dichlorophenoldisodium dihydrate and water to make 200 mL, and filter. Prepare before use.

Standardization—Weigh accurately about 50 mg of Ascorbic Acid RS, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in metaphosphoric acid-acetic acid TS to make exactly 100 mL. Pipet 2 mL of this solution, shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS, and titrate with 2,6-dichlorophenoldisodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction. Calculate the quantity (A mg) of L-ascorbic acid (C₆H₈O₆) equivalent to 1 mL of this test solution.

Containers and storage Containers—Tight containers.

L-Aspartic Acid

L-アスパラギン酸

C₆H₇NO₄: 133.10
(2S)-2-Aminobutanedioic acid [56-84-8]

L-Aspartic Acid, when dried, contains not less than 98.5% and not more than 101.0% of C₆H₇NO₄.

Description L-Aspartic Acid occurs as white, crystals or crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid and in 0.2 mol/L sodium hydroxide TS.

Identification Determine the infrared absorption spectrum of L-Aspartic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D: +24.0° to +26.0° (2 g, after drying, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 0.4 g of L-Aspartic Acid in 100 mL of water by warming, and allow to cool: between 2.5 and 3.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Aspartic Acid in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of L-Aspartic Acid in 6 mL of dilute nitric acid and 20 mL of water, add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.6 g of L-Aspartic Acid in 5 mL of dilute hydrochloric acid and 30 mL of water, add water to make 45 mL, and add 5 mL of barium chloride TS. Perform the test with this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS, add 5 mL of dilute hydrochloric acid and water to make 45 mL, and add 5 mL of barium chloride (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Aspartic Acid. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Aspartic Acid according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <1.10>—Prepare the test solution with 1.0 g of L-Aspartic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.20 g of L-Aspartic Acid in 10 mL of 0.2 mol/L sodium hydroxide TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100),Request rewriting of content here and heat at 80°C for 10 minutes: the spot other than the principal spot is not more intense than the spot obtained with the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of L-Aspartic Acid, previously dried, dissolve in 50 mL of water by warming. After cooling, titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 13.31 mg of C₆H₇NO₄

Containers and storage Containers—Tight containers.
Aspirin

Acetylsalicylic Acid

**Aspirin**

**Acetylsalicylic Acid**

Aspirin occurs as white crystals, granules or powder. It is odorless, and has a slight acid taste.

It is freely soluble in ethanol (95) and in acetone, soluble in diethyl ether, and slightly soluble in water.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

In moist air, it gradually hydrolyzes to salicylic acid and acetic acid.

Melting point: about 136°C (bath fluid is heated at 130°C previously).

**Identification** (1) Boil 0.1 g of Aspirin in 5 mL of water for 5 to 6 minutes, cool, and add 1 to 2 drops of iron (III) chloride TS: a red-purple color is produced.

(2) Boil 0.5 g of Aspirin in 10 mL of sodium carbonate TS for 5 minutes, and add 10 mL of dilute sulfuric acid: the odor of acetic acid is perceptible, and a white precipitate is produced. Filter the precipitate, add 3 mL of ethanol (95) and 3 mL of sulfuric acid to the filtrate, and heat: the odor of ethyl acetate is perceptible.

**Purity** (1) Clarity of solution—Dissolve 0.5 g of Aspirin in 10 mL of warm sodium carbonate TS: the solution is clear.

(2) Salicylic acid—Dissolve 2.5 g of Aspirin in 25 mL of ethanol (95), and add 1.0 mL of this solution to a solution which is prepared by transferring 1 mL of a freshly prepared dilute ammonium iron (III) chloride TS to the filtrate: a red-violet color develops.

**Assay**

Weigh accurately about 1.5 g of Aspirin, previously dried, add exactly 50 mL of 0.5 mol/L sodium hydroxide VS, and boil gently for 10 minutes under a reflux condenser with a carbon dioxide-absorbing tube (soda lime). Cool, and titrate 2.50 mL immediately the excess sodium hydroxide with 0.25 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS = 45.04 mg of C9H8O4

**Containers and storage** Containers—Well-closed containers.

**Aspirin Tablets**

**Acetylsalicylic Acid Tablets**

Aspirin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of aspirin (C9H8O4: 180.16).

**Method of preparation** Prepare as directed under Tablets, with Aspirin.

**Identification** (1) Weigh a quantity of powdered Aspirin Tablets, equivalent to 0.1 g of Aspirin according to the labeled amount, add 10 mL of water, and boil for 5 to 6 minutes. After cooling, filter, and add 1 to 2 drops of iron (III) chloride TS to the filtrate: a red-violet color develops.

(2) Weigh a portion of powdered Aspirin Tablets, equivalent to 0.5 g of Aspirin according to the labeled amount, extract with two 10-mL portions of warm ethanol (95), and filter the combined extracts. Evaporate the filtrate to dryness, and boil the residue with 10 mL of sodium carbonate TS for 5 minutes. Proceed as directed in the Identification (2) under Aspirin.

**Purity** Salicylic acid—Take a portion of the powdered Aspirin Tablets, equivalent to 1.0 g of Aspirin according to the labeled amount, shake with 15 mL of ethanol (95) for 5 minutes, filter, discard the first 5 mL of the filtrate, and add 1.0 mL of the subsequent filtrate to a solution which is prepared by transferring 1 mL of freshly prepared dilute am-
Containers and storage  Containers—Well-closed containers.

Assay  Weigh accurately and powder not less than 20 Aspirin Tablets. Weigh accurately a portion of the powder, equivalent to about 1.5 g of aspirin (C9H8O4), add exactly 50 mL of 0.5 mol/L sodium hydroxide VS, and proceed as directed in the Assay under Aspirin.

Each mL of 0.5 mol/L sodium hydroxide VS = 45.04 mg of aspirin (C9H8O4)

Containers and storage  Containers—Well-closed containers.

**Aspirin Aluminum**

**Aluminum Acetylsalicylate**

アルセリンアルミニウム

Aspirin Aluminum contains not less than 83.0% and not more than 90.0% of aspirin (C9H8O4: 180.16), and not less than 6.0% and not more than 7.0% of aluminum (Al: 26.98), calculated on the anhydrous basis.

**Description**  Aspirin Aluminum occurs as a white, crystalline powder. It is odorless or has a slight, acetic odor. It is practically insoluble in water, in methanol, in ethanol (95) and in diethyl ether. It dissolves, with decomposition, in sodium hydroxide TS and in sodium carbonate TS.

**Identification (1)**  Dissolve 0.1 g of Aspirin Aluminum in 10 mL of sodium hydroxide TS by heating, if necessary. Neutralize 2 mL of the solution with hydrochloric acid, and add 1 to 2 drops of iron (III) chloride TS: a red-purple color develops.

(2)  Determine the absorption spectrum of the sample solution obtained in the Assay (1) as directed under Ultraviolet-visible Spectrophotometry <2,24>: it exhibits a maximum between 277 nm and 279 nm.

(3)  Place 2 g of Aspirin Aluminum in a platinum crucible, and ignite until charred. To the residue add 1 g of anhydrous sodium carbonate, and ignite for 20 minutes. After cooling, to the residue add 15 mL of dilute hydrochloric acid, shake, and filter: the filtrate responds to the Qualitative Tests <1,09> for aluminum salt.

**Purity (1)**  Salicylate—Using $A_{T1}$ and $A_{S2}$ obtained in the Assay (1), calculate the amount of salicylate [as salicylic acid (C9H8O4: 180.16)] by the following equation: salicylate content is not more than 7.5%, calculated on the anhydrous basis.

$$\text{Amount (mg) of aspirin (C9H8O4)} = M_S \times \frac{A_{T1} - A_{S2} \times A_{S2}}{A_{S3}} \times \frac{1}{4}$$

$M_S$: Amount (mg) of salicylic acid for assay

(2)  Heavy metals <1.07>—Place 2.0 g of Aspirin Aluminum in a porcelain crucible, cover the crucible loosely, and ignite at a low temperature until charred. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid to the content of the crucible, heat gently the crucible until white fumes are evolved, and continue the heating until white fumes are no longer evolved, then ignite between 500°C and 600°C until the carbon is incinerated. When the incineration is not completed, add 2 mL of nitric acid and 1 mL of sulfuric acid, and heat gently in the same manner, then ignite between 500°C and 600°C to incinerate completely. After cooling, add 2 mL of hydrochloric acid, and proceed as directed in Method 2, and perform the test. Prepare the control solution by using the same quantities of the same reagents as directed for the preparation of the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 10 ppm).

(3)  Arsenic <1.11>—Dissolve 1.0 g of Aspirin Aluminum in 15 mL of sodium hydroxide TS. To this solution add 1 drop of phenolphthalein TS, and with stirring, add dropwise hydrochloric acid until the red color of the solution disappears. Then add 2 mL of hydrochloric acid, cool with occasional shaking for 10 minutes, and filter with a glass filter (G3). Wash the residue with two 5 mL portions of 1 mol/L hydrochloric acid TS, and combine the filtrate and the washings. Use this solution as the test solution, and perform the test (not more than 2 ppm).

**Water <2.48>**  Not more than 4.0% (0.15 g, direct titration).

**Assay (1)**  Aspirin—Weigh accurately about 0.1 g of Aspirin Aluminum, add 40 mL of sodium fluoride TS, and shake for 5 minutes. Allow the solution to stand for 10 minutes with frequent shaking. Extract the solution with six 20-mL portions of chloroform. Combine all chloroform extracts, and add chloroform to make exactly 200 mL. Measure exactly 10 mL of this solution, add chloroform to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.09 g of salicylic acid for assay, previously dried in a desicator (silica gel) for 3 hours, and dissolve in chloroform to make exactly 200 mL. Measure exactly 5 mL of this solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution (1). Then weigh accurately about 0.09 g of aspirin for assay, previously dried in a desicator (silica gel) for 5 hours, and dissolve in chloroform to make exactly 200 mL. Measure exactly 10 mL of this solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2,24>. Determine the absorbances, $A_{T1}$ and $A_{S1}$, of the sample solution and standard solution (1) at 278 nm, and absorbances, $A_{T2}$ and $A_{S2}$, of these solutions, at 308 nm, respectively. Then determine the absorbance $A_{S3}$ of the standard solution (2) at 278 nm.

$$\text{Amount (mg) of aspirin (C9H8O4)} = M_S \times \left[ \frac{A_{T1}}{A_{S1}} - \frac{A_{T2} \times A_{S1}}{A_{S2}} \times \frac{1}{4} \right]$$
Aspoxicillin Hydrate / Official Monographs

M₅: Amount (mg) of aspirin for assay

(2) Aluminum—Weigh accurately about 0.4 g of Aspirin Aluminum, and dissolve in 10 mL of sodium hydroxide TS. Add dropwise 1 mol/L hydrochloric acid TS to adjust the solution to a pH of about 1, add 20 mL of acetic acid-ammonium acetate buffer solution, pH 3.0, and 0.5 mL of Cu-PAN TS, and heat. While boiling, titrate with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to yellow and persists for 1 minute. Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.349 mg of Al

Containers and storage Containers—Well-closed containers.

Aspoxicillin Hydrate

Aspoxicillin Hydrate occurs as a white, crystalline powder. It is freely soluble in N,N-dimethylformamide, sparingly soluble in water, and practically insoluble in acetonitrile, in methanol and in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Aspoxicillin Hydrate (1 in 4000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or spectrum of a solution of Aspoxicillin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aspoxicillin Hydrate as directed in the potassium bromide method under Infrared Spectrophotometry <2.55>. Both spectral exhibit similar intensities of absorption at the same wave numbers.

Containers—Well-closed containers.

Optical rotation <2.49> [α] <2.49> C₁₂H₁₇N₅O₇S: +170° – +185° (0.2 g calculated on the anhydrous bases, water, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Aspoxicillin Hydrate in 50 mL of water: the pH of the solution is between 4.2 and 5.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Aspoxicillin Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Aspoxicillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Aspoxicillin Hydrate according to Method 5, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.05 g of Aspoxicillin Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the area of each peak other than aspoxicillin from the sample solution is not larger than 3/10 times the peak area of aspoxicillin from the standard solution, and the total of peak areas other than aspoxicillin from the sample solution is not larger than the peak area of aspoxicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of aspoxicillin.

System suitability—

Test for required detection: Pipet 2 mL of the standard solution, add the mobile phase to make exactly 10 mL. Confirm that the peak area of aspoxicillin obtained from 10 μL of this solution is equivalent to 15 to 25% of that of aspoxicillin obtained from 10 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution, resist the operating conditions, the relative standard deviation of the peak areas of aspoxicillin is not more than 5%.

Water <2.48> Not less than 95.0% and not more than 13.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Aspoxicillin Hydrate and Aspoxicillin RS, equivalent to about 0.1 g (potency), dissolve each in a suitable amount of water, add exactly 10 mL of the internal standard solution, 6.5 mL of acetonitrile and water to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁, and Q₂, of the peak area of aspoxicillin to that of the internal standard of each solution.

C₁₂H₁₇N₅O₇S: 547.58
(2S,5R,6R)-6-[(2R)-2-[2(R)-2-Amino-3-methylcarbamoylpropanoylamino]-2-(4-hydroxyphenyl)acetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate [63358-49-6, anhydride]

Aspoxicillin Hydrate contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Aspoxicillin Hydrate is expressed as mass (potency) of aspoxicillin (C₁₂H₁₇N₅O₇S: 493.53).

Description Aspoxicillin Hydrate occurs as a white, crystalline powder.

Optical rotation <2.49> [α] <2.49> C₁₂H₁₇N₅O₇S: +170° – +185° (0.2 g calculated on the anhydrous bases, water, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Aspoxicillin Hydrate in 50 mL of water: the pH of the solution is between 4.2 and 5.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Aspoxicillin Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Aspoxicillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Aspoxicillin Hydrate according to Method 5, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.05 g of Aspoxicillin Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the area of each peak other than aspoxicillin from the sample solution is not larger than 3/10 times the peak area of aspoxicillin from the standard solution, and the total of peak areas other than aspoxicillin from the sample solution is not larger than the peak area of aspoxicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of aspoxicillin.

System suitability—

Test for required detection: Pipet 2 mL of the standard solution, add the mobile phase to make exactly 10 mL. Confirm that the peak area of aspoxicillin obtained from 10 μL of this solution is equivalent to 15 to 25% of that of aspoxicillin obtained from 10 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution, resist the operating conditions, the relative standard deviation of the peak areas of aspoxicillin is not more than 5%.

Water <2.48> Not less than 95.0% and not more than 13.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Aspoxicillin Hydrate and Aspoxicillin RS, equivalent to about 0.1 g (potency), dissolve each in a suitable amount of water, add exactly 10 mL of the internal standard solution, 6.5 mL of acetonitrile and water to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁, and Q₂, of the peak area of aspoxicillin to that of the internal standard of each solution.
Amount [µg (potency)] of aspoxicillin (C_{21}H_{27}N_{5}O_{7}S) = M_{S} \times Q_{T} / Q_{S} \times 1000

M_{S}: Amount [µg (potency)] of Aspoxicillin RS

Internal standard solution—A solution of N-(3-hydroxyphenyl)acetamide (1 in 1000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To 130 mL of acetonitrile add potassium dihydrogen phosphate TS, pH 3.0 to make 1000 mL.
Flow rate: Adjust the flow rate so that the retention time of aspoxicillin is about 3 minutes.

System suitability—
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, aspoxicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of aspoxicillin to that of the internal standard is not more than 0.8%.

Containers and storage—Containers—Tight containers.

### Atenolol

Atenolol occurs as a white to pale yellow crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (99.5), and slightly soluble in water.

A solution of Atenolol in methanol (1 in 25) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Atenolol in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Atenolol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Point <2.60> 152 – 156°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Atenolol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Atenolol in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than atenolol is not larger than 1/2 times the peak area of atenolol obtained with the standard solution, and the total area of the peaks other than atenolol is not larger than the peak area of atenolol with the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 226 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 3.0 with phosphoric acid. To 40 volume of this solution add 9 volume of methanol and 1 volume of tetrahydrofuran. Dissolve 1 g of sodium 1-octanesulfonate and 0.4 g of tetrabutylammonium hydrogensulfate in 1000 mL of this solution.
Flow rate: Adjust the flow rate so that the retention time of atenolol is about 8 minutes.

Time span of measurement: About 4 times as long as the retention time of atenolol.

System suitability—
Test for required detectability: To exactly 10 mL of the standard solution add the mobile phase to make exactly 50 mL. Confirm that the peak area of atenolol obtained with 10 µL of this solution is equivalent to 14 to 26% of that obtained with 10 µL of the standard solution.
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atenolol are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atenolol is not more than 1.0%.

Loss on drying <2.4I> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.3 g of Atenolol, previously dried, dissolve in 100 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentio-
metric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 26.63 mg of \( \text{Ca}_6\text{H}_{22}\text{N}_4\text{O}_8 \)

Containers and storage Containers—Tight containers.

Atorvastatin Calcium Hydrate

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\[
\text{C}_{66}\text{H}_{68}\text{CaF}_{2}\text{N}_4\text{O}_{10}\cdot 3\text{H}_2\text{O}: 1209.39 \\
\text{Monocalcium bis}((3R,5R)-7-[2-(4-fluorophenyl]-5-(1-methylpropyl)-3-phenyl-4(phenylcarbamoyl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoate trihydrate} \\
[344423-98-9]
\]

Atorvastatin Calcium Hydrate contains not less than 98.0% and not more than 102.0% of atorvastatin calcium (\( \text{C}_{66}\text{H}_{68}\text{CaF}_{2}\text{N}_4\text{O}_{10} \)): 1155.34), calculated on the anhydrous basis.

Description Atorvastatin Calcium Hydrate occurs as a white or pale yellowish white crystalline powder.

It is very soluble in methanol, freely soluble in dimethylsulfoxide, and very slightly soluble in water and in ethanol (99.5).

It gradually turns yellowish white on exposure to light.

Identification (1) Determine the absorption spectrum of a solution of Atorvastatin Calcium Hydrate in methanol (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Atorvastatin Calcium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Atorvastatin Calcium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Atorvastatin Calcium RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the reference standard according to the method otherwise specified, filter, dry the crystals, and perform the test with the crystals.

(3) A gruel-like liquid of Atorvastatin Calcium Hydrate prepared by adding a small amount of dilute hydrochloric acid responds to the Qualitative Tests <1.09> (1) for calcium salt. A solution of Atorvastatin Calcium Hydrate in a mixture of methanol and water (7:3) (1 in 250) is also responds to the Qualitative Tests <1.09> (3) for calcium salt.

Optical rotation \(<2.49> [\alpha]_D^2: -7 - -10^\circ (0.2 \text{g}, \text{calculated on the anhydrous basis}, \text{dimethylsulfoxide}, 20 \text{mL}, 100 \text{mm})

Purity (1) Heavy metals \(<1.07>—\text{Proceed with 1.0 g of Atorvastatin Calcium Hydrate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).}

(2) Related substances—Dissolve 20 mg of Atorvastatin Calcium Hydrate in 20 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.87> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.8 to atorvastatin, obtained from the sample solution is not larger than 3/10 times the peak area of atorvastatin from the standard solution, the area of the peak other than atorvastatin and peak mentioned above from the sample solution is not larger than 1/10 times the peak area of atorvastatin from the standard solution, and the total area of the peaks other than atorvastatin from the sample solution is not larger than the peak area of atorvastatin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 10.5 g of citric acid monohydrate in 900 mL of water, adjust to pH 5.0 with ammonia solution (28), and add water to make 1000 mL. To 400 mL of this solution add 100 mL of acetonitrile and 100 mL of tetrahydrofuran.

Mobile phase B: A mixture of acetonitrile and tetrahydrofuran (1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 40</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>40 – 80</td>
<td>93 → 60</td>
<td>7 → 40</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of atorvastatin is about 16 minutes.

Time span of measurement: About 5 times as long as the retention time of atorvastatin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. Confirm that the peak area of atorvastatin obtained with 20 \( \mu \)L of this solution is equivalent to 3.5 to 6.5% of that with 20 \( \mu \)L of the standard solution.

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating con-
ditions, the number of theoretical plates and the symmetry factor of the peak of atorvastatin are not less than 8000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atorvastatin is not more than 2.0%.

(3) Residual solvents—Being specified separately.

Water ≤2.48  3.5 – 5.5% (50 mg, coulometric titration).

Assay Weigh accurately about 20 mg each of Atorvastatin Calcium Hydrate and Atorvastatin Calcium RS (separately determine the water ≤2.48 in the same manner as Atorvastatin Calcium Hydrate), dissolve each in an adequate amount of a mixture of water and acetonitrile (1:1), add exactly 10 mL of the internal standard solution, then add a mixture of water and acetonitrile (1:1) to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ≤2.01 according to the following conditions, and calculate the ratios, QT and QS, of the peak area of atorvastatin to that of the internal standard.

Amount (mg) of atorvastatin calcium (C66H68CaF2N4O10) = Mx × Qt/Qt

M5: Amount (mg) of Atorvastatin Calcium RS, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of water and acetonitrile (1:1) (1 in 1500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 10.5 g of citric acid monohydrate in 900 mL of water, adjust to pH 4.0 with ammonia solution (28), and add water to make 1000 mL. To 530 mL of this solution add 270 mL of acetonitrile and 200 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of atorvastatin is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and atorvastatin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of atorvastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Atorvastatin Calcium Tablets

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Atorvastatin Calcium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of atorvastatin calcium hydrate (C66H68CaF2N4O10·3H2O: 1209.39).

Method of preparation Prepare as directed under Tablets, with Atorvastatin Calcium Hydrate.

Identification To a quantity of powdered Atorvastatin Calcium Tablets, equivalent to 10 mg of Atorvastatin Calcium Hydrate according to the labeled amount, add 50 mL of methanol, shake thoroughly, and centrifuge. To 2.5 mL of the supernatant liquid add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ≤2.24: it exhibits a maximum between 244 nm and 248 nm.

Uniformity of dosage units ≤6.02 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Atorvastatin Calcium Tablets add 3/5/5 mL of a mixture of water and methanol (1:1), and disintegrate the tablet by shaking. Add exactly V/10 mL of the internal standard solution, and add a mixture of water and methanol (1:1) to make V mL so that each mL contains about 0.1 mg of atorvastatin calcium hydrate (C66H68CaF2N4O10·3H2O). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 22 mg of Atorvastatin Calcium RS (separately determine the water ≤2.48 in the same manner as Atorvastatin Calcium Hydrate), and dissolve in a mixture of water and methanol (1:1) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ≤2.01 according to the following conditions, and calculate the ratios, QT and QS, of the peak area of atorvastatin to that of the internal standard.

Amount (mg) of atorvastatin calcium hydrate (C66H68CaF2N4O10·3H2O) = M5 × Qt/Qt

M5: Amount (mg) of Atorvastatin Calcium RS, calculated on the anhydrous basis

Internal standard solution—A solution of 1,3-dinitrobenzene in methanol (1 in 2500).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and atorvastatin are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times
with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of atorvastatin to that of the internal standard is not more than 1.0%.

**Dissolution** 6.10 When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Atorvastatin Calcium Tablets is not less than 80%.

Start the test with 1 tablet of Atorvastatin Calcium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 6 µg of atorvastatin calcium hydrate \((\text{C}_{66}\text{H}_{68}\text{CaF}_{2}\text{N}_{4}\text{O}_{10}.3\text{H}_{2}\text{O})\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Atorvastatin Calcium RS (separately determine the water in the sample solution. Separately, weigh accurately about 44 mg of Atorvastatin Calcium RS (separately determine the water and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, \(Q_7\) and \(Q_9\), of the peak area of atorvastatin to that of the internal standard.

\[
\text{Amount (mg) of atorvastatin calcium hydrate (C}_{66}\text{H}_{68}\text{CaF}_{2}\text{N}_{4}\text{O}_{10}.3\text{H}_{2}\text{O})
\]
\[
\text{in 1 tablet of Atorvastatin Calcium Tablets = } M_S \times Q_7/Q_9 \times V/400 \times 1.047
\]

\(M_S\): Amount (mg) of Atorvastatin Calcium RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of 1,3-dinitrobenzene in methanol (1 in 125).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 244 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadeycliclsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: Dissolve 10.5 g of citric acid monohydrate in 900 mL of water, adjust to pH 4.0 with ammonia solution (28), and add water to make 1000 mL. To 530 mL of this solution add 270 mL of acetonitrile and 200 mL of tetrahydrofuran.
Flow rate: Adjust the flow rate so that the retention time of atorvastatin is about 9 minutes.

**System suitability**—
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the internal standard and atorvastatin are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of atorvastatin to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.
Atropine Sulfate Hydrate

Atropine Sulfate Hydrate, when dried, contains not less than 98.0% of atropine sulfate [(C17H23NO3)2.H2SO4.H2O: 676.82].

Description Atropine Sulfate Hydrate occurs as colorless crystals or a white, crystalline powder. It is odorless. It is very soluble in water and in acetic acid (100), freely soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: 188 – 194°C (with decomposition). Introduce a capillary tube charged with dried sample into a bath previously heated to 180°C, and continue to heat at a rate of rise of about 3°C per minute. It is affected by light.

Identification (1) To 1 mg of Atropine Sulfate Hydrate add 3 drops of fuming nitric acid, and evaporate the mixture on a water bath to dryness. Dissolve the residue in 1 mL of N,N-dimethylformamide, and add 5 to 6 drops of tetrachloroethylene (III) hydrate: a red-purple color develops.

(2) To 2 mL of a solution of Atropine Sulfate Hydrate (1 in 50) add 4 to 5 drops of hydrogen tetrachloroaurate (III) TS: a lusterless, yellowish white precipitate is formed.

(3) To 5 mL of a solution of Atropine Sulfate Hydrate (1 in 25) add 2 mL of ammonia TS, and allow to stand for 2 to 3 minutes. Collect the precipitate, wash with water, and dry in a desiccator (in vacuum, silica gel) for 4 hours: it melts <2.60° between 115°C and 118°C.

(4) A solution of Atropine Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for sulfate.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Atropine Sulfate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 1.0 g of Atropine Sulfate Hydrate in 20 mL of water, and add 0.30 mL of 0.02 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.

(3) Related substances—Dissolve 0.25 g of Atropine Sulfate Hydrate in 1 mL of dilute hydrochloric acid (1 in 10), add water to make 15 mL, and use this solution as the sample solution.

(i) To 5 mL of the sample solution add 2 to 3 drops of hydrogen hexachloroplatinate (IV) TS: no precipitate is formed.

(ii) To 5 mL of the sample solution add 2 mL of ammonia TS, and shake vigorously: the turbidity of the solution is not greater than that of the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL. To this solution add 1 mL of silver nitrate TS, and allow 7 mL of the mixture to stand for 5 minutes.

(4) Hyoscyamine—Weigh accurately about 1 g of Atropine Sulfate Hydrate, previously dried, and dissolve in water to make exactly 10 mL: the specific optical rotation [α]D<2.49> of this solution in a 100-mm cell is between −0.60° and +0.10°.

(5) Readily carbonizable substances <1.17>—Take 0.2 g of Atropine Sulfate Hydrate, and perform the test: the solution has no more color than Matching Fluid A.

Loss on drying <2.47> Not more than 4.0% (0.5 g, in vacuum, phosphorus (V) oxide, 110°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Dissolve about 0.25 g of Atropine Sulfate Hydrate, previously dried and accurately weighed, in 30 mL of acetic acid (100). If necessary, dissolve it by warming, and cool. Titrate <2.50> with 0.05 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 33.84 mg of atropine sulfate [(C17H23NO3)2.H2SO4].

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Atropine Sulfate Injection

Atropine Sulfate Injection is an aqueous solution for injection. It contains not less than 93.0% and not more than 107.0% of the labeled amount of atropine sulfate hydrate [(C17H23NO3)2.H2SO4.H2O: 676.83].

Method of preparation Prepare as directed under Injections, with Atropine Sulfate Hydrate.

Description Atropine Sulfate Injection is a clear, colorless liquid.

pH: 4.0 – 6.0

Identification (1) Evaporate a volume of Atropine Sulfate Injection, equivalent to 1 mg of Atropine Sulfate Hydrate according to the labeled amount, on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Atropine Sulfate Hydrate.

(2) Evaporate an exactly measured volume of Atropine Sulfate Injection, equivalent to 5 mg of Atropine Sulfate Hydrate according to the labeled amount, on a water bath to dryness. After cooling, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. If insoluble substance remains, crush it, allow to stand, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Atropine Sulfate RS in 2 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer
Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28:90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff’s TS for spraying on the plate; the spots obtained from the sample solution and the standard solution show an orange color and the same Rf value.

(3) Atropine Sulfate Injection responds to the Qualitative Tests <1.09> for sulfate.

Bacterial endotoxins <4.04> Less than 75 EU/mg.

Extractable volume <6.07> It meets the requirements.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test: it meets the requirement.

Assay

To an exactly measured volume of Atropine Sulfate Injection, equivalent to about 5 mg of atropine sulfate hydrate \([\text{C}_{17}\text{H}_{23}\text{NO}_{3}\text{H}_{2}\text{SO}_{4}\text{H}_{2}\text{O}]\), add exactly 3 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate RS, separately determine its loss on drying <2.41> in the same conditions as for Atropine Sulfate Hydrate, and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 3 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_s\), of the peak area of atropine to that of the internal standard.

Amount (mg) of atropine sulfate hydrate
\[
\text{Amount (mg)} = M_S \times \frac{Q_T}{Q_s} \times 1/5 \times 1.027
\]

\(M_S\): Amount (mg) of Atropine Sulfate RS, calculated based on the dried basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 1000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To 0.4 g of sodium lauryl sulfate add 500 mL of diluted phosphoric acid (1 in 1000) to dissolve, and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.
Flow rate: Adjust the flow rate so that the retention time of atropine is about 16 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and atropine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of atropine to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Azathioprine

\(\text{C}_9\text{H}_7\text{N}_7\text{O}_2\text{S}\) 277.26
6-(1-Methyl-4-nitro-1H-imidazol-5-ylthio)purine [446-86-6]

Azathioprine, when dried, contains not less than 98.5% of \(\text{C}_9\text{H}_7\text{N}_7\text{O}_2\text{S}\).

Description Azathioprine is light yellow crystals or crystalline powder. It is odorless.
It is sparingly soluble in \(N, N\)-dimethylformamide and in pyridine, very slightly soluble in water and in ethanol (99.5), and practically insoluble in chloroform and in diethyyl ether.
It dissolves in sodium hydroxide TS and in ammonia TS. It is gradually colored by light.
Melting point: about 240°C (with decomposition).

Identification (1) Dissolve 0.01 g of Azathioprine in 50 mL of water by warming. To 5 mL of this solution add 1 mL of dilute hydrochloric acid and 0.01 g of zinc powder, and allow to stand for 5 minutes: a yellow color is produced. Filter this solution: the filtrate responds to the Qualitative Tests <1.09> for primary aromatic amines, and a red color is produced.

(2) Dissolve 0.01 g of Azathioprine in 50 mL of water by warming. To 1 mL of this solution add 0.5 mL of phosphotungstic acid TS and 0.5 mL of dilute hydrochloric acid: a white precipitate is formed.

(3) Prepare the test solution by proceeding with 0.03 g of Azathioprine according to the Oxygen Flask Combustion Method <1.06>, using 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <1.09> (1) for sulfate.

(4) Dissolve 0.01 g of Azathioprine in 2 mol/L hydrochloric acid TS to make 100 mL. Dilute 5 mL of the solution with water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.26>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Azathioprine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.
**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Azathioprine in 50 mL of N,N-dimethylformamide: the solution is clear and shows a light yellow color.

(2) Acidity or alkalinity—Add 100 mL of water to 2.0 g of Azathioprine, shake well for 15 minutes, centrifuge for 5 minutes at 10,000 revolutions per minute, and filter. Discard the first 20 mL of the filtrate, add 2 drops of methyl red TS to 40 mL of the subsequent filtrate, and use this solution as the sample solution.

(i) Add 0.10 mL of 0.02 mol/L hydrochloric acid VS to 20 mL of the sample solution: a yellow color develops.

(ii) Add 0.10 mL of 0.02 mol/L sodium hydroxide VS to 20 mL of the sample solution: a yellow color develops.

(3) Sulfate \(<1.14^\circ\)—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the sample solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals \(<1.07^\circ\)—Proceed with 2.0 g of Azathioprine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic \(<1.17^\circ\)—Prepare the test solution with 1.0 g of Azathioprine, according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 10 mg of Azathioprine in 80 mL of the mobile phase by warming, cool, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07^\circ\) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than that of azathioprine from the sample solution is not larger than 1/2 times the peak area of azathioprine from the standard solution.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 296 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclysilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Adjust to pH 2.5 of a solution of 0.05 mol/L potassium dihydrogenphosphate TS (1 in 2) with diluted phosphoric acid (3 in 2000). To 800 mL of this solution add 200 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of azathioprine is about 8 minutes.
Time span of measurement: About three times as long as the retention time of azathioprine beginning after the solvent peak.

**System suitability**—
Test for required detection: To exactly 5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of azathioprine obtained from 20 μL of this solution is equivalent to 8 to 12% of that of azathioprine obtained from 20 μL of the standard solution.
System performance: Dissolve 10 mg of Azathioprine in 80 mL of water by warming, cool, and add water to make 100 mL. To 2 mL of this solution add 2 mL of a solution, separately prepared by dissolving 0.06 g of benzoic acid in 3 mL of methanol and diluting with water to make 10 mL, and add the mobile phase to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, azathioprine and benzoic acid are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of azathioprine is not more than 2.0%.

**Loss on drying** \(<2.4^\circ\) Not more than 0.5% (1 g, 105°C, 5 hours).

**Residue on ignition** \(<2.4^\circ\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Azathioprine, previously dried, add 80 mL of N,N-dimethylformamide, and warm to dissolve. After cooling, titrate \(<2.5^\circ\) with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through yellow-green to blue-green (indicator: 1 mL of thymol blue-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 27.73 mg of C9H7O7O2S

**Containers and storage** Containers—Well-closed containers.
Storage—Light-resistant.

### Azathioprine Tablets

アザチオプリン錠

Azathioprine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of azathioprine (C9H7O7O2S; 277.26).

**Method of preparation** Prepare as directed under Tablets, with Azathioprine.

**Identification** (1) Weigh a quantity of powdered Azathioprine Tablets, equivalent to 0.01 g of Azathioprine according to the labeled amount. Add 50 mL of water, shake well while warming, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Azathioprine.

(2) Proceed with 1 mL of the filtrate obtained in (1) as directed in the Identification (2) under Azathioprine.

(3) Determine the absorption spectrum of the sample solution in the Assay as directed under Ultraviolet-visible Spectrophotometry \(<2.24^\circ\): it exhibits a maximum between 278 nm and 282 nm.

(4) Weigh a quantity of powdered Azathioprine Tablets, equivalent to 0.1 g of Azathioprine to the labeled amount. Add 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 0.1 g of Azathioprine RS in 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.07^\circ\). Spot 5 μL each of the sample
solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, a solution of ammonia solution (28) in methanol (1 in 10), n-butyl formate and 1,2-dichloroethane (15:10:5:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show the same Rf value.

**Uniformity of dosage units**<6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Azathioprine Tablets add 1 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry per 5 mg of azathioprine (C₉H₇N₇O₂S), shake well, add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.2 mg of azathioprine (C₉H₇N₇O₂S), and filter. Discard the first 20 mL of the filtrate, measure exactly 3 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of azathioprine (C₉H₇N₇O₂S)} = M_S \times A_T/A_S \times V/500
\]

\[M_S: \text{Amount (mg) of Azathioprine RS}\]

**Assay** Weigh accurately and powder not less than 20 Azathioprine Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of azathioprine (C₉H₇N₇O₂S), add 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.2 mg of azathioprine (C₉H₇N₇O₂S), and filter. Discard the first 20 mL of the filtrate, measure exactly 3 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Azathioprine RS, previously dried at 105°C for 5 hours, dissolve in 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Measure exactly 3 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Amount (mg) of azathioprine (C₉H₇N₇O₂S)} = M_S \times A_T/A_S
\]

\[M_S: \text{Amount (mg) of Azathioprine RS}\]

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Azelastine Hydrochloride**

アゼラスチン塩酸塩

C₂₂H₂₄ClN₃O.HCl: 418.36
4-[(4-Chlorophenyl)methyl]-2-[(4RS)-
(1-methylazepan-4-yl)phthalazin-1(2H)-one
monohydrochloride [79307-93-0]

Azelastine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C₂₂H₂₄ClN₃O.HCl.

**Description** Azelastine Hydrochloride occurs as a white crystalline powder.

It is freely soluble in formic acid, and slightly soluble in water and in ethanol (99.5).

Melting point: about 225°C (with decomposition).

A solution of Azelastine Hydrochloride (1 in 200) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Azelastine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum:

- Both spectra exhibit similar intensities of absorption at the same wavelengths.

**Determination of impurities**

-**Arsenic**<1.17>—Prepare the test solution with 1.0 g of Azelastine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm). 
-**Related substances**—Dissolve 50 mg of Azelastine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: each peak area other than azelastine obtained from the sam-
ple solution is not larger than 1/10 times the peak area of azelastine from the standard solution, and the total area of the peaks other than the peak of azelastine from the sample solution is not larger than 1/2 times the peak area of azelastine from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of water, acetonitrile and perchloric acid (660:340:1).

Flow rate: Adjust the flow rate so that the retention time of azelastine is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of azelastine, beginning after the solvent peak.

**System suitability**—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of azelastine obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of azelastine is not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of azelastine is not more than 1.0%.

**Loss on drying** <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.6 g of previously dried Azelastine Hydrochloride, dissolve in 5 mL of formic acid, add 70 mL of acetic anhydride, and titrate according to the following titration procedure. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 41.84 mg of C22H24ClN3O.HCl

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Azelastine Hydrochloride Granules**

アゼラスチン塩酸塩顆粒

Azelastine Hydrochloride Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of azelastine hydrochloride (C22H24ClN3O.HCl: 418.36).

**Method of preparation** Prepare as directed under Granules, with Azelastine Hydrochloride.

**Identification** To a quantity of Azelastine Hydrochloride Granules, equivalent to 2 mg of Azelastine Hydrochloride according to the labeled amount, add 30 mL of 0.1 mol/L hydrochloric acid TS, and treat with ultrasonic waves for 30 minutes. After cooling, add 0.1 mol/L hydrochloric acid TS to make 50 mL, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm.

**Dissolution** <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the dissolution medium, the dissolution rate in 45 minutes of Azelastine Hydrochloride Granules is not less than 80%.

Start the test with accurately weighed amount of Azelastine Hydrochloride Granules, equivalent to about 1 mg of azelastine hydrochloride (C22H24ClN3O.HCl) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of azelastine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the dissolution medium to make exactly 250 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A1 and A2, of azelastine in each solution.

Dissolution rate (%) with respect to the labeled amount of azelastine hydrochloride (C22H24ClN3O.HCl) = M2/M1 × A1/A2 × 1/C × 9/5

M2: Amount (mg) of azelastine hydrochloride for assay
M1: Amount (g) of Azelastine Hydrochloride Granules
C: Labeled amount (mg) of azelastine hydrochloride (C22H24ClN3O.HCl) in 1 g

**Operating conditions**—

Proceed as directed in the operating conditions in the Assay.

**System suitability**—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of azelastine are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of azelastine is not more than 2.0%.

**Assay** Weigh accurately an amount of Azelastine Hydrochloride Granules, equivalent to about 2 mg of azelastine hydrochloride (C22H24ClN3O.HCl), add 50 mL of 0.1 mol/L hydrochloric acid TS, treat with ultrasonic waves for 20 minutes, add 40 mL of ethanol (99.5), add exactly 5 mL of the internal standard solution, and add ethanol (99.5) to
make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of azelastine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, and add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS and 40 mL of ethanol (99.5), add exactly 5 mL of the internal standard solution, add ethanol (99.5) to make 100 mL, and use this solution as the standard solution.

Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_1$ and $Q_0$, of the peak area of azelastine to that of the internal standard.

$$M_S = \frac{M_5 \times Q_1}{Q_0} \times \frac{1}{25}$$

$M_S$: Amount (mg) of azelastine hydrochloride for assay

Internal standard solution—Dissolve 0.2 g of 2-ethylhexyl parahydroxybenzoate in ethanol (99.5) to make 100 mL.

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilsanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and a solution of sodium lauryl sulfate in diluted acetic acid (100) (1 in 250) (1 in 500) (11:9).

Flow rate: Adjust the flow rate so that the retention time of azelastine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, azelastine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of azelastine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Azithromycin Hydrate

アジスロマイシン水和物

C$_{38}$H$_{72}$N$_2$O$_{12}$.2H$_2$O: 785.02
(2R,3S,4S,5R,6R,8R,11R,12R,13S,14R)-5-
(3,4,6-Trideoxy-3-dimethylamino-β-D-xylo-
heptamethylhexadecan-14-olide dihydrate
[117772-70-0]

Azithromycin Hydrate is the derivative of erythromycin.

It contains not less than 945 µg (potency) and not more than 1030 µg (potency) per mg, calculated on the anhydrous basis. The potency of Azithromycin Hydrate is expressed as mass (potency) of azithromycin

$\text{C}_{34} \text{H}_{61} \text{N}_{10} \text{O}_{15} \cdot 2 \text{H}_{2} \text{O}$: 748.98.

Description

Azithromycin Hydrate occurs as a white crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification

Determine the infrared absorption spectrum of Azithromycin Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Azithromycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]$_D^{20}$: −45° to −49° (0.4 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Purity

(1) Heavy metals <1.07>—Proceed with 1.0 g of Azithromycin Hydrate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Being specified separately.

(3) Residual solvent—Being specified separately.

Water <2.45> Not less than 4.0% and not more than 5.0% (0.4 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay

Weigh accurately an amount of Azithromycin Hydrate and Azithromycin RS, equivalent to about 50 mg (potency), dissolve each in an adequate amount of a mixture of acetonitrile and water (3:2), add exactly 2 mL of the internal
standard solution and the mixture of acetonitrile and water (3:2) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL of each of the sample solution and standard solution as directed under Liquid Chromatography under Nuclear Magnetic Resonance Spectroscopy according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of azithromycin to that of the internal standard.

$$M_S = M_S \times \frac{Q_T}{Q_S} \times 1000$$

$M_S$: Amount [mg (potency)] of Azithromycin RS

Internal standard solution—A solution of 4,4'-bis(diethylamino)benzophenone in acetonitrile (3 in 4000).

Operating conditions—
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 6.97 g of dipotassium hydrogen phosphate in about 750 mL of water, adjust the pH to 11.0 with potassium hydroxide TS, and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile for liquid chromatography.
Flow rate: Adjust the flow rate so that the retention time of azithromycin is about 10 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, azithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of azithromycin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Aztreonam

アズトレオナム

C₁₃H₁₁N₅O₈S₂: 435.43
2-[(Z)-(2-Aminothiazol-4-yl)-[25S]-2-methyl-4-oxo-1-sulfoazetidin-3-ylcarbamoyl]methyleneaminoxy]-2-methyl-1-propanoic acid

【78110-38-0】

Aztreonam contains not less than 920 μg (potency) and not more than 1030 μg (potency) per mg, calculated on the anhydrous basis. The potency of Aztreonam is expressed as mass (potency) of aztreonam (C₁₃H₁₁N₅O₈S₂).

Description Aztreonam occurs as a white to yellowish white crystalline powder.
It is freely soluble in dimethylsulfoxide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Aztreonam (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Aztreonam RS: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Aztreonam in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10), using a light hydrogen substance existing in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy as an internal reference compound and 2.50 ppm for its chemical shift, as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits a multiple signal at around δ 2.49 ppm, and a single signal at around δ 2.15 ppm. The ratio of integrated intensity of each signal is 9:1.

Optical rotation <2.49> [α]D²⁰: −26° – −32° (0.25 g calculated on the anhydrous bases, water, 50 mL, 100 mm).

pH <2.45> Dissolve 0.05 g of Aztreonam in 10 mL of water: the pH of this solution is between 2.2 and 2.8.

Purity (1) Clarity and color of solution—Dissolve 0.1 g of Aztreonam in 20 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Aztreonam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 2 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Aztreonam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.04 g of Aztreonam in 100 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make
Aztreenam for Injection

Aztreenam for Injection is a preparation for injection which is dissolved before use. It contains not less than 93.0% and not more than 107.0% of the labeled amount of aztreenam (C₁₃H₁₇N₅O₈S₂: 435.43).

Method of preparation— Prepare as directed under Injections, with Aztreenam.

Description— Aztreenam for Injection is white to yellowish white masses or powder.

Identification—(1) Dissolve an amount of Aztreenam for Injection, equivalent to 6 mg (potency) of Aztreenam according to the labeled amount, in 1 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and mix: a red-brown color develops.

(2) Dissolve an amount of Aztreenam for Injection, equivalent to 3 mg (potency) of Aztreenam according to the labeled amount, in 100 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 289 nm and 293 nm.

pH— The pH of a solution prepared by dissolving an amount of Aztreenam for Injection, equivalent to 1.0 g (potency) of Aztreenam according to the labeled amount, in 10 mL of water is 4.5 to 7.0.

Purity—Clarity and color of solution—Dissolve an amount of Aztreenam for Injection, equivalent to 1.0 g (potency) of Aztreenam according to the labeled amount, in 10 mL of water: the solution is clear, and its absorbance at 450 nm is not more than 0.06.

System suitability—

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of aztreenam to that of the internal standard is not more than 1.5%.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Time span of measurement: About 4 times as long as the retention time of aztreenam beginning after the solvent peak.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of aztreenam to that of the internal standard is not more than 2.0%.

Water—Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition—Not more than 0.1% (1 g).

Assay—Weigh accurately an amount of Aztreenam and Aztreenam RS, equivalent to about 20 mg (potency), dissolve each in 70 mL of water, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 25 μL each of these solutions as directed under Liquid Chromatography: according to the following conditions, and calculate the areas of each peak by the automatic integration method: the area of each peak is not more than the peak area of aztreenam from the standard solution, and the total area of peaks other than aztreenam from the sample solution is not larger than 2.5 times the peak area of aztreenam from the standard solution.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of aztreenam to that of the internal standard is not more than 1.5%.

Containers and storage—Containers—Tight containers.

Storage—Light-resistant.

Aztreenam for Injection

注射用アズトレオナム

Aztreenam for Injection is a preparation for injection which is dissolved before use. It contains not less than 93.0% and not more than 107.0% of the labeled amount of aztreenam (C₁₃H₁₇N₅O₈S₂: 435.43).

Method of preparation— Prepare as directed under Injections, with Aztreenam.

Description—Aztreenam for Injection is white to yellowish white masses or powder.

Identification—(1) Dissolve an amount of Aztreenam for Injection, equivalent to 6 mg (potency) of Aztreenam according to the labeled amount, in 1 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and mix: a red-brown color develops.

(2) Dissolve an amount of Aztreenam for Injection, equivalent to 3 mg (potency) of Aztreenam according to the labeled amount, in 100 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 289 nm and 293 nm.

pH—The pH of a solution prepared by dissolving an amount of Aztreenam for Injection, equivalent to 1.0 g (potency) of Aztreenam according to the labeled amount, in 10 mL of water is 4.5 to 7.0.

Purity—Clarity and color of solution—Dissolve an amount of Aztreenam for Injection, equivalent to 1.0 g (potency) of Aztreenam according to the labeled amount, in 10 mL of water: the solution is clear, and its absorbance at 450 nm is not more than 0.06.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Time span of measurement: About 4 times as long as the retention time of aztreenam beginning after the solvent peak.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of aztreenam to that of the internal standard is not more than 1.5%.

Containers and storage—Containers—Tight containers.

Storage—Light-resistant.
**Water** <2.40> Not more than 2.0% (0.5 g, volumetric titration, direct titration).

**Bacterial endotoxins** <4.01> Less than 0.10 EU/mg (potency).

**Uniformity of dosage units** <6.02> It meets the requirement of the Mass variation test.

**Foreign insoluble matter** <6.06> Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Take an amount of Aztreonam for Injection, equivalent to about 5 g (potency) of Aztreonam, dissolve the contents with a suitable amount of water, and transfer to a 100-mL volumetric flask. Wash each container with water, combine the washings and the solution, and add water to make exactly 100 mL. Pipet 10 mL of this solution, and add water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Aztreonam RS, equivalent to about 20 mg (potency), dissolve in a suitable amount of water, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Aztreonam.

\[
\text{Amount [mg (potency)] of aztreonam (C}_{13}\text{H}_{17}\text{N}_{5}\text{O}_{8}\text{S}_{2}) = M_5 \times \frac{Q_T}{Q_s} \times 250
\]

\(M_5:\) Amount [mg (potency)] of Aztreonam RS

**Internal standard solution**—A solution of 4-aminobenzoic acid (1 in 6250).

**Containers and storage** Containers—Hermetic containers.

**Storage**—Light-resistant.

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**Bacampicillin Hydrochloride**

**Ampicillin Ethoxycarbonyloxyethyl Hydrochloride**

バカンピシリン塩酸塩

\[
\text{C}_{13}\text{H}_{20}\text{N}_{5}\text{O}_{8}\text{S} \cdot \text{HCl:} \ 501.98
\]

1-Ethoxycarbonyloxyethyl (2S,5R,6R)-6-[(2R)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [37661-08-8]

Bacampicillin Hydrochloride is a hydrochloride of ampicillin ethoxycarbonyloxyethyl ester.

It contains not less than 626 μg (potency) per mg, calculated on the anhydrous basis. The potency of Bacampicillin Hydrochloride is expressed as mass (potency) of ampicillin (C\(_{16}\)H\(_{19}\)N\(_{3}\)O\(_{4}\)S: 349.40).

**Description** Bacampicillin Hydrochloride occurs as a white to pale yellow crystalline powder. It has a characteristic odor.

It is freely soluble in methanol and in ethanol (95), and soluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Bacampicillin Hydrochloride in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of Bacampicillin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bacampicillin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Bacampicillin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bacampicillin Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

**Optical rotation** <2.49> [α] = +140° to +170° (0.1 g calculated on the anhydrous basis, ethanol (95), 25 mL, 100 mm).

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Bacampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 1.0 g of Bacampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Free ampicillin—Weigh accurately about 0.1 g of Bacampicillin Hydrochloride, transfer into a 100-mL separator, add exactly 15 mL of ice-cold water to dissolve, add and mix with exactly 10 mL of ice-cold 0.05 mol/L phosphate buffer solution, pH 7.0, then add 25 mL of ice-cold chloroform, shake, and abandon the chloroform layer. Repeat the procedure twice with two 25-mL portions of ice-cold chloroform. Centrifuge the water layer, filter the supernatant, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 20 mg, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add 10 mL of 0.05 mol/L phosphate buffer solution, pH 7.0 and water to make exactly 25 mL, and use this solution as the standard solution. To exactly 10 mL of the sample solution and standard solution add exactly 2 mL of sodium hydroxide TS, allow to stand for exactly 15 minutes, add exactly 2 mL of 1 mol/L hydrochloric acid TS, exactly 10 mL of 0.3 mol/L potassium hydrogen phthalate buffer solution, pH 4.6, and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 20 minutes without exposure to light. Titrate <2.50> these solutions with 0.01 mol/L sodium thiosulfate VS until the color of the solution changes to colorless. Separately, to exactly 10 mL each of the sample solution and the standard solution add exactly 10 mL of 0.3 mol/L potassium hydrogen phthalate buffer solution, pH 4.6 and exactly 10 mL of 0.005 mol/L iodine VS, and perform a blank determination
with the same manner. Determine the consumed amounts (mL) of 0.005 mol/L iodine VS, $V_1$ and $V_S$, of the sample solution and the standard solution: the amount of ampicillin is not more than 1.0%.

\[
\text{Amount (mg) of ampicillin (C₁₆H₁₉N₃O₄S)} = M_S \times V_1 / V_S \times 1/20
\]

$M_S$: Amount (mg) of Ampicillin RS

**Water** <2.48> Not more than 1.0% (0.5 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 1.5% (1 g).

**Assay** Weigh accurately an amount of Bacampicillin Hydrochloride and Bacampicillin Hydrochloride RS, equivalent to about 40 mg (potency), dissolve each in water to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of bacampicillin of these solutions.

\[
\text{Amount [µg (potency)] of ampicillin (C₁₆H₁₉N₃O₄S)} = M_S \times A_T / A_S \times 1000
\]

$M_S$: Amount [mg (potency)] of Bacampicillin Hydrochloride RS

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of diluted 2 mol/L sodium dihydrogen phosphate TS (1 in 100), add dilute 0.05 mol/L disodium hydrogen phosphate TS (2 in 5) to adjust the pH to 6.8. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of bacampicillin is about 6.5 minutes.

**System suitability**—

System performance: When the procedure is run with 20 µL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bacampicillin are not less than 10,000 and not more than 2, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of peak areas of bacampicillin is not more than 2.0%.

**Containers and storage** Containers—Tight containers.

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### Bacitracin

バシトラシン

[1405-87-4]

Bacitracin is a mixture of peptide substances having antibacterial activity including bacitracin A as the main component produced by the growth of *Bacillus subtilis* or *Bacillus licheniformis*.

It contains not less than 60 Units per mg. The potency of Bacitracin is expressed as unit calculated from the amount of bacitracin A (C₆₆H₁₀₃N₁₇O₁₆S: 1422.69). One unit of Bacitracin is equivalent to 23.8 µg of bacitracin A (C₆₆H₁₀₃N₁₇O₁₆S).

**Description** Bacitracin occurs as a white to light brown powder. It is freely soluble in water, and slightly soluble in ethanol (99.5).

**Identification** (1) To 3 mL of a solution of Bacitracin (1 in 100) add 3 mL of 4-dimethylaminobenzaldehyde TS, shake until red-rosy to red-purple color appears, then add several drops of a solution of sodium nitrite (1 in 100), and shake: a green to dark green color is produced.

(2) Dissolve 60 mg each of Bacitracin and Bacitracin RS in 10 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

**Residue on ignition** <2.44> Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Micrococcus luteus* ATCC 10240.

(ii) Culture medium—Use the medium iii in 3) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Bacitracin RS, equivalent to about 400 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL,
and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 10°C and use within 2 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Bacitracin, equivalent about 400 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage  Containers—Tight containers.

Storage—In a cold place.

**Baclofen**

パクロフェン

C\textsubscript{10}H\textsubscript{12}ClNO\textsubscript{2}: 213.66

(3RS)-4-Amino-3-(4-chlorophenyl)butanoic acid

[B134-47-0]

Baclofen contains not less than 98.5% of C\textsubscript{10}H\textsubscript{12}ClNO\textsubscript{2}, calculated on the anhydrous basis.

Description  Baclofen occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, very slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid.

Identification  (1) To 5 mL of a solution of Baclofen (1 in 1000) add 1 mL of ninhydrin TS, and heat on a water bath for 3 minutes: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Baclofen in 0.1 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Baclofen RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Baclofen as directed under Flame Coloration Test \(<1.04\rangle\) (2); a green color appears.

(4) Assay  Weigh accurately about 0.5 g of Baclofen, dissolve in 80 mL of acetic acid (100), and titrate \(<2.50\rangle\) with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

\[= 21.37 \text{ mg of C}_{10}\text{H}_{12}\text{ClNO}_2\]
Baclofen Tablets

Baclofen Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of baclofen (C\textsubscript{10}H\textsubscript{12}ClNO\textsubscript{2}: 213.66).

**Method of preparation** Prepare as directed under Tablets, with Baclofen.

**Identification (1)** To a portion of powdered Baclofen Tablets, equivalent to 0.01 g of Baclofen according to the labeled amount, add 10 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and proceed as directed in the Identification (1) under Baclofen.

**Identification (2)** To a portion of powdered Baclofen Tablets, equivalent to 25 mg of Baclofen according to the labeled amount, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake for 15 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> 2.24: it exhibits maxima between 257 nm and 261 nm, between 264 nm and 268 nm, and between 272 nm and 276 nm.

**Identification (3)** To a portion of powdered Baclofen Tablets, equivalent to 0.1 g of Baclofen according to the labeled amount, add 2 mL of a mixture of methanol and acetic acid (100:1), shake well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.01 g of Baclofen RS in 2 mL of a mixture of methanol and acetic acid (100:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 20 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and propanol (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same \(R_f\) value.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Baclofen Tablets add 5 mL of 0.1 mol/L hydrochloric acid TS, disperse the tablet into small particles with the aid of ultrasonic waves, then shake for 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly \(V\) mL so that each mL contains about 0.5 mg of baclofen (C\textsubscript{10}H\textsubscript{12}ClNO\textsubscript{2}). Centrifuge, pipet 5 mL of the supernatant liquid, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, then add water to make exactly 50 mL, and use this solution as the standard solution. Separately, weigh accurately about 25 mg of Baclofen RS (separately determine the water content <2.48> in the same manner as Baclofen), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, then add water to make exactly 50 mL, and use this solution as the standard solution. To exactly 2 mL each of the sample solution and standard solution add 4 mL of ninhydrin-tin (II) chloride TS, mix, heat on a water bath for 20 minutes, then immediately shake vigorously for 2 minutes. After cooling, add a mixture of water and 1-propanol (1:1) to make them exactly 25 mL, and determine the absorbances, \(A_T\) and \(A_S\), of them at 570 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution obtained with 2 mL of water by the same procedure as above as the blank.

\[
\text{Amount (mg) of baclofen (C}_{10}\text{H}_{12}\text{ClNO}_{2}) = M_S \times A_T/A_S \times V/50
\]

where:
- \(M_S\): Amount (mg) of Baclofen RS, calculated on the anhydrous basis
- \(A_T\): Absorbance of the sample solution at 570 nm
- \(A_S\): Absorbance of the standard solution at 570 nm
- \(V\): Volume of the standard solution

**Dissolution** <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 500 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Baclofen Tablets is not less than 70%.

Start the test with 1 tablet of Baclofen Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent, add water to make exactly \(V\) mL so that each mL contains about 10 \(\mu\)g of baclofen (C\textsubscript{10}H\textsubscript{12}ClNO\textsubscript{2}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baclofen RS (separately determine the water <2.48> in the same manner as Baclofen), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), of the sample solution and the standard solution at 220 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Dissolution rate (\%)} = (A_T/A_S) \times (1/C \times 50)
\]

where:
- \(C\): Labeled amount (mg) of baclofen (C\textsubscript{10}H\textsubscript{12}ClNO\textsubscript{2}) in 1 tablet

**Assay** Weigh accurately and powder not less than 20 Baclofen Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of baclofen (C\textsubscript{10}H\textsubscript{12}ClNO\textsubscript{2}), add 130 mL of 0.1 mol/L hydrochloric acid TS, shake for 10 minutes, add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of Baclofen RS (separately determine the water content <2.48> in the same manner as Baclofen), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, add water to make exactly 50 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and the standard solution, to each add 4 mL of ninhydrin-stannous chloride TS, shake, heat on a water bath for
Bamethan Sulfate

Control solution: To 1.5 mL of Matching Fluid O add diluted hydrochloric acid (1 in 40) to make 200 mL.

(2) Chloride $<1.05>$.—Perform the test with 3.5 g of Bamethan Sulfate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.002%).

(3) Heavy metals $<1.07>$.—Proceed with 2.0 g of Bamethan Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic $<1.11>$.—Prepare the test solution with 1.0 g of Bamethan Sulfate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Bamethan Sulfate in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.02>$. Spot 2 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (7:2) in a developing vessel saturated with ammonia vapor to a distance of about 12 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate, air-dry for 15 minutes, spray Dragendorff’s TS for spraying again, then, after 1 minute, spray evenly a solution of sodium nitrite (1 in 20), and immediately put a glass plate on the plate. Examine the plate after 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $<2.41>$. Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $<2.44>$. Not more than 0.1% (1 g).

Assay Weigh accurately about 0.75 g of Bamethan Sulfate, previously dried, dissolve in 80 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

$= 51.67$ mg of $(C_{12}H_{19}NO_{2})_2.H_2SO_4$.

Barbital

Barbital, when dried, contains not less than 99.0% of $C_8H_{12}N_2O_3$.

Description Barbital occurs as colorless or white crystals or
Barium Sulfate / Official Monographs

420

Barium Sulfate

硫酸バリウム

BaSO₄: 233.39

Description Barium Sulfate occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It does not dissolve in hydrochloric acid, in nitric acid and in sodium hydroxide TS.

Identification (1) Mix 0.5 g of Barium Sulfate with 2 g each of anhydrous sodium carbonate and potassium carbonate in a crucible, heat the mixture until fusion is complete, treat the cooled mass with hot water, and filter. The filtrate, acidified with hydrochloric acid, responds to the Qualitative Tests <1.09> for sulfate.

(2) Wash the hot water-insoluble residue obtained in (1) with water, dissolve in 2 mL of acetic acid (31), and filter, if necessary: the solution responds to the Qualitative Tests <1.09> for barium salt.

Purity (1) Acidity or alkalinity—Agitate 1.0 g of Barium Sulfate with 20 mL of water for 5 minutes: the solution is neutral.

(2) Phosphate—Boil 1.0 g of Barium Sulfate with 3 mL of nitric acid and 5 mL of water for 5 minutes, cool, and add water to restore the original volume. Filter through a filter paper, previously washed with dilute nitric acid, to the filtrate add an equal volume of hexaammonium heptamolybdic acid TS, and allow to stand between 50°C and 60°C for 1 hour: no yellow precipitate is produced.

(3) Sulfide—Place 10 g of Barium Sulfate in a 250-mL conical flask, add 10 mL of dilute hydrochloric acid and water to make 100 mL, and boil for 10 minutes: the gas evolved does not darken moistened lead (II) acetate paper.

(4) Heavy metals <1.07>—Boil 5.0 g of Barium Sulfate with 2.5 mL of acetic acid (100) and 50 mL of water for 10 minutes, cool, add 0.5 mL of ammonia TS and water to make 100 mL, and filter. Perform the test with a 50-mL portion of this filtrate. Prepare the control solution with 2.5 mL of Standard Lead Solution, 1.25 mL of acetic acid (100), 0.25 mL of ammonia TS and water to make 50 mL (not more than 10 ppm).

(5) Arsenic <1.11>—Proceed the test solution with 2.0 g of Barium Sulfate according to Method 1, and perform the test (not more than 1 ppm).

(6) Hydrochloric acid-soluble substances and soluble barium salts—Cool the solution obtained in (3), add water to
make 100 mL, and filter. Evaporate 50 mL of the filtrate on a water bath to dryness, add 2 drops of hydrochloric acid and 10 mL of warm water, filter through paper for assay, and wash with 10 mL of warm water. Evaporate the combined filtrate and washings on a water bath to dryness, and dry the residue at 105°C for 1 hour: the residue weighs not more than 15 mg. Shake the residue, if any, with 10 mL of water, and filter. To the filtrate add 0.5 mL of dilute sulfuric acid, and allow to stand for 30 minutes: no turbidity is produced.

**Containers and storage** Containers—Well-closed containers.

**Freeze-dried BCG Vaccine**

(オールド BCG ワクチン)

Freeze-dried BCG Vaccine (for Percutaneous Use) is a preparation for injection which is dissolved before use.

It contains live bacteria derived from a culture of the bacillus of Calmette and Guérin.

It conforms to the requirements of Freeze-dried BCG Vaccine (for Percutaneous Use) in the Minimum Requirements for Biological Products.

**Description** Freeze-dried BCG Vaccine (for Percutaneous Use) becomes a white to light yellow, turbid liquid on addition of solvent.

**Beclometasone Dipropionate**

(ベクロメタゾンプロピオン酸エステル)

Beclometasone Dipropionate, when dried, contains not less than 97.0% and not more than 103.0% of \(C_{28}H_{37}ClO_{7}\).

**Description** Beclometasone Dipropionate occurs as a white to pale yellow powder. It is odorless.

It is freely soluble in chloroform, soluble in methanol, sparingly soluble in ethanol (95%) and in 1,4-dioxane, slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 208°C (with decomposition).

**Identification** (1) Dissolve 2 mg of Beclometasone Dipropionate in 2 mL of sulfuric acid: initially a yellowish color develops, and gradually changes through orange to dark red-brown. To this solution add carefully 10 mL of water: the color changes to bluish green, and a flocculent precipitate is formed.

(2) Dissolve 0.01 g of Beclometasone Dipropionate in 1 mL of methanol, add 1 mL of Fehling’s TS, and heat: a red to red-brown precipitate is formed.

(3) Prepare the test solution with 0.02 g of Beclometasone Dipropionate as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 1 mL of sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests <1.09> for chloride.

(4) Determine the infrared absorption spectrum of Beclometasone Dipropionate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Beclometasone Dipropionate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Beclometasone Dipropionate and Beclometasone Dipropionate RS in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

**Optical rotation** <2.49> \([\alpha]_D^2 + 88° \pm 94°\) (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

**Purity** (1) Heavy metals <1.07>—Proceed with 0.5 g of Beclometasone Dipropionate according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 20 mg of Beclometasone Dipropionate in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (475:25:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 20 mg each of Beclometasone Dipropionate and Beclometasone Dipropionate RS, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and methanol to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_t\) and \(Q_b\), of the peak area of beclometasone dipropionate to that.
Beef Tallow

Sevum Bovinum

牛脂

Beef Tallow is a purified fat obtained by wet steam rendering from the fresh fatty tissues of Bos taurus Linné var. domesticus Gmelin (Bovidae).

Description Beef Tallow occurs as a white, uniform mass. It has a characteristic odor and a mild taste.

It is freely soluble in diethyl ether and in petroleum ether, very slightly soluble in ethanol (95), and practically insoluble in water.

It is breakable at a low temperature, but softens above 9°C.

Acid value <1.13> Not more than 2.0.

Saponification value <1.13> 193 – 200

Iodine value <1.13> 33 – 50 (When the sample is insoluble in 20 mL of cyclohexane, dissolve it by shaking a glass-stoppered flask in warm water. Then, if insoluble, increase the volume of solvent.)

Purity (1) Moisture and coloration—Beef Tallow (5.0 g), melted by heating on a water bath, forms a clear liquid, from which no water separates. In a 10-mm thick layer of the liquid, it is colorless or slightly yellow.

(2) Alkalinity—To 2.0 g of Beef Tallow add 10 mL of water, melt by heating on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: no color develops.

(3) Chloride—To 1.5 g of Beef Tallow add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the turbidity of the mixture does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, then add 5 drops of an ethanolic solution of silver nitrate (1 in 50).

Containers and storage Containers—Well-closed containers.

White Beeswax

Cera Alba

サラシミツロウ

White Beeswax is bleached Yellow Beeswax.

Description White Beeswax occurs as white to yellowish white masses. It has a characteristic odor. It is comparatively brittle when cooled, and the fractured surface is granular, and non-crystalline.

It is slightly soluble in diethyl ether, and practically insoluble in water and in ethanol (99.5).

Acid value <1.13> 5 – 9 or 17 – 22 Weigh accurately about 6 g of White Beeswax, place in a glass-stoppered 250-mL flask, and add 50 mL of ethanol (99.5). Warm the mixture to dissolve the wax, add 1 mL of phenolphthalein TS, and proceed as directed in the Acid value. Perform a blank determination using solvent which is not previously neutralized, and make any necessary correction.

Saponification value <1.13> 80 – 100 Weigh accurately about 3 g of White Beeswax, place in a glass-stoppered 250-mL flask, and add exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS and 50 mL of ethanol (95), heat for 4 hours on a water bath under a reflux condenser, and proceed as directed in the Saponification value.

Melting point <1.13> 60 – 67°C.

Purity Paraffin, fat, Japan wax or resin—Melt White Beeswax at the lowest possible temperature, drip the liquid into a vessel containing ethanol (95) to form granules, and allow them to stand in air for 24 hours. Drop the granules into two mixtures of ethanol (95) and water, one adjusted so as to have a specific gravity of 0.95 and the other 0.97: the granules sink or are suspended in the mixture with the specific gravity of 0.95, and float or are suspended in the other mixture.

Containers and storage Containers—Well-closed containers.
Yellow Beeswax

Cera Flava

ミツロウ

Yellow Beeswax is the purified wax obtained from honeycombs such as those of Apis mellifera Linné or Apis cerana Fabricius (Apidae).

**Description** Yellow Beeswax occurs as light yellow to brownish yellow masses. It has a characteristic odor, which is not rancid. It is comparatively brittle when cooled, and the fractured surface is granular, and non-crystalline.

**Acid value**: Weigh accurately about 6 g of Yellow Beeswax, place in a glass-stoppered 250-mL flask, and add 50 mL of ethanol (99.5). Warm the mixture to dissolve the wax, add 1 mL of phenolphthalein TS, and proceed as directed in the Acid value. Perform a blank determination using solvent which is not previously neutralized, and make any necessary correction.

**Saponification value**: Weigh accurately about 3 g of Yellow Beeswax, place in a 250-mL glass-stoppered flask, and add 25 mL of 0.5 mol/L potassium hydroxide-ethanol and 50 mL of ethanol (95), insert a reflux condenser, heat for 4 hours on a water bath, and proceed as directed in the Saponification value.

**Melting point**: Melt Yellow Beeswax at the lowest possible temperature, drip the liquid into a glass vessel containing ethanol (95) to form granules, and allow them to stand in air for 24 hours. Drop the granules into two mixtures of ethanol (95) and water, one adjusted so as to have a specific gravity of 0.95 and the other 0.97: the granules sink or are suspended in the mixture with the specific gravity of 0.95, and float or are suspended in the other mixture.

**Containers and storage**: Well-closed containers.

Bekanamycin Sulfate

ベカナマイシン硫酸塩

C$_{18}$H$_{37}$N$_{5}$O$_{10}$·xH$_{2}$SO$_{4}$

3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-[2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine sulfate

[70550-99-1]

Bekanamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of the mutant of *Streptomycetes kanamyceticus*.

It contains not less than 680 mg (potency) and not more than 770 mg (potency) per mg, calculated on the dried basis. The potency of Bekanamycin Sulfate is expressed as mass (potency) of bekamycin (C$_{18}$H$_{37}$N$_{5}$O$_{10}$: 483.51).

**Description** Bekanamycin Sulfate occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

**Identification**

(1) Dissolve 20 mg of Bekanamycin Sulfate in 2 mL of 1/15 mol/L phosphate buffer solution, pH 5.6, add 1 mL of ninhydrin TS, and boil: a blue-purple color develops.

(2) Dissolve 30 mg each of Bekanamycin Sulfate and Bekanamycin Sulfate RS in 5 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same Rf value.

(3) To a solution of Bekanamycin Sulfate (1 in 5) add 1 drop of barium chloride TS: a white turbidity is produced.

**Optical rotation**: [α]$_{D}$: +102° to +116° (after drying, 0.25 g, water, 25 mL, 100 mm).

**pH**: The pH of a solution obtained by dissolving 0.50 g of Bekanamycin Sulfate in 10 mL of water is between
distance of about 10 cm, and air-dry the plate. Spray evenly a solution of potassium dihydrogen phosphate (3 in 40) to a gel for thin-layer chromatography. Develop the plate with a solvent. Test the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solvent.

Perform the test according to the Cylinder-plate Assay method as directed under Microbial Assay for Antibiotics.

(b) Test organism—Bacillus subtilis ATCC 6633

(i) Test organism—Bacillus subtilis ATCC 6633
(ii) Culture medium—Use the medium i in 1) under (1)

(iii) Agar media for seed and base layer having pH 6.0 and 8.5.

(iv) Sample solutions—Weigh accurately an amount of Bekanamycin Sulfate RS, previously dried, equivalent to 2.0 g (potency), dissolve in diluted phosphate buffer solution (pH 8.0) to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 10 µg (potency) and 2.5 µg (potency), and use these solutions as the high concentration sample solution and low concentration standard solution, respectively.

(v) Standard solutions—Weigh accurately an amount of Bekanamycin Sulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15 °C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 10 µg (potency) and 2.5 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

Containers and storage—Containers—Tight containers.

Benidipine Hydrochloride

ベニジピン塩酸塩

\[
\text{C}_{28}\text{H}_{31}\text{N}_{3}\text{O}_{6}\cdot\text{HCl}: 542.02
\]

3-[(3RS)-1-Benzylpiperidin-3-yl]-5-methyl (4RS)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate monohydrochloride [91599-74-5]

Benidipine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of \(\text{C}_{28}\text{H}_{31}\text{N}_{3}\text{O}_{6}\cdot\text{HCl}\).

Description Benidipine Hydrochloride occurs as a yellow crystalline powder.

It is very soluble in formic acid, soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Benidipine Hydrochloride in methanol (1 in 100) shows no optical rotation.

Melting point: about 200°C (with decomposition).

Identification

(1) Determine the absorption spectrum of a solution of Benidipine Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benidipine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Benidipine Hydrochloride (1 in 10) add 5 mL of ammonia TS, heat on a water bath for 5 minutes, cool, and filter. The filtrate, which is acidified with dilute nitric acid, responds to the Qualitative Tests <1.69> (2) for chloride.

Purity

(1) Heavy metals <1.07>—Proceed with 1.0 g of Benidipine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Benidipine Hydrochloride in 100 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and methanol (1:1) to make exactly 500 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of bisbenzyl-
piperidyl ester having the relative retention time of about 0.35 with respect to benidipine, dehydro derivative having the relative retention time of about 0.75 and other related substances are not larger than 1/2 times the peak area of benidipine with the standard solution, and the total area of the peaks other than benidipine is not larger than the peak area of benidipine with the standard solution. For this calculation, use the peak areas of bisbenzylpiperidyl ester and dehydro derivative after multiplying by their response factors, 1.6, respectively.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0, methanol and tetrahydrofuran (65:27:8).

Flow rate: Adjust the flow rate so that the retention time of benidipine is about 20 minutes.

Time span of measurement: About 2 times as long as the retention time of benidipine beginning after the solvent peak.

**System suitability—**

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mixture of water and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of benidipine obtained with 10 μL of this solution is equivalent to 18 to 32% of that with 10 μL of the standard solution.

System performance: Dissolve 6 mg of Benidipine Hydrochloride and 5 mg of benzoin in 200 mL of the mixture of water and methanol (1:1). When the procedure is run with 10 mL of this solution under the above operating conditions, benzoin and benidipine are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benidipine is not more than 3.5%.

**Loss on drying** <2.41> Not more than 0.5% (0.5 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.7 g of Benidipine Hydrochloride, previously dried, dissolve in 10 mL of formic acid, add 70 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 54.20 mg of C$_2$H$_2$N$_2$O$_6$.HCl

**Containers and storage** Containers—Tight containers.
with 10 \mu L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benidipine is not more than 2.0%.

**Uniformity of dosage units**

Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Benidipine Hydrochloride Tablets add 40 mL of a mixture of diluted phosphoric acid (1 in 500) and methanol (1:1), shake to disintegrate, and add a suitable amount of the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly V mL of a solution, containing 40 \mu g of benidipine hydrochloride (C_{28}H_{31}N_{3}O_{6}.HCl) per mL. Centrifuge the solution, pipet 20 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of benidipine hydrochloride (C}_{28}\text{H}_{31}\text{N}_{3}\text{O}_{6}.\text{HCl)} = M_S \times Q_T / Q_S \times V / 1000
\]

\( M_S \): Amount (mg) of benidipine hydrochloride for assay

**Internal standard** solution—A solution of benzoin in a mixture of water and methanol (1:1) (13 in 200,000).

**Dissolution**

Perform the test at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate of a 2-mg tablet and a 4-mg tablet in 30 minutes is not less than 80%, and that of a 8-mg tablet in 45 minutes is not less than 85%.

Start the test with 1 tablet of Benidipine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \mu m. Discard the first 10 mL of the filtrate pipet the subsequent V mL, and add the dissolution medium to make exactly V mL so that each mL contains about 2.2 \mu g of benidipine hydrochloride (C_{28}H_{31}N_{3}O_{6}.HCl) according to the labeled amount. Pipet 5 mL of this solution, add exactly 5 mL of the mobile phase, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of benidipine hydrochloride for assay, previously dried at 105°C and cooled, and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the mobile phase, and use this solution as the standard solution. Perform the test with exactly 50 \mu L of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of benidipine.

Dissolution rate (%) of benidipine hydrochloride (C_{28}H_{31}N_{3}O_{6}.HCl) with respect to the labeled amount

\[
= M_S \times A_T / A_S \times V' / V \times 1 / C \times 9
\]

\( M_S \): Amount (mg) of benidipine hydrochloride for assay

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \mu m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of benidipine is about 5 minutes.

**System suitability—**

System performance: When the procedure is run with 50 \mu L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benidipine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 \mu L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benidipine is not more than 1.5%.

**Assay**

Weigh accurately the mass of not less than 20 Benidipine Hydrochloride Tablets, and power using an agate mortar. Weigh accurately a part of the powder, equivalent to about 8 mg of benidipine hydrochloride (C_{28}H_{31}N_{3}O_{6}.HCl), add about 150 mL of a mixture of diluted phosphoric acid (1 in 500) and methanol (1:1), shake, then add the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 200 mL, and centrifuge. Pipet 20 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of benidipine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 \mu L each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of benidipine to that of the internal standard.

\[
\text{Amount (mg) of benidipine hydrochloride (C}_{28}\text{H}_{31}\text{N}_{3}\text{O}_{6}.\text{HCl)} = M_S \times Q_T / Q_S \times 1/5
\]

\( M_S \): Amount (mg) of benidipine hydrochloride for assay

**Internal standard** solution—A solution of benzoin in a mixture of water and methanol (1:1) (13 in 200,000).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 \mu m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0, methanol and tetrahydrofuran (65:27:8).
Identification (1) Determine the absorption spectrum of a solution of Benserazide Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance of this solution at 430 nm is not more than 0.10.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Benserazide Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.25 g of Benserazide Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL and 3 mL of the sample solution, add methanol to make exactly 200 mL, and use these solutions as the standard solution (1) and (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.06>. Spot 2 μL each of the sample solution and standard solutions (1) and (2) on a plate of cellulose for thin-layer chromatography. Develop the plate with a solution of formic acid in sodium chloride TS (1 in 1000) to a distance of about 10 cm, and air-dry the plate. Spray evenly sodium carbonate TS, air-dry, and then spray evenly Folin’s TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (2), and the number of the spots which intense more than the spot from the standard solution (1) are not more than 2.

Water <2.45> Not more than 2.5% (0.5 g, direct titration). Use a solution of salicylic acid in methanol for Karl Fischer method (3 in 20) instead of methanol for Karl Fischer method.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Benserazide Hydrochloride, dissolve in 5 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> immediately with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 29.37 mg of C₁₀H₁₅N₃O₅.HCl

Containers and storage Containers—Well-closed containers.

Bentonite

Bentonite is a natural, colloidal, hydrated aluminum silicate.

Description Bentonite occurs as a very fine, white to light yellow-brown powder. It is odorless. It has a slightly earthy taste.

It is practically insoluble in water and in diethyl ether. It swells in water.

Identification (1) Add 0.5 g of Bentonite to 3 mL of diluted sulfuric acid (1 in 3), and heat until white fumes are evolved. Cool, add 20 mL of water, and filter. To 5 mL of the filtrate add 3 mL of ammonia TS: a white, gelatinous
precipitate is produced, which turns red on the addition of 5 drops of alizarin red S TS.

(2) Wash the residue obtained in (1) with water, add 2 mL of a solution of methylene blue trihydrate (1 in 10,000), and wash again with water: the residue is blue in color.

**pH** <2.54> To 1.0 g of Bentonite add 50 mL of water, and shake: the pH of the suspension is between 9.0 and 10.5.

**Purity** (1) Heavy metals <1.07>—To 1.5 g of Bentonite add 80 mL of water and 5 mL of hydrochloric acid, and boil gently for 20 minutes with thorough stirring. Cool, centrifuge, collect the supernatant liquid, wash the residue with two 10-mL portions of water, and centrifuge each. Combine the supernatant liquid and the washings, and add dropwise ammonia solution (28). When a precipitate is produced, add dropwise dilute hydrochloric acid with vigorous stirring, and dissolve. To the solution add 0.45 g of hydroxyaluminium chloride, and heat. Cool, and add 0.45 g of sodium acetate trihydrate, 6 mL of dilute acetic acid and water to make 150 mL. Pipet 50 mL of the solution, and perform the test using this solution as the test solution. Prepare the control solution as follows: mix 2.5 mL of Standard Lead Solution, 0.15 g of hydroxyaluminium chloride, 0.15 g of sodium acetate trihydrate, and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 50 ppm).

(2) Arsenic <1.17>—To 1.0 g of Bentonite add 5 mL of dilute hydrochloric acid, and gently heat to boil while stirring. Cool immediately, and centrifuge. To the residue add 5 mL of dilute hydrochloric acid, shake well, and centrifuge. To the residue add 10 mL of water, and perform the same operations. Combine all the extracts, and heat on a water bath to concentrate to 5 mL. Perform the test with this solution as the test solution (not more than 2 ppm).

(3) Foreign matter—Place 2.0 g of Bentonite in a mortar, add 20 mL of water to swell, disperse evenly with a pestle, and dilute with water to 100 mL. Pour the suspension through a No. 200 (74 μm) sieve, and wash the sieve thoroughly with water. No grit is felt when the fingers are rubbed over the wire mesh of the sieve.

**Loss on drying** <2.47> 5.0 – 10.0% (2 g, 105°C, 2 hours).

**Gel formation** Mix 6.0 g of Bentonite with 0.30 g of magnesium oxide. Add the mixture, in several portions, to 200 mL of water contained in a glass-stoppered 500-mL cylinder. Agitate for 1 hour, transfer 100 mL of the suspension to a 100-mL graduated cylinder, and allow to stand for 24 hours: not more than 2 mL of supernatant appears on the surface.

**Swelling power** To 100 mL of water in a glass-stoppered 100-mL cylinder add 2.0 g of Bentonite in ten portions, allowing each portion to settle before adding the next, and allow to stand for 24 hours: the apparent volume of the sediment at the bottom is not less than 20 mL.

**Containers and storage** Containers—Well-closed containers.

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**Benzalkonium Chloride**

Benzalkonium Chloride is represented by the formula \([\text{C}_6\text{H}_5\text{CH}_2\text{N(CH}_3\text{)}_2\text{R}]\text{Cl}\), in which \(R\) extends from C\(_8\)H\(_{17}\) to C\(_{18}\)H\(_{37}\), with C\(_{12}\)H\(_{25}\) and C\(_{14}\)H\(_{29}\) comprising the major portion.

It contains not less than 95.0% and not more than 105.0% of benzalkonium chloride (as \(\text{C}_{22}\text{H}_{40}\text{ClN:} \text{C}_{540.01}\)), calculated on the anhydrous basis.

**Description** Benzalkonium Chloride occurs as a white to yellowish white powder, colorless to light yellow, gelatinous pieces, or jelly-like fluid or mass. It has a characteristic odor.

It is very soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Benzalkonium Chloride foams strongly when shaken.

**Identification** (1) Dissolve 0.2 g of Benzalkonium Chloride in 1 mL of sulfuric acid, add 0.1 g of sodium nitrate, and heat for 5 minutes on a water bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool, and filter; the filtrate responds to the Qualitative Tests <1.09> for primary aromatic amines. The color of the solution is red.

(2) To 2 mL of a solution of Benzalkonium Chloride (1 in 1000) add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color develops. Add 4 mL of chloroform to this solution, and shake vigorously: the blue color shifts to the chloroform layer. Collect the chloroform layer, and add dropwise, with stirring, a solution of sodium lauryl sulfate (1 in 1000): the chloroform layer turns colorless.

(3) Determine the absorption spectrum of a solution of Benzalkonium Chloride in 0.1 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 1 mL of a solution of Benzalkonium Chloride (1 in 100) add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. This precipitate does not dissolve on the addition of dilute nitric acid, but dissolves on the addition of ammonia TS.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Benzalkonium Chloride in 10 mL of water: the solution is clear and colorless to light yellow.

(2) Petroleum ether-soluble substances—To 3.0 g of Benzalkonium Chloride add water to make 50 mL, then add 50 mL of ethanol (99.5) and 5 mL of 0.5 mol/L sodium hydroxide TS, and extract with three 50-mL portions of petroleum ether. Combine the petroleum ether extracts, and wash with three 50-mL portions of dilute ethanol. After shaking well with 10 g of anhydrous sodium sulfate, filter through a dry filter paper, and wash the filter paper with two 10-mL portions of petroleum ether. Evaporate the petroleum ether on a water bath by heating, and dry the residue at 105°C for 1 hour: the residue is not more than 1.0%.
**Benzalkonium Chloride Solution**

Benzalkonium Chloride Solution is an aqueous solution containing not more than 50.0 w/v% of benzalkonium chloride.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of benzalkonium chloride (C\(_{22}\)H\(_{40}\)ClN: 354.01).

**Method of preparation**
Dissolve Benzalkonium Chloride in Water, Purified Water or Purified Water in Containers. It is also prepared by diluting Concentrated Benzalkonium Chloride Solution 50 with Water, Purified Water or Purified Water in Containers.

**Description**
Benzalkonium Chloride Solution is a clear, colorless to light yellow liquid, having a characteristic odor. It foams strongly on shaking.

**Identification (1)**
Evaporate a volume of Benzalkonium Chloride Solution, equivalent to 0.2 g of Benzalkonium Chloride according to the labeled amount, on a water bath to dryness, and proceed with the residue as directed in the Identification (1) under Benzalkonium Chloride.

(2) To a volume of Benzalkonium Chloride Solution, equivalent to 0.01 g of Benzalkonium Chloride according to the labeled amount, add water to make 10 mL. Proceed with 2 mL of this solution as directed in the Identification (2) under Benzalkonium Chloride.

(3) To a volume of Benzalkonium Chloride Solution, equivalent to 1 g of Benzalkonium Chloride according to the labeled amount, add water or concentrate on a water bath, if necessary, to make 10 mL. To 1 mL of this solution add 0.1 mol/L hydrochloric acid VS to make 200 mL, and proceed as directed in the Identification (3) under Benzalkonium Chloride.

(4) To a volume of Benzalkonium Chloride Solution, equivalent to 0.1 g of Benzalkonium Chloride according to the labeled amount, add water or concentrate on a water bath, if necessary, to make 10 mL. Proceed with 1 mL of this solution as directed in the Identification (4) under Benzalkonium Chloride.

**Assay**
Pipet a volume of Benzalkonium Chloride Solution, equivalent to about 0.15 g of benzalkonium chloride (C\(_{22}\)H\(_{40}\)ClN), dilute with water to make 75 mL, if necessary, and proceed as directed in the Assay under Benzalkonium Chloride.

Each mL of 0.02 mol/L sodium tetraphenylboron VS = 7.080 mg of benzalkonium chloride (C\(_{22}\)H\(_{40}\)ClN)
to make 50 mL, then add 50 mL of ethanol (99.5) and 5 mL of 0.5 mol/L sodium hydroxide TS, and extract with three 50-mL portions of petroleum ether. Combine the petroleum ether extracts, and wash with three 50-mL portions of dilute ethanol. After shaking well with 10 g of anhydrous sodium sulfate, filter through a dry filter paper, and wash the filter paper with two 10-mL portions of petroleum ether.

Evaporate the petroleum ether on a water bath by heating, and dry the residue at 105°C for 1 hour: the residue is not more than 1.0%.

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Assay**

Weigh accurately about 0.3 g of Benzalkonium Chloride Concentrated Solution 50, and dissolve in 75 mL of water. Adjust the pH to between 2.6 and 3.4 by adding dropwise diluted dilute hydrochloric acid (1 in 2), add 1 drop of methyl orange TS, and titrate <2.50> with 0.02 mol/L sodium tetraphenylboron VS until the color of the solution becomes red.

Each mL of 0.02 mol/L sodium tetraphenylborate VS = 7.080 mg of benzalkonium chloride (C₂₂H₄₀ClN)

**Containers and storage** Containers—Tight containers.

### Benzbramarone

ベンズブロマロン

**C₁₇H₁₂Br₂O₃**: 424.08
3,5-Dibromo-4-hydroxyphenyl 2-ethylbenzo[6]furan-3-yl ketone

3562-84-2

Benzbramarone, when dried, contains not less than 98.5% and not more than 101.0% of C₁₇H₁₂Br₂O₃.

**Description**

Benzbramarone occurs as a white to light yellow, crystalline powder.

It is very soluble in N,N-dimethylformamide, freely soluble in acetone, sparingly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

**Identification**

(1) Determine the absorption spectrum of a solution of Benzbramarone in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzbramarone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point**<2.60> 149 – 153°C

**Purity**

(1) Sulfate <1.14>—Dissolve 1.0 g of Benzbramarone in 40 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 40 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.019%).

(2) Soluble halides—Dissolve 0.5 g of Benzbramarone in 40 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Proceed with this solution as directed under Chloride Limit Test <1.05>. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Benzbramarone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Iron <1.10>—Prepare the test solution with 1.0 g of Benzbramarone according to Method 3, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 20 ppm).

(5) Related substances—Dissolve 0.10 g of Benzbramarone in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, 4-methyl-2-pentanone, ethanol (99.5) and acetic acid (100:20:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**<2.41> Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 50°C, 4 hours).

**Residue on ignition**<2.44> Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.6 g of Benzbramarone, previously dried, dissolve in 30 mL of N,N-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (indicator: 5 drops of thymol blue-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 42.41 mg of C₁₇H₁₂Br₂O₃

**Containers and storage** Containers—Light-resistant.

**Storage**—Light-resistant.
Benzethonium Chloride
ベンゼトニウム塩化物

\[
C_{27}H_{42}ClNO_2: 448.08
\]

Benzethonium Chloride, when dried, contains not less than 97.0% of \(C_{27}H_{42}ClNO_2\).

**Description** Benzethonium Chloride occurs as colorless or white crystals. It is odorless.

It is very soluble in ethanol (95), freely soluble in water, and practically insoluble in diethyl ether.

A solution of Benzethonium Chloride foams strongly when shaken.

**Identification (1)** Dissolve 0.2 g of Benzethonium Chloride in 1 mL of sulfuric acid, add 0.1 g of sodium nitrate, and heat for 5 minutes on a water bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests \(<1.09\) for primary aromatic amines, developing a red color.

(2) To 2 mL of a solution of Benzethonium Chloride (1 in 1000) add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color develops. Add 4 mL of chloroform to this solution, and shake vigorously: the blue color shifts to the chloroform layer. Collect the chloroform layer, and add dropwise a solution of sodium lauryl sulfate (1 in 1000) with stirring: the chloroform layer turns colorless.

(3) Determine the absorption spectrum of a solution of Benzethonium Chloride in 0.1 mol/L hydrochloric acid TS (1 in 5000) as directed in the Identification (4) under Benzethonium Chloride. (4) To 1 mL of a solution of Benzethonium Chloride (1 in 100) add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. This precipitate does not dissolve on addition of dilute nitric acid, but dissolves on addition of ammonia TS.

**Melting point** \(<2.60\) 158 – 164°C (after drying).

**Purity** Ammonium—Dissolve 0.10 g of Benzethonium Chloride in 5 mL of water, and boil with 3 mL of sodium hydroxide TS: the evolving gas dose not change moistened red litmus paper to blue.

**Loss on drying** \(<2.41\) Not more than 5.0% (1 g, 105°C, 4 hours).

**Residue on ignition** \(<2.44\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Benzethonium Chloride, previously dried, dissolve in 75 mL of water, add diluted dilute hydrochloric acid (1 in 2) dropwise to adjust the pH to 2.6–3.4, then add 1 drop of methyl orange TS, and titrate \(<2.50\) with 0.02 mol/L tetrabutylinylboron VS until the solution develops a red.

Each mL of 0.02 mol/L sodium tetrabutylinylboron VS = 8.962 mg of \(C_2H_2ClINO_2\).

**Containers and storage** Containers—Tight containers.

**Storage**—Light-resistant.

Benzethonium Chloride Solution
ベンゼトニウム塩化物液

Benzethonium Chloride Solution contains not less than 93.0% and not more than 107.0% of the labeled amount of benzethonium chloride \((C_{27}H_{42}ClNO_2: 448.08)\).

**Method of preparation** Dissolve Benzethonium Chloride in Water, Purified Water or Purified Water in Containers.

**Description** Benzethonium Chloride Solution is a clear, colorless liquid. It is odorless.

It foams strongly when shaken.

**Identification (1)** Evaporate a volume of Benzethonium Chloride Solution, equivalent to 0.2 g of Benzethonium Chloride according to the labeled amount, on a water bath to dryness, and proceed with the residue as directed in the Identification (1) under Benzethonium Chloride.

(2) To a volume of Benzethonium Chloride Solution, equivalent to 0.01 g of Benzethonium Chloride according to the labeled amount, add water to make 10 mL, proceed with 2 mL of this solution as directed in the Identification (2) under Benzethonium Chloride.

(3) To a volume of Benzethonium Chloride Solution, equivalent to 1 g of Benzethonium Chloride according to the labeled amount, and add water or concentrate on a water bath to make 10 mL. To 1 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 500 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry \(<2.24\): it exhibits maxima between 262 nm and 264 nm, between 268 nm and 270 nm, and between 274 nm and 276 nm.

(4) To a volume of Benzethonium Chloride Solution, equivalent to 0.1 g of Benzethonium Chloride according to the labeled amount, add water, or concentrate on a water bath, if necessary, to make 10 mL, and proceed with 1 mL of this solution as directed in the Identification (4) under Benzethonium Chloride.

**Purity (1)** Nitrite—Add 1.0 mL of Benzethonium Chloride Solution to a mixture of 1 mL of a solution of glycine (1 in 10) and 0.5 mL of acetic acid (31): no gas is evolved.

(2) Oxidizing substances—To 5 mL of Benzethonium Chloride Solution add 0.5 mL of potassium iodide TS and 2 to 3 drops of dilute hydrochloric acid: no yellow color is produced.

**Assay** Pipet a volume of Benzethonium Chloride Solution, equivalent to about 0.2 g of benzethonium chloride \((C_{27}H_{42}ClNO_2)\), dilute with water to make 75 mL, if necessary, and proceed as directed in the Assay under Benzetho-
Benzoic Acid

**Description**
Benzoic Acid occurs as white crystals or crystalline powder. It is odorless, or has a faint, benzaldehyde-like odor.

It is freely soluble in ethanol (95), in acetone and in diethyl ether, soluble in hot water, and slightly soluble in water.

**Identification**
Dissolve 1 g of Benzoic Acid in 8 mL of sodium hydroxide TS, and add water to make 100 mL. This solution responds to the Qualitative Tests (1.09) (2) for benzoate.

**Melting point**
\(<2.60\) 121 – 124°C

**Purity**
(1) Heavy metals \(<1.07\)—Dissolve 1.0 g of Benzoic Acid in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, 25 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

(2) Chlorinated compounds—Take 0.5 g of Benzoic Acid and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water, and dry. Ignite it at about 600°C, dissolve in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washing, add water to make 50 mL, and add 0.5 mL of silver nitrate TS: this solution has not more turbid than the following control solution.

Control solution: Dissolve 0.7 g of calcium carbonate in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, add 1.2 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL, and add 0.5 mL of silver nitrate TS.

(3) Potassium permanganate-reducing substances—Add 0.02 mol/L potassium permanganate VS dropwise to a boiling mixture of 100 mL of water and 1.5 mL of sulfuric acid, until a red color persists for 30 seconds. Dissolve 1.0 g of Benzoic Acid in this boiling solution, and add 0.50 mL of 0.02 mol/L potassium permanganate VS: a red color persists for at least 15 seconds.

(4) Phthalic acid—To 0.10 g of Benzoic Acid add 1 mL of water and 1 mL of resorcinol-sulfuric acid TS, and heat the mixture in an oil bath heated at a temperature between 120°C and 125°C. After evaporating the water, heat the residue for 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of the solution add 10 mL of a solution of sodium hydroxide (43 in 500), shake, then examine under light at a wavelength between 470 nm and 490 nm: the green fluorescence of the solution is not more intense than that of the following control solution.

Control solution: Dissolve 61 mg of potassium hydrogen phthalate in water to make exactly 1000 mL. Measure exactly 1 mL of the solution, add 1 mL of resorcinol-sulfuric acid TS, and proceed as directed above.

(5) Readily carbonizable substances \(<1.15\)—Perform the test with 0.5 g of Benzoic Acid. The solution is not more colored than Matching Fluid Q.

**Loss on drying**
\(<2.41\) Not more than 0.5% (1 g, silica gel, 3 hours).

**Residue on ignition**
\(<2.44\) Not more than 0.05% (1 g).

**Assay**
Weigh accurately about 0.5 g of Benzoic Acid, previously dried, dissolve in 25 mL of neutralized ethanol and 25 mL of water, and titrate \(\leq 2.50\) with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS = 12.21 mg of C₇H₆O₂

**Containers and storage**
Containers—Well-closed containers. Storage—Light-resistant.

Benzy1 Alcohol

**Description**
Benzy1 Alcohol is a clear, colorless oily liquid.

It is miscible with ethanol (95), with fatty oils and with essential oils.

It is soluble in water.

Specific gravity \(d_{20}^{20}\): 1.043 – 1.049

**Identification**
Determine the infrared absorption spectrum of Benzy1 Alcohol as directed in the liquid film method under Infrared Spectrophotometry \(\leq 2.25\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**RefRACTive index**
\(\leq 2.45\) \(n_{D}^{20}\): 1.538 – 1.541

**Purity**
(1) Clarity and color of solution—Dissolve 2.0
mL of Benzyl Alcohol in 60 mL of water: the solution is clear and colorless.

(2) Acidity—To 10 mL of Benzyl Alcohol add 10 mL of ethanol (95) and 2 drops of phenolphthalein TS, and add dropwise 0.1 mol/L sodium hydroxide VS until the solution acquires a light red color: the amount of 0.1 mol/L sodium hydroxide VS used is not more than 1.0 mL.

(3) Benzaldehyde and other related substances—Use Benzyl Alcohol as the sample solution. Separately, dissolve exactly 0.100 g of ethylbenzene in Benzyl Alcohol to make exactly 10 mL. Pipet 2 mL of this solution, add Benzyl Alcohol to make exactly 20 mL, and use this solution as the ethylbenzene stock solution. Separately, dissolve exactly 2.000 g of dicyclohexyl in Benzyl Alcohol to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of ethylbenzene stock solution and exactly 3 mL of dicyclohexyl stock solution, then add Benzyl Alcohol to make exactly 20 mL, and use this solution as the standard solution (1). Perform the test with exactly 0.1 mL of each of the sample solution and standard solution (1) as directed under Gas Chromatography \( <2.02 \) according to the following conditions, and when the peak having the retention time corresponding to ethylbenzene and dicyclohexyl appears on the chromatogram obtained with the sample solution, correct the peak areas of ethylbenzene and dicyclohexyl obtained with the standard solution (1) by deducting the relevant peak area obtained with the sample solution, the peak area of benzaldehyde obtained with the sample solution is not more than the difference between the peak areas of benzaldehyde of the sample solution and the standard solution (2) (0.0%)

relating to benzaldehyde and cyclohexylmethanol obtained with the sample solution is not more than 2 times the peak area or the corrected peak area of ethylbenzene with the standard solution (2) (0.02%). For these calculation the peak areas less than 1/100 times the peak area or the corrected peak area of ethylbenzene with the standard solution (2) are excluded.

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated inside with polyethylene glycol 20 M for gas chromatography in 0.5 \( \mu \)m thickness.

Column temperature: Inject at a constant temperature of about 50°C, raise the temperature at a rate of 5°C per minute to 220°C, and maintain at 220°C for 35 minutes.

Temperature of injection port: A constant temperature of about 200°C.

Temperature of detector: A constant temperature of about 310°C.

Carrier gas: Helium.
Flow rate: 25 cm/second.
Split ratio: Splitless.
Detection sensitivity: When 0.1 \( \mu \)L of the standard solution (1) is injected, adjust the sensitivity of the detector so that the height of the peak of ethylbenzene is not less than 30% of the full scale of the recorder. For Benzyl Alcohol labeled to use for injection, use the standard solution (2) instead of the standard solution (1).

System suitability—
System performance: When the procedure is run with the standard solution (1) under the above operating conditions, the retention time of benzyl alcohol is about 26 minutes, the relative retention times of ethylbenzene, dicyclohexyl, benzaldehyde and cyclohexylmethanol with respect to benzyl alcohol are about 0.28, about 0.59, about 0.68 and about 0.71, respectively, and the resolution between the peaks of benzaldehyde and cyclohexylmethanol is not less than 3.0. In the case of Benzyl Alcohol labeled to use for injection, proceed with the standard solution (2) instead of the standard solution (1).

(4) Peroxide value—Weigh accurately about 5 g of Benzyl Alcohol, and dissolve in 30 mL of a mixture of acetic acid (100) and chloroform (3:2) in a 250-mL glass-stoppered conical flask. Add 0.5 mL of saturated potassium iodide solution, shake for exactly 1 minute, add 30 mL of water, and titrate \( <2.50 \) with 0.01 mol/L sodium thiosulfate VS, adding the titrant slowly with continuous vigorous shaking, until the
blue color of the solution disappears after addition of 5 mL of starch TS near the end point where the solution is a pale yellow color. Perform a blank determination in the same manner. Calculate the amount of peroxide by the following formula: not more than 5. In the determination, the required amount of 0.01 mol/L sodium thiosulfate VS must not exceed 0.1 mL.

\[
\text{Amount (mEq/kg) of peroxide} = \frac{10 \times (V_1 - V_0)}{M}
\]

\(V_1\): Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed in the test

\(V_0\): Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed in the blank determination

\(M\): Amount (g) of the sample

(5) Residue on evaporation—Perform the test after confirmation that the sample meets the requirement of the peroxide value. Transfer 10.0 g of Benzyl Alcohol to a porcelain or quartz crucible or platinum dish, previously weighed accurately, and heat on a hot-plate at not exceeding 200°C, taking care to avoid boiling, to evaporate to dryness. Dry the residue on the hot-plate for 1 hour, and allow to cool in a desiccator: not more than 5 mg.

**Assay** Weigh accurately about 0.9 g of Benzyl Alcohol, add exactly 15 mL of a mixture of pyridine and acetic anhydride (7:1), and heat on a water bath under a reflux condenser with 2.50 mL of neutralized ethanol, and add 0.5 mL of 0.1 mol/L phenolphthalein TS. Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS = 108.1 mg of C\(_7\)H\(_8\)O

*•Containers and storage* Containers—Tight containers.

Storage—Light-resistant.

**Benzyl Benzoate**

ベンジルベンゾート

\[\text{C}_{14}\text{H}_{12}\text{O}_2: 212.24}\n
Benzyl benzoate

[120-51-4]

Benzyl Benzoate contains not less than 99.0% of C\(_{14}\)H\(_{12}\)O\(_2\).

**Description** Benzyl Benzoate is a colorless, clear, viscous liquid. It has a faint, aromatic odor and a pungent, burning taste.

It is miscible with ethanol (95) and with diethyl ether. It is practically insoluble in water.

Congealing point: about 17°C

Specific gravity of \(\rho_20\): about 1.123

Boiling point: about 323°C

**Identification (1)** Heat gently 1 mL of Benzyl Benzoate with 5 mL of sodium carbonate TS and 2 mL of potassium permanganate TS: the odor of benzaldehyde is perceptible.

(2) Warm the titrated mixture obtained in the Assay on a water bath to remove ethanol, and add 0.5 mL of iron (III) chloride TS: a light yellow-red precipitate is produced, which turns white on the addition of dilute hydrochloric acid.

**Refractive index** \(\eta_\text{D}: 1.568 – 1.570\)

**Purity** Acidity—Dissolve 5.0 mL of Benzyl Benzoate in 25 mL of neutralized ethanol, and add 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

**Residue on ignition** Not more than 0.05% (2 g).

**Assay** Weigh accurately about 2 g of Benzyl Benzoate, add exactly 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and boil gently for 1 hour under a reflux condenser with a carbon dioxide-absorbing tube (soda lime). Cool, and titrate 2.50 mL of the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L potassium hydroxide-ethanol VS = 106.1 mg of C\(_7\)H\(_8\)O

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Benzylpenicillin Benzathine Hydrate**

ペンジルペニシリンベンザチン水和物

(Benzylpenicillin Benzathine Hydrate)

[C\(_{16}\)H\(_{19}\)N\(_2\)O\(_4\)S\(_2\).C\(_{16}\)H\(_{20}\)N\(_2\).4H\(_2\)O: 981.18](2S,5R,6R)-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid hemi(N,N'-dibenzylethylenediamine) dihydrate

[41372-02-5]

Benzylpenicillin Benzathine Hydrate is the \(N,N'\)-dibenzylethylenediamine salt of a penicillin compound having antibacterial activity produced by the growth of *Penicillium* species.

It contains not less than 1213 Units and not more than 1333 Units per mg, calculated on the anhydrous basis. The potency of Benzylpenicillin Benzathine Hydrate is expressed as unit calculated from the amount of benzylpenicillin sodium (C\(_{16}\)H\(_{17}\)N\(_2\)NaO\(_4\)S: 356.37). 1 Unit of Benzylpenicillin Benzathine Hydrate is equivalent to 0.6 μg of benzylpenicillin sodium (C\(_{16}\)H\(_{17}\)N\(_2\)NaO\(_4\)S). It contains not less than 24.0% and not more than 27.0% of \(N,N'\)-dibenzylethylenediamine (C\(_{16}\)H\(_{30}\)N\(_2\): 240.34), calculated on the anhydrous basis.

**Description** Benzylpenicillin Benzathine Hydrate occurs as a white crystalline powder.

It is slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification (1)** Determine the absorption spectrum of a
solution of Benzylpenicillin Benzathine Hydrate in methanol (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzylpenicillin Benzathine Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [α]D 20: +217 – +233° (0.1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Benzylpenicillin Benzathine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Benzylpenicillin Benzathine Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 70 mg of Benzylpenicillin Benzathine Hydrate in 25 mL of methanol, add a solution prepared by dissolving 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 2.4 with respect to benzylpenicillin is not larger than 2 times the total area of the peaks of benzylpenicillin and N,N'-dibenzylethlenediamine obtained from the standard solution, and the area of the peak other than benzylpenicillin, N,N'-dibenzylethlenediamine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 25.

**System suitability**—
Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase A to make exactly 20 mL. Confirm that the peak area of benzylpenicillin obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from the standard solution.

**System performance:** When the procedure is run with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of benzylpenicillin is not more than 2.0%.

**Water** <2.48> 5.0–8.0% (1 g, volumetric titration, direct titration).

**Assay** (1) Benzylpenicillin—Weigh accurately an amount of Benzylpenicillin Benzathine Hydrate, equivalent to about 85,000 Units, dissolve in 25 mL of methanol, and add a solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Benzylpenicillin Potassium RS, equivalent to about 85,000 Units, and about 25 mg of N,N'-dibenzylethlenediamine diacetate, dissolve in 25 mL of methanol, and add the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A1 and A5, of benzylpenicillin.

Amount (unit) of benzylpenicillin sodium (C_{16}H_{17}N_{2}NaO_{4}S) = M_{5} \times A_{1}/A_{5}

M_{5}: Amount (unit) of Benzylpenicillin Potassium RS

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>10 – 20</td>
<td>75 → 0</td>
<td>25 → 100</td>
</tr>
<tr>
<td>20 – 55</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL/min
Time span of measurement: About 3 times as long as the retention time of benzylpenicillin beginning after the solvent peak.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of water, methanol and 0.25 mol/L potassium dihydrogen phosphate TS, pH 3.5 (11:7:2).

Flow rate: Adjust the flow rate so that the retention time of benzylpenicillin is about 18 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, N,N'-dibenzylethlenediamine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of N,N'-dibenzylethlenediamine and benzylpenicillin are not more than 2.0%, respectively.

(2) N,N'-Dibenzylethlenediamine—Determine the areas, \( A_1 \) and \( A_2 \), of the peak corresponding to N,N'-dibenzylethlenediamine on the chromatograms obtained in (1) with the sample solution and standard solution.

\[
\text{Amount (%)} \text{ of N,N'-dibenzylethlenediamine (C}_{16}\text{H}_{20}\text{N}_{2}\text{N}_{2}) = \frac{M_2}{M_1} \times \frac{A_1}{A_2} \times 100 \times 0.667
\]

\( M_1 \): Amount (mg) of N,N'-dibenzylethlenediamine diacetate

\( M_2 \): Amount (mg) of the sample

0.667: Conversion factor for the molecular mass of N,N'-dibenzylethlenediamine diacetate (C_{16}H_{20}N_{2}2CH_{3}COOH) to that of N,N'-dibenzylethlenediamine (benzathine, C_{16}H_{20}N_{2}N_{2})

Containers and storage—Containers—Tight containers.

Storage—Light-resistant.

**Benzylpenicillin Potassium**

**Penicillin G Potassium**

ベンジルペニシリンカリウム

\[ \text{C}_{16}\text{H}_{17}\text{KN}_{2}\text{O}_{4}\text{S} : 372.48 \]

Monopotassium (2S,5R,6R)-3,3-dimethyl-7-oxo-6-[[phenylacetyl]amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [113-98-4]

Benzylpenicillin Potassium is the potassium salt of a penicillin substance having antibacterial activity produced by the growth of *Penicillium* species.

It contains not less than 1430 units and not more than 1630 units per mg, calculated on the dried basis. The potency of Benzylpenicillin Potassium is expressed as mass unit of benzylpenicillin potassium (C_{16}H_{17}KN_{2}O_{4}S). One unit of Benzylpenicillin Potassium is equivalent to 0.57 μg of benzylpenicillin potassium.

**Description**

Benzylpenicillin Potassium occurs as white, crystals or crystalline powder.

It is very soluble in water, and slightly soluble in ethanol (99.5).

**Identification**

(1) Determine the absorption spectrum of a solution of Benzylpenicillin Potassium (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Benzylpenicillin Potassium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzylpenicillin Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum or the spectrum of Benzylpenicillin Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Benzylpenicillin Potassium responds to the Qualitative Tests \(<1.09\rangle\) (1) for potassium salt.

**Optical rotation** \(<2.49\rangle\ [α]_{D}^{27}: +270 – +300°\ (1.0 g calculated on the dried basis, water, 50 mL, 100 mm).

**pH** \(<2.54\rangle\ The pH of a solution obtained by dissolving 1.0 g of Benzylpenicillin Potassium in 100 mL of water is between 5.0 and 7.5.

**Purity**

(1) Clarity and color of solution—A solution obtained by dissolving 1 g of Benzylpenicillin Potassium in 10 mL of water is clear, and colorless or light yellow.

(2) Heavy metals \(<1.07\rangle\—Proceed with 2.0 g of Benzylpenicillin Potassium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic \(<1.17\rangle\—Prepare the test solution by incinerating 1.0 g of Benzylpenicillin Potassium according to Method 4, and perform the test. In the incineration, use a crucible of porcelain, and after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) add 1 mL of hydrogen peroxide (30), then burn the ethanol (not more than 2 ppm).

(4) Related substances—Dissolve 40 mg of Benzylpenicillin Potassium in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\rangle\ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than benzylpenicillin obtained from the sample solution is not larger than the peak area of benzylpenicillin from the standard solution, and the total area of the peaks other than benzylpenicillin from the sample solution is not larger than 3 times the peak area of benzylpenicillin from the standard solution.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of diammonium hydrogen phosphate (33 in 5000) and acetonitrile (19:6), adjusted the pH to 8.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of benzylpenicillin is about 7.5 minutes.

Time span of measurement: About 5 times as long as the retention time of benzylpenicillin.

**System suitability**—

Test for required detection: Pipet 10 mL of the standard solution, and add water to make exactly 100 mL. Confirm that the peak area of benzylpenicillin obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: Dissolve 40 mg of Benzylpenicillin Potassium in 20 mL of water. Separately, dissolve 10 mg of methyl parahydroxybenzoate in 20 mL of acetonitrile. To 1 mL of this solution add water to make 20 mL. Mix 1 mL of each of these solutions, and add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, benzylpenicillin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benzylpenicillin is not more than 2.0%.

**Loss on drying** (2.41) Not more than 1.0% (3 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics (4.02) according to the following conditions.

(i) Test organism—Staphylococcus aureus ATCC 6538 P
(ii) Culture medium—Use the medium iii in 3) under (1) Agar media for seed and base layer.
(iii) Standard solutions—Weigh accurately an amount of Benzylpenicillin Potassium RS, equivalent to about 107.0 Units of Benzylpenicillin Potassium for Injection, equivalent to 1.0 × 10^6 Units of Benzylpenicillin Potassium according to the labeled amount, in 10 mL of water is 5.0 to 7.5.

**Purity** Clarity and color of solution—A solution prepared by dissolving an amount of Benzylpenicillin Potassium for Injection, equivalent to 1.0 × 10^6 Units of Benzylpenicillin Potassium according to the labeled amount, in 10 mL of water is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): the absorbance at 400 nm is not more than 0.10.

**Bacterial endotoxins** (4.01) Less than 1.25 × 10^-4 EU/Unit.

**Uniformity of dosage units** (6.02) It meets the requirement of the Mass variation test.

**Foreign insoluble matter** (6.06) Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** (6.07) It meets the requirement.

**Sterility** (4.06) Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Weigh accurately the mass of the contents of not less than 10 containers of Benzylpenicillin Potassium for Injection. Weigh accurately an amount of a portion of the contents, equivalent to about 6 × 10^4 Units of Benzylpenicillin Potassium, dissolve in water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Benzylpenicillin Potassium RS, equivalent to about 6 × 10^4 Units, dissolve in water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions. Determine the peak area of benzylpenicillin, A_1 and A_2, from Benzylpenicillin Potassium for Injection

注射用ベンジルペニシリンカリウム

Benzylpenicillin Potassium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of benzylpenicillin potassium (C_{16}H_{17}KN_2O_4S: 372.48).

**Method of preparation** Prepare as directed under Injections, with Benzylpenicillin Potassium.

**Description** Benzylpenicillin Potassium for Injection occurs as white crystals or crystalline powder.

**Identification** Proceed as directed in the Identification (2) under Benzylpenicillin Potassium.

**Osmotic pressure ratio** Being specified separately.

**pH** (2.54) The pH of a solution prepared by dissolving an amount of Benzylpenicillin Potassium for Injection, equivalent to 1.0 × 10^6 Units of Benzylpenicillin Potassium according to the labeled amount, in 10 mL of water is 5.0 to 7.5.

**Foreign insoluble matter** (6.07) It meets the requirement.

**Insoluble particulate matter** (6.07) It meets the requirement.

**Sterility** (4.06) Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Weigh accurately the mass of the contents of not less than 10 containers of Benzylpenicillin Potassium for Injection. Weigh accurately an amount of a portion of the contents, equivalent to about 6 × 10^4 Units of Benzylpenicillin Potassium, dissolve in water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Benzylpenicillin Potassium RS, equivalent to about 6 × 10^4 Units, dissolve in water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions. Determine the peak area of benzylpenicillin, A_1 and A_2, from
Beraprost Sodium

ベラプロストナトリウム

- C₂₃H₂₉NaO₅: 420.47
- Monosodium (1RS,2RS,3aSR,8bSR)-2,3,3a,8b-tetrahydro-2-hydroxy-1-[(1E,3SR,4RS)-3-hydroxy-4-methyloct-1-en-6-yn-1-yl]-1H-cyclopenta[b]benzofuran-5-butanoate
- Monosodium (1RS,2RS,3aSR,8bSR)-2,3,3a,8b-tetrahydro-2-hydroxy-1-[(1E,3SR,4RS)-3-hydroxy-4-methyloct-1-en-6-yn-1-yl]-1H-cyclopenta[b]benzofuran-5-butanoate (88475-69-8)

Beraprost Sodium, when dried, contains not less than 98.5% and not more than 101.0% of C₂₃H₂₉NaO₅.

**Description** Beraprost Sodium occurs as a white powder.

It is very soluble in methanol, and freely soluble in water and in ethanol (99.5).

It is hygroscopic.

A solution of Beraprost Sodium (1 in 200) shows no optical rotation.

**Identification** (1) Determine the absorption spectrum of a solution of Beraprost Sodium in methanol (3 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of previously dried Beraprost Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Beraprost Sodium in methanol (1 in 1000) responds to the Qualitative Tests <1.09> (1) for sodium salt.

**Purity** (1) Related substances—Dissolve 20 mg of Beraprost Sodium in 2 mL of methanol, and use this solution as the sample solution. Perform the test with 15 µL of the sample solution as directed under Liquid Chromatography <2.01>, and compare the sample solution with the Reference Solution: the amount of the peak, other than the two peaks of beraprost and the peaks mentioned above, is not exceeding 0.1%, and the total amount of the peaks, other than the two peaks of beraprost, is not more than 1.5%.

**Operating conditions**—


Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 µm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: A mixture of methanol and acetic acid (100) (640:330:30:1).

Mobile phase B: A mixture of acetonitrile, water and acetic acid (100) (900:100:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>30 – 45</td>
<td>100 → 56</td>
<td>0 → 44</td>
</tr>
<tr>
<td>45 – 60</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>60 – 70</td>
<td>56 → 0</td>
<td>44 → 100</td>
</tr>
<tr>
<td>70 – 80</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of the second peak of beraprost is about 23 minutes.

Each solution.

Amount (unit) of Benzylpenicillin Potassium (C₁₆H₁₇KN₂O₄S) = Mₛ × A₁/ₐₛ

Mₛ: Amount (unit) of Benzylpenicillin Potassium RS

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To a mixture of diammonium hydrogen phosphate solution (33 in 5000) and acetonitril (19:6), add phosphoric acid to adjust the pH of this solution to 8.0.

Flow rate: Adjust the flow rate so that the retention time of benzylpenicillin is about 7.5 minutes.

System suitability—

System performance: When the procedure is run with 5 theoretical plates and the symmetry factor of the peak of benzylpenicillin are not less than 6000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benzylpenicillin is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.

**Purity (2)** Related substances—Dissolve 20 mg of Beraprost Sodium in 2 mL of methanol, and use this solution as the sample solution. Perform the test with 15 µL of the sample solution as directed under Liquid Chromatography <2.01>, and compare the sample solution with the Reference Solution: the amount of the peak, other than the two peaks of beraprost and the adjacent two peaks having the relative retention time of about 2.0 are not more than 0.3%, respectively, the amount of the peak having the relative retention time of about 1.7 and another adjacent two peaks having the relative retention time of about 2.0 are not more than 0.2%, respectively, the amount of the peak having the relative retention time of about 1.2 is not more than 0.3%, the amount of the peak, other than the two peaks of beraprost and the peaks mentioned above, is not exceeding 0.1%, and the total amount of the peaks, other than the two peaks of beraprost, is not more than 1.5%.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To a mixture of diammonium hydrogen phosphate solution (33 in 5000) and acetonitril (19:6), add phosphoric acid to adjust the pH of this solution to 8.0.

Flow rate: Adjust the flow rate so that the retention time of benzylpenicillin is about 7.5 minutes.
Time span of measurement: For 80 minutes after injection, beginning after the solvent peak.

**System suitability**—
Test for required detectability: To 1 mL of the sample solution add methanol to make 20 mL. To 1 mL of this solution add methanol to make 20 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add methanol to make exactly 10 mL. Confirm that the total area of the two peaks of beraprost obtained with 15 µL of this solution is equivalent to 14 to 26% of that with 15 µL of the solution for system suitability test.

System performance: When the procedure is run with 15 µL of the solution for system suitability test under the above operating conditions, the resolution between the two peaks of beraprost is not less than 1.5.

System repeatability: When the test is repeated 6 times with 15 µL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the total area of the two peaks of beraprost is not more than 2.0%.

(2) Residual solvent—Being specified separately.

**Loss on drying** <2.41> Not more than 3.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, silica gel, 60°C, 5 hours).

**Isomer ratio** Dissolve 10 mg of Beraprost Sodium in 5 mL of methanol, and use this solution as the sample solution. Perform the test with 15 µL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas, A1 of the peak which appears at the retention time about 25 minutes, and A2 of the peak which appears at about 27 minutes: A1/A2 is between 0.90 and 1.10.

**Operating conditions**—
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of methanol, water and acetic acid (100:600:400:1).
Flow rate: Adjust the flow rate so that the retention time of the second eluting peak of beraprost is about 27 minutes.

**System suitability**—
System performance: When the procedure is run with 15 µL of the sample solution under the above operating conditions, the resolution between the two peaks of beraprost is not less than 1.2.
System repeatability: When the test is repeated 6 times with 15 µL of the sample solution under the above operating conditions, the relative standard deviation of the total area of the two peaks of beraprost is not more than 2.0%.

**Assay** Weigh accurately about 0.1 g of Beraprost Sodium, previously dried, dissolve in 30 mL of diluted ethanol with freshly boiled and cooled water (7 in 10), add exactly 2 mL of 0.2 mol/L hydrochloric acid TS, and titrate <2.50> with 0.025 mol/L sodium hydroxide-ethanol VS from the first equivalence point to the second equivalence point (potentiometric titration). Each mL of 0.025 mol/L sodium hydroxide-ethanol VS = 10.51 mg of C24H29NaO5.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

### Beraprost Sodium Tablets

Beraprost Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of beraprost sodium (C24H29NaO5: 420.47).

**Method of preparation** Prepare as directed under Tablets, with Beraprost Sodium.

**Identification** Powder Beraprost Sodium Tablets. To a portion of the powder, equivalent to 0.2 mg of Beraprost Sodium according to the labeled amount, add 10 mL of water, shake, and filter through a membrane filter with a pore size not exceeding 0.45 µm. To the filtrate add 1 mL of 0.1 mol/L hydrochloric acid TS, extract with two 50-mL portions of ethyl acetate, combine the extracts, and evaporate in reduced pressure at 40°C. Dissolve the residue in 1 mL of methanol, use this solution as the sample solution. Separately, dissolve 1 mg of beraprost sodium in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 µL of each sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with the upper layer of a mixture of 11 volumes of ethyl acetate, 10 volumes of water, 4 volumes of isooctane and 2 volumes of acetic acid (100) to a distance of about 10 cm, air-dry the plate, and heat at 120°C for 30 minutes. After cooling, spray evenly a mixture of ethanol (99.5), water, sulfuric acid and 4-methoxybenzaldehyde (17:2:1:1) on the plate, and heat at 120°C for 3 minutes: the principal spot obtained from the sample solution and the spot from the standard solution show the same RF value.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Beraprost Sodium Tablets add exactly V mL of the internal standard solution so that each mL contains about 2 µg of beraprost sodium (C24H29NaO5), shake at 30°C for 30 minutes, filter through a membrane filter with a pore size not exceeding 0.45 µm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of beraprost sodium (C24H29NaO5) = M3 × Q2/Q1 × V/10,000

M3: Amount (mg) of beraprost sodium for assay

**Internal standard solution**—A mixture of water and 4-isopropylphenol in methanol (1 in 250,000) (1:1).

**Dissolution** <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Beraprost Sodium Tablets is not less than 85%.
Start the test with 1 tablet of Beraprost Sodium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 22 ng of beraprost sodium (C₂₄H₂₉NaO₅) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of beraprost sodium for assay, previously dried in reduced pressure not exceeding 0.67 kPa at 60°C for 5 hours using silica gel as a desiccant, and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, and add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the total areas, A₁ and A₃, of the two peaks of beraprost of both solutions.

Dissolution rate (%) with respect to the labeled amount of beraprost sodium (C₂₄H₂₉NaO₅)  
\[ \text{Dissolution rate} = \frac{M_S}{M_i} \times \frac{A_t}{A_s} \times \frac{V'}{V} \times \frac{1}{C} \times 9/100 \]

Where:
- \( M_S \): Amount (mg) of beraprost sodium for assay
- \( M_i \): Labeled amount (mg) of beraprost sodium (C₂₄H₂₉NaO₅) in 1 tablet

**Operating conditions**
- Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: A mixture of methanol, water and acetic acid (100) (650:350:1).
- Flow rate: Adjust the flow rate so that the retention time of the first eluting peak of beraprost is about 15 minutes.

**System suitability**
- System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and beraprost are eluted in this order and the resolution between the internal standard peak and the first eluting peak of beraprost is not less than 11, and the resolution between the two peaks of beraprost is not less than 1.5.
- System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the total area of the two peaks of beraprost to the peak area of the internal standard is not more than 2.0%.

**Containers and storage** Containers—Well-closed containers.

**Berberine Chloride Hydrate**

ベルベリン塩化物水和物

\[ \text{C}_9\text{H}_8\text{ClNO}_4\cdot\text{xH}_2\text{O} \]

9,10-Dimethoxy-5,6-dihydro[1,3]dioxolo[4,5-g]isoquinol[3,2-a]isoquinolin-7-ium chloride hydrate [633-65-8, anhydride]

Berberine Chloride Hydrate contains not less than 95.0% and not more than 102.0% of berberine chloride (C₉H₈ClNO₄ 371.81), calculated on the anhydrous basis.

**Description** Berberine Chloride Hydrate occurs as yellow crystals or crystalline powder. It is odorless or has a faint,
characteristic odor. It has a very bitter taste.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Berberine Chloride Hydrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Berberine Chloride RS prepared in the same manner as the sample solution; both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Berberine Chloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Berberine Chloride RS; both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.1 g of Berberine Chloride Hydrate in 20 mL of water by warming, add 0.5 mL of nitric acid, cool, and filter after allowing to stand for 10 minutes. To 3 mL of the filtrate add 1 mL of silver nitrate TS, and collect the produced precipitate: the precipitate does not dissolve in dilute nitric acid, but it dissolves in an excess amount of ammonia TS.

Purity (1) Acidity—Shake thoroughly 0.10 g of Berberine Chloride Hydrate with 30 mL of water, and filter. To the filtrate add 2 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L sodium hydroxide VS: the yellow color changes to an orange to red color.

(2) Sulfate <1.14>—Shake 1.0 g of Berberine Chloride Hydrate with 48 mL of water and 2 mL of dilute hydrochloric acid for 1 minute, and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid, 5 to 10 drops of bromophenol blue TS and water to make 50 mL (not more than 0.04%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Berberine Chloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Related substances—Dissolve 10 mg of Berberine Chloride Hydrate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 4 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total of the peak of the areas other than berberine of the sample solution is not larger than the peak area of berberine of the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 345 nm).
Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 3.4 g of monobasic potassium phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).
Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.
Selection of column: Dissolve each 1 mg of berberine chloride and palmatin chloride in the mobile phase to make 10 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of palmatin and berberine in this order with the resolution between these peaks being not less than 1.5.
System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of berberine is not more than 1.5%.

Containers and storage—Containers—Tight containers.
Storage—Light-resistant.

**Berberine Tannate**

**タンニン酸ベルベリン**

Berberine Tannate is a compound of berberine and tannic acid.

It contains not less than 27.0% and not more than 33.0% of berberine (C_{20}H_{19}NO_{5}: 353.37), calculated on the anhydrous basis.

**Description** Berberine Tannate occurs as a yellow to light...
yellow-brown powder. It is odorless or has a faint, characteristic odor, and is tasteless.

It is practically insoluble in water, in acetonitrile, in methanol and in ethanol (95).

**Identification (1)** To 0.1 g of Berberine Tannate add 10 mL of ethanol (95), and heat in a water bath for 3 minutes with shaking. Cool, filter, and to 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a blue-green color is produced, and on allowing to stand, a bluish black precipitate is formed.

(2) Dissolve 0.01 g of Berberine Tannate in 10 mL of methanol and 0.4 mL of 1 mol/L hydrochloric acid TS, and add water to make 200 mL. To 8 mL of the solution add water to make 25 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.4>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Berberine Tannate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity (1)** Acidity—To 0.10 g of Berberine Tannate add 30 mL of water, and filter after shaking well. To the filtrate add 2 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L sodium hydroxide VS: the color of the solution changes from yellow to orange to red.

(2) Chloride <1.03>—Shake 1.0 g of Berberine Tannate with 38 mL of water and 12 mL of dilute nitric acid for 5 minutes, and filter. Discard the first 5 mL of the filtrate, to 25 mL of the subsequent filtrate add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.01 mol/L hydrochloric acid VS by adding 6 mL of dilute nitric acid, 10 to 15 drops of bromophenol blue TS and water to make 50 mL (not more than 0.035%).

(3) Sulfate <1.14>—Shake 1.0 g of Berberine Tannate with 48 mL of water and 2 mL of dilute hydrochloric acid for 1 minute, and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid, 5 to 10 drops of bromophenol blue TS and water to make 50 mL (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Berberine Tannate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(5) Related substances—Dissolve 10 mg of Berberine Tannate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 4 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total of the peak areas other than berberine of the sample solution is not larger than the peak area of berberine of the standard solution.

**Operating conditions—**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of berberine beginning after the solvent peak.

**System suitability—**

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of berberine obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 3.0%.

**Water <2.48>** Not more than 6.0% (0.7 g, direct titration).

**Residue on ignition <2.44>** Not more than 1.0% (1 g).

**Assay** Weigh accurately about 30 mg of Berberine Tannate, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately, determine the water content <2.48> in the same manner as Berberine Chloride Hydrate), dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A₁ and A₂, of berberine in each solution.

\[
\text{Amount (mg) of berberine} (C_{20}H_{18}NO_3) = M_s \times \frac{A_1}{A_2} \times 0.950
\]

M₅: Amount (mg) of Berberine Chloride RS, calculated on the dehydrated basis.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

**System suitability—**

System performance: Dissolve 1 mg each of berberine chloride and palmatin chloride in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, palmatin and berberine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak
area of berberine is not more than 1.5%.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Betahistine Mesilate**

ベタヒスチンメシル酸塩

\[
\text{C}_8\text{H}_{12}\text{N}_2\cdot 2\text{C}_2\text{H}_4\text{O}_3\text{S} : 328.41
\]

\[\text{N-Methyl-2-pyridin-2-ylethylamine dimethanesulfonate} \]

[5638-76-6, Betahistine]

Betahistine Mesilate, when dried, contains not less than 98.0% and not more than 101.0% of \( \text{C}_8\text{H}_{12}\text{N}_2\cdot 2\text{C}_2\text{H}_4\text{O}_3\text{S} \).

**Description** Betahistine Mesilate occurs as white crystals or crystalline powder.

- It is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).
- It dissolves in dilute hydrochloric acid.
- It is hygroscopic.

**Identification (1)** Determine the absorption spectrum of a solution of Betahistine Mesilate in 0.1 mol/L hydrochloric acid (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Betahistine Mesilate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** (2.60) 110–114°C (after drying).

**Purity (1)** Heavy metals (1.07)—Proceed with 1.0 g of Betahistine Mesilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Dissolve 50 mg of Betahistine Mesilate in 10 mL of a mixture of water and acetonitrile (63:37), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (63:37) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography (2.07) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than betahistine with the sample solution is not larger than 1/10 times the peak area of betahistine with the standard solution, and the total area of the peaks other than the peak of betahistine with the sample solution is not larger than 1/2 times the peak area of betahistine with the standard solution.

**Operating conditions**—

- Detector: An ultraviolet absorption photometer (wavelength: 261 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 35°C.
- Mobile phase: To 5 mL of diethyl amine and 20 mL of acetic acid (100) add water to make 1000 mL. Dissolve 2.3 g of sodium lauryl sulfate in 630 mL of this solution, and add 370 mL of acetonitrile.
- Flow rate: Adjust the flow rate so that the retention time of betahistine is about 5 minutes.
- Time span of measurement: About 3 times as long as the retention time of betahistine, beginning after the solvent peak.

**System suitability**—

Test for required detectability: To exactly 5 mL of the standard solution add the mixture of water and acetonitrile (63:37) to make exactly 50 mL. Confirm that the peak area of betahistine obtained with 20 \( \mu \)L of this solution is equivalent to 7 to 13% of that with 20 \( \mu \)L of the standard solution.

System performance: Dissolve 10 mg of betahistine mesilate and 10 mg of 2-vinylpyridine in 50 mL of the mixture of water and acetonitrile (63:37). To 2 mL of this solution add the mixture of water and acetonitrile (63:37) to make 50 mL. When the procedure is run with 20 \( \mu \)L of this solution under the above operating conditions, 2-vinylpyridine and betahistine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 1.0%.

**Loss on drying** (2.41) Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 70°C, 24 hours).

**Residue on ignition** (2.42) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Betahistine Mesilate, previously dried, dissolve in 1 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate (2.50) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 16.42 mg of \( \text{C}_8\text{H}_{12}\text{N}_2\cdot 2\text{C}_2\text{H}_4\text{O}_3\text{S} \).

**Containers and storage** Containers—Tight containers.

**Betahistine Mesilate Tablets**

ベタヒスチンメシル酸塩錠

Betahistine Mesilate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of betahistine mesilate (\( \text{C}_8\text{H}_{12}\text{N}_2\cdot 2\text{C}_2\text{H}_4\text{O}_3\text{S} : 328.41 \)).

**Method of preparation** Prepare as directed under Tablets, with Betahistine Mesilate.
Uniformity of dosage units

Content uniformity test.

To perform the test according to the following method: it meets the requirement of the official monograph regardless of the labeled amount, and use this solution as the sample solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry. With a detector and reference cell, measure the absorbance at the specified wavelength within the specified range, and determine the peak areas. When the procedure is run with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine obtained with 20 μL of the standard solution and the total area of the peaks other than betahistine is not larger than 3/5 times the peak area of betahistine from the standard solution.

System suitability

- Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the Assay.
- Time span of measurement: About 8 times as long as the retention time of betahistine beginning after the solvent peak.
- System performance: Dissolve 10 mg of betahistine mesilate and 10 mg of 2-vinylpyridine in 50 mL of the mixture of water and acetonitrile (63:37). To 2 mL of this solution add the mixture of water and acetonitrile (63:37) to make exactly 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 2-vinylpyridine and betahistine are eluted in this order with the resolution between these peaks being not less than 5.
- System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 1.0%.

Uniformity of dosage units (6.02) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Betahistine Mesilate Tablets add exactly V mL of 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of betahistine obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

Dissolution (6.10) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Betahistine Mesilate Tablets is not less than 85%.

Start the test with 1 tablet of Betahistine Mesilate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 6.7 μg of betahistine mesilate (C8H12N2.2CH4O3S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of betahistine mesilate for assay, previously dried under reduced pressure with phosphorous (V) oxide at 70°C for 24 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography. With a detector and reference cell, measure the absorbance at the specified wavelength within the specified range, and determine the peak areas, A1 and A3, of betahistine.

Dissolution rate (%) with respect to the labeled amount of betahistine mesilate (C8H12N2.2CH4O3S) = M5 × A1/A3 × V/V × 1/C × 36

M5: Amount (mg) of betahistine mesilate for assay

C: Labeled amount (mg) of betahistine mesilate (C8H12N2.2CH4O3S) in 1 tablet

Operating conditions—

Proceed as directed in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betahistine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Beta-histine Mesilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of betahistine mesilate (C8H12N2.2CH4O3S), add 40 mL of 0.1 mol/L hydrochloric acid TS, agitate for 10 minutes with the aid of ultrasonic waves, and add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.1 g of betahistine mesilate for assay, previously dried under reduced pressure with phosphorous (V) oxide at 70°C for 24 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography, according to the following conditions, and determine the peak areas, A1 and A3, of betahistine.
Amount (mg) of betahistine mesilate \((C_{22}H_{29}FO_5\)S) = \(M_s \times A_1/A_s \times 1/5\)

\(M_s\): Amount (mg) of betahistine mesilate for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 261 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 5 mL of diethylamine and 20 mL of acetic acid (100) add water to make 1000 mL. In 630 mL of this solution dissolve 2.3 g of sodium lauryl sulfate, and add 370 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the detention time of betahistine is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 \(\mu\)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betahistine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Betamethasone

ベタメタゾン

\(C_{22}H_{29}FO_5: 392.46\)

9-Fluoro-11\(\beta\),17,21-trihydroxy-16\(\beta\)-methylpregna-1,4-diene-3,20-dione

\([378-44-9]\)

Betamethasone, when dried, contains not less than 96.0% and not more than 103.0% of \(C_{22}H_{29}FO_5\).

Description Betamethasone occurs as a white to pale yellowish white, crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in acetone, and practically insoluble in water.

Melting point: about 240°C (with decomposition).

Identification (1) Proceed 10 mg of Betamethasone as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid, and prepare the test solution: the test solution so obtained responds to the Qualitative Tests <1.09> for fluoride.

(2) Dissolve 1.0 mg of Betamethasone in 10 mL of ethanol (95). Mix 2.0 mL of the solution with 10 mL of phenylhydrazinium hydrochloride TS, heat in a water bath at 60°C for 20 minutes, and cool the solution. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using as the blank the solution prepared with 2.0 mL of ethanol (95) in the same manner as the former solution, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Betamethasone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Betamethasone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Betamethasone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Betamethasone and Betamethasone RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <2.49> \([\alpha]_D^{20}: +118 +/ - 126°\) (after drying, 0.1 g, methanol, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 0.5 g of Betamethasone according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 10 mg of Betamethasone in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (0.1 g, platinum crucible).

Assay Dissolve about 20 mg each of Betamethasone and Betamethasone RS, previously dried and accurately weighed, in methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 \(\mu\)L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_t\) and \(Q_s\), of the peak area of betamethasone to that of the internal standard, respectively.

Amount (mg) of \(C_{22}H_{29}FO_5\) = \(M_s \times Q_t/Q_s\)

\(M_s\): Amount (mg) of Betamethasone RS
Betamethasone Tablets

**Internal standard solution**—A solution of butyl parahydroxybenzoate in methanol (2 in 3500).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column about 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of water and acetonitrile (3:2).
Flow rate: Adjust the flow rate so that the retention time of betamethasone is about 4 minutes.

**System suitability**—
- System performance: When the test with 10 µL of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
- System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.
Storage—Light-resistant.

**Betamethasone Tablets**

ベタメタゾン錠

Betamethasone Tablets contain not less than 90.0% and not more than 107.0% of the labeled amount of betamethasone (C₂₂H₂₉FO₅: 392.46).

**Method of preparation**—Prepare as directed under Tablets, with Betamethasone.

**Identification**—Pulverize Betamethasone Tablets. To a portion of the powder, equivalent to 2 mg of Betamethasone according to the labeled amount, add 20 mL of methanol, shake for 5 minutes, and filter. Evaporate the filtrate on a water bath to dryness, dissolve the residue after cooling in 2 mL of methanol, filter if necessary, and use this as the sample solution. Separately, dissolve 2 mg of Betamethasone RS in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.05). Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic anhydride (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained with the sample solution and the spot with the standard solution show the same *Rf* value.

**Uniformity of dosage units**—Perform the test according to the following method: it meets the requirement of the Content uniformity test.
To 1 tablet of Betamethasone Tablets add *V* mL of water so that each mL contains about 50 µg of betamethasone (C₂₂H₂₉FO₅). Add exactly 2 *V* mL of the internal standard solution, shake vigorously for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Betamethasone RS, previously dried for 4 hours in a desiccator (in vacuum, phosphorus (V) oxide), dissolve in acetonitrile to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, add 5 mL of water, and use this solution as the standard solution. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, *Q₁* and *Q₂*, of the peak area of betamethasone to that of the internal standard.

Amount (mg) of betamethasone (C₂₂H₂₉FO₅) = *M₃* × *Q₁*/*Q₂* × *V*/400

*M₃*: Amount (mg) of Betamethasone RS

**Internal standard solution**—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 40,000).

**Operating conditions**—
Proceed as directed in the operating conditions in the Assay.

**System suitability**—
- System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
- System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone to that of the internal standard is not more than 1.0%.

**Dissolution**—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Betamethasone Tablets is not less than 85%.
Start the test with 1 tablet of Betamethasone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet the subsequent *V* mL of the filtrate, add water to make exactly *V* mL so that each mL contains about 0.56 µg of betamethasone (C₂₂H₂₉FO₅). Add 5 mL of this solution, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, *A₁* and *A₃*, of betamethasone.
Dissolution rate (%) with respect to the labeled amount of betamethasone (C<sub>21</sub>H<sub>33</sub>FO<sub>7</sub>)

\[
M_S = \frac{M_S}{A_T / A_S} \times \frac{V'}{V} \times \frac{1}{C} \times \frac{9}{5}
\]

- **M<sub>S</sub>:** Amount (mg) of Betamethasone RS
- **C:** Labeled amount (mg) of betamethasone (C<sub>21</sub>H<sub>33</sub>FO<sub>7</sub>) in 1 tablet

**Operating conditions—**

**Detector:** An ultraviolet absorption photometer (wavelength: 241 nm).

**Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

**Column temperature:** A constant temperature of about 25°C.

**Mobile phase:** A mixture of water and acetonitrile (3:2).

**Flow rate:** Adjust the flow rate so that the retention time of betamethasone is about 7 minutes.

**System suitability—**

- **System performance:** When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betamethasone are not less than 3000 and not more than 2.0, respectively.

- **System repeatability:** When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betamethasone is not more than 2.0%.

**Assay** Weigh accurately the mass of not less than 20 Betamethasone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of betamethasone (C<sub>21</sub>H<sub>33</sub>FO<sub>7</sub>), add 25 ml of water, then add exactly 50 ml of the internal standard solution, and shake vigorously for 10 minutes. Filter through a membrane filter with pore size of not more than 0.5 μm, discard the first 5 ml of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of Betamethasone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in acetonitrile to make exactly 50 ml. Pipet 5 ml of this solution, add exactly 20 ml of the internal standard solution and 5 ml of water, and use this solution as the standard solution. Perform the test with 20 μl each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q1 and Q8, of the peak area of betamethasone to that of the internal standard.

- **Amount (mg) of betamethasone (C<sub>21</sub>H<sub>33</sub>FO<sub>7</sub>):**

\[
M_S = \frac{M_S \times Q_1}{Q_8} \times \frac{1}{4}
\]

- **M<sub>S</sub>:** Amount (mg) of Betamethasone RS

**Internal standard solution—** A solution of butyl parahydroxybenzoate in acetonitrile (1 in 10,000).

**Operating conditions—**

**Detector:** An ultraviolet absorption photometer (wavelength: 241 nm).

**Column:** A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

**Column temperature:** A constant temperature of about 25°C.

**Mobile phase:** A mixture of water and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of betamethasone is about 4 minutes.

**System suitability—**

- **System performance:** When the procedure is run with 20 μL of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

- **System repeatability:** When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Betamethasone Dipropionate**

ベタメタゾンジプロピオン酸エステル

C<sub>29</sub>H<sub>37</sub>FO<sub>7</sub>: 504.59

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropanoate [5593-20-4]

Betamethasone Dipropionate, when dried, contains not less than 97.0% and not more than 103.0% of C<sub>29</sub>H<sub>37</sub>FO<sub>7</sub>, and not less than 3.4% and not more than 4.1% of fluorine (F:19.00).

**Description** Betamethasone Dipropionate occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetone, in 1,4-dioxane and in chloroform, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water and in hexane.

It is affected gradually by light.

**Identification**

1. To 1 mL of a solution of Betamethasone Dipropionate in methanol (1 in 10,000) add 4 mL of isoniazid TS, and heat on a water bath for 2 minutes: a yellow color develops.

2. Proceed with 0.01 g of Betamethasone Dipropionate as directed under Oxygen Flask Combustion Method <1.067>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution so obtained responds to the Qualitative Tests <1.099> for fluorine.

3. Determine the absorption spectrum of a solution of Betamethasone Dipropionate in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(4) Determine the infrared absorption spectrum of Betamethasone Dipropionate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** 2.60> 176 – 180°C

**Optical rotation** 2.49: αD: +63 ± 70° (after drying, 50 mg, 1,4-dioxane, 10 mL, 100 mm).

**Purity** (1) Fluoride—To 0.10 g of Betamethasone Dipropionate add 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), shake for 10 minutes, and filter through a membrane filter (0.4-µm pore size). Place 5.0 mL of the filtrate in a 20-mL volumetric flask, and add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS (1:1:1), add water to make 20 mL, allow to stand for 1 hour, and use this solution as the sample solution. Separately, place 1.0 mL of Standard Fluorine Solution in a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), then 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS (1:1:1), proceed in the same manner as the preparation of the sample solution, and use this solution as the standard solution. Place 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in a 20-mL volumetric flask, and proceed in the same manner as the preparation of the sample solution. Using this solution as the blank, determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance of the sample solution is not greater than that of the standard solution (not more than 0.012%).

(2) Heavy metals <1.07>—Proced with 1.0 g of Betamethasone Dipropionate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.010 g of Betamethasone Dipropionate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 0.010 g of Betamethasone Dipropionate in 10 mL of chloroform, and use this solution as the standard solution. Place 0.01 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in a 20-mL volumetric flask, and proceed in the same manner as the preparation of the sample solution. Using this solution as the blank, determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance of the sample solution is not greater than that of the standard solution (not more than 0.012%).

**Assay** (1) Betamethasone dipropionate—Weigh accurately about 15 mg of Betamethasone Dipropionate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and dilute with methanol to exactly 50 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 239 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>:

\[ \text{Amount (mg) of C}_2\text{H}_{19}\text{FNa}_2\text{O}_8\text{P} = A/312 \times 10,000 \]

(2) Fluorine—Weigh accurately about 10 mg of Betamethasone Dipropionate, previously dried, and proceed as directed in the procedure of determination for fluorine under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid.

**Containers and storage** Containers—Light-resistant.

**Betamethasone Sodium Phosphate**

![Chemical Structure](image)

Betamethasone Sodium Phosphate contains not less than 97.0% and not more than 103.0% of C22H28FNa2O8P, calculated on the anhydrous basis.

**Description** Betamethasone Sodium Phosphate occurs as white to pale yellowish white, crystalline powder or masses. It is odorless.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

**Melting point** about 213°C (with decomposition).

**Identification** (1) Dissolve 2 mg of Betamethasone Sodium Phosphate in 2 mL of sulfuric acid: a brown color develops, and gradually changes to blackish brown.

(2) Prepare the test solution with 0.01 g of Betamethasone Sodium Phosphate as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests <1.09> (2) for fluorine.

(3) Take 40 mg of Betamethasone Sodium Phosphate in a platinum crucible, and carbonize by heating. After cooling, add 5 drops of nitric acid, and incinerate by heating. To the residue add 10 mL of diluted nitric acid (1 in 50), and boil for several minutes. After cooling, filter if necessary, and use this solution as the sample solution. The sample solution responds to the Qualitative Tests <1.09> for sodium salt, and
to the Qualitative Tests <1.0> (1) and (3) for phosphate.

(4) Determine the infrared absorption spectrum of Betamethasone Sodium Phosphate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Betamethasone Sodium Phosphate RS; both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.40> \[\alpha]^n_D + 99° to +105° (0.1 g, calculated on the anhydrous basis, water, 10 mL, 100 mm).

**pH** <2.54> Dissolve 0.10 g of Betamethasone Sodium Phosphate in 20 mL of water: the pH of this solution is between 7.5 and 9.0.

**Purity** (1) Clarity and color of solution—Dissolve 0.25 g of Betamethasone Sodium Phosphate in 10 mL of water: the solution is clear and colorless.

(2) Free phosphoric acid—Weigh accurately about 20 mg of Betamethasone Sodium Phosphate, dissolve in 20 mL of water, and use this solution as the sample solution. Separately, pipet 4 mL of Standard Phosphoric Acid Solution, add 20 mL of water, and use this solution as the standard solution. To each of the sample solution and the standard solution add exactly 7 mL of dilute sulfuric acid, exactly 2 mL of hexaammonium heptamolybdate-sulfuric acid TS and exactly 2 mL of methylaminophenol sulfate TS, shake well, and allow to stand at 20 ± 1°C for 15 minutes. To each add water to make exactly 50 mL, and allow to stand at 20 ± 1°C for 15 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.34>, using a solution prepared with 20 mL of water in the same manner as the blank. Determine the absorbances, \(A_1\) and \(A_2\), of each solution from the sample solution and standard solution at 730 nm: the amount of free phosphoric acid is not more than 0.5%.

\[
\text{Amount (mg) of free phosphoric acid (H}_3\text{PO}_4) = A_1/A_2 \times 1/M \times 10.32
\]

\(M\): Amount (mg) of Betamethasone Sodium Phosphate, calculated on the anhydrous basis.

(3) Betamethasone—Dissolve 20 mg of Betamethasone Sodium Phosphate in exactly 2 mL of methanol, and use this solution as the sample solution. Separately, dissolve 20 mg of Betamethasone RS in exactly 10 mL of methanol. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 \(\mu\)L of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a freshly prepared mixture of 1-butanol, water and acetic anhydride (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

**Water** <2.48> Not more than 10.0% (0.2 g, back titration).

**Assay** Weigh accurately about 20 mg each of Betamethasone Sodium Phosphate and Betamethasone Sodium Phosphate RS (determine the water <2.48> before using in the same manner as Betamethasone Sodium Phosphate), and dissolve each in methanol to make exactly 20 mL. Pipet 5 mL each of these solutions, and exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of betamethasone phosphate to that of the internal standard, respectively.

\[
\text{Amount (mg) of C}_{22}\text{H}_{28}\text{FNa}_2\text{O}_8\text{P} = M \times Q_T/Q_S
\]

\(M\): Amount (mg) of Betamethasone Sodium Phosphate RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of butyl parahydroxybenzoate in methanol (1 in 5000).

**Operating conditions**—
- **Detector:** An ultraviolet absorption photometer (wavelength: 254 nm).
- **Column:** A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 \(\mu\)m in particle diameter).
- **Column temperature:** A constant temperature of about 25°C.

Mobile phase: Dissolve 1.6 g of tetra-n-butylammonium bromide, 3.2 g of disodium hydrogen phosphate dodecahydrate and 6.9 g of potassium dihydrogen phosphate in 1000 mL of water, and add 1500 mL of methanol.

**Flow rate:** Adjust the flow rate so that the retention time of betamethasone phosphate is about 5 minutes.

**System suitability**—
- **System performance:** When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, betamethasone phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
- **System repeatability:** When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of betamethasone phosphate to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

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**Betamethasone Valerate**

ベタメタゾン吉草酸エステル

\(\text{C}_{27}\text{H}_{37}\text{FO}_6\): 476.58

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-pentanoate

[2152-44-5]

Betamethasone Valerate, when dried, contains not less than 97.0% and not more than 103.0% of
Betamethasone Valerate and Gentamicin Sulfate Cream

**Betamethasone Valerate** occurs as a white, crystalline powder. It is odorless. It is freely soluble in chloroform, soluble in ethanol (95), sparingly soluble in methanol, slightly soluble in diethyl ether, and practically insoluble in water. Melting point: about 190°C (with decomposition).

**Description**

Betamethasone Valerate can be dissolved in 0.1 g methanol, 20 mL 100 mm) methanol (9:1) to make exactly 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of ethyl acetate, mix, and use as the sample solution.

**Purity** Related substances—Conduct this procedure without exposure to daylight. Dissolve 0.02 g of Betamethasone Valerate as directed under Oxygen Flask Combustion Method <1.00>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide solution and 20 mL of water as the absorbing liquid, and prepare the test solution: the test solution so obtained responds to the Qualitative Tests <1.00> for fluoride.

(2) Determine the infrared absorption spectrum of Betamethasone Valerate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Betamethasone Valerate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** 

\[\beta_{D}^{2.49}\] [α]D9: +77 – +83° (after drying, 0.1 g, methanol, 20 mL, 100 mm).

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.2% (0.5 g, platinum crucible).

**Assay** Dissolve about 10 mg each of Betamethasone Valerate and Betamethasone Valerate RS, previously dried and accurately weighed, in methanol to make exactly 100 mL. Pipet 10 mL each of these solutions, add 10 mL each of the internal standard solution, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_t\) and \(Q_S\), of the peak area of betamethasone valerate to that of the internal standard, respectively.

\[\text{Amount (mg) of } C_{27}H_{37}FO_{6} = M_S \times Q_t/Q_S \]

\[M_S: \text{Amount (mg) of Betamethasone Valerate RS} \]

**Internal standard solution—**A solution of isoamyl benzoate in methanol (1 in 1000).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of betamethasone valerate is about 10 minutes.

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

**Betamethasone Valerate and Gentamicin Sulfate Cream**

Betamethasone Valerate and Gentamicin Sulfate Cream contains not less than 90.0% and not more than 110.0% of the labeled amount of betamethasone valerate \((C_{27}H_{37}FO_6; 476.58)\) and not less than 90.0% and not more than 115.0% of the labeled amount of gentamicin \(C_1(C_{21}H_{43}N_5O_7; 477.60)\).

**Method of preparation** Prepare as directed under Creams, with Betamethasone Valerate and Gentamicin Sulfate.

**Identification** (1) To a quantity of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1.2 mg of Betamethasone Valerate according to the labeled amount, add 20 mL of methanol and 20 mL of hexane, shake vigorously for 10 minutes, and allow to stand. Take 15 mL of the lower layer, evaporate the layer to dryness on a water bath under a current of nitrogen. To the residue add 1 mL of ethyl acetate, mix, and use as the sample solution. Separately, dissolve about 18 mg of Betamethasone Valerate RS in 20 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate; the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

\[\text{Amount (mg) of } C_{27}H_{37}FO_{6} = M_S \times Q_t/Q_S \]

\[M_S: \text{Amount (mg) of Betamethasone Valerate RS} \]

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of betamethasone valerate is about 10 minutes.

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
Purity Related substances—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1 mg of Betamethasone Valerate according to the labeled amount, add 15 mL of water, and mix while warming on a water bath to make a milky liquid: the pH of the cooled liquid is between 4.0 and 6.0.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 45°C.
Mobile phase: A mixture of water, acetonitrile and methanol (12:7:1) in the ratios, Q1, Q2, Q3.
Flow rate: Adjust the flow rate so that the retention time of betamethasone valerate is about 16 minutes.
Time span of measurement: About 2.5 times as long as the retention time of betamethasone valerate beginning after the solvent peak. The peaks of the compounding ingredients are not determined.

System suitability—
Test for required detectability: Dissolve 20 mg of Betamethasone Valerate in 100 mL of a mixture of methanol and water (7:3). To exactly 1 mL of this solution add the mixture of methanol and water (7:3) to make exactly 100 mL, and use this solution as the solution for system suitability test. To exactly 2.5 mL of this solution add the mixture of methanol and water (7:3) to make exactly 25 mL. Pipet 5 mL of this solution, and add the mixture of methanol and water (7:3) to make exactly 50 mL. Pipet 10 mL of this solution, and add the mixture of methanol and water (7:3) to make exactly 100 mL. Perform the test with 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography to determine the number of theoretical plates and the symmetry factor of the peak of betamethasone valerate.

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betamethasone valerate is not more than 2.0%.

System repeatability: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betamethasone valerate is not more than 2.0%.

Assay (1) Betamethasone valerate—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1 mg of betamethasone valerate (C27H37FO6), add 10 mL of a mixture of methanol and water (7:3), and add exactly 10 mL of the internal standard solution. After warming in a water bath at 60°C for 5 minutes, shake vigorously for 20 minutes. Repeat this procedure twice, cool with ice for 15 minutes, centrifuge for 5 minutes, then filter the supernatant liquid, discard the first 5 mL of filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Betamethasone Valerate RS, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, and add the mixture of methanol and water (7:3) to make exactly 50 mL. Pipet 10 mL of this solution, and add the mixture of methanol and water (7:3) to make exactly 100 mL. Perform the test with 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography to determine the ratios, Q1 and Q2, of the peak area of betamethasone valerate to that of the internal standard.

Amount (mg) of betamethasone valerate (C27H37FO6) = M5 × Q1/Q2 × 1/25

M5: Amount (mg) of Betamethasone Valerate RS

Internal standard solution—Dissolve 20 mg of beclometasone dipropionate in 10 mL of methanol, and add the mixture of methanol and water (7:3) to make 200 mL.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 2.1 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of methanol and water (13:7).
Flow rate: Adjust the flow rate so that the retention time of betamethasone valerate is about 16 minutes.

System suitability—
System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

(2) Gentamicin sulfate—Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics. Use Lactobacillus fermentum L-20 as test organism, agar media for base layer and seed layer, agar medium for transferring test organisms, and standard solutions—Follow the directions as directed in the Assay under Gentamicin Sulfate.
(ii) Sample solutions—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1 mg (potency) of Gentamicin Sulfate, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 8.0, previously warmed to about 85°C, and shake well to dissolve. After cooling, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 250 mL to make the high concentration sample solution, which contains 4 μg (potency) per mL. Pipet a suitable amount of the high concentration sample solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 so that each mL contains 1 μg (potency), and use this solution as the low concentration sample solution.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Betamethasone Valerate and Gentamicin Sulfate Ointment ベタメタゾン吉草酸エステル・ゲンタマイシン硫酸塩軟膏

Betamethasone Valerate and Gentamicin Sulfate Ointment contains not less than 95.0% and not more than 110.0% of the labeled amount of betamethasone valerate (C_{27}H_{37}FO_{6} : 476.58) and not less than 90.0% and not more than 115.0% of the labeled potency of gentamicin C_{1} (C_{27}H_{37}NO_{7} : 477.60).

Method of preparation Prepare as directed under Ointment, with Betamethasone Valerate and Gentamicin Sulfate.

Identification (1) To a quantity of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to 1.2 mg of Betamethasone Valerate according to the labeled amount, add 20 mL of methanol and 20 mL of hexane, and disperse the ointment with the aid of ultrasonic. Shake vigorously for 5 minutes, centrifuge for 5 minutes, cool for 15 minutes with ice, and take 15 mL of the lower layer. Evaporate the layer to dryness on a water bath under a current of nitrogen. To the residue add 1 mL of ethyl acetate, apply ultrasonic waves, filter, if necessary, and use the filtrate as the sample solution. Separately, dissolve 18 mg of Betamethasone Valerate RS in 20 mL of ethyl acetate, and use this solution as the sample solution. After warming in a water bath at 75°C for 5 minutes, shake vigorously for 10 minutes. Repeat this procedure once more, cool with ice for 15 minutes, filter, discard the first 5 mL of filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Betamethasone Valerate RS, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, and add the mixture of methanol and water (7:3) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 3 μL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.02 according to the following conditions, and calculate the ratios, Q_{1} and Q_{6}, of the peak area of betamethasone valerate to that of the internal standard.

\[ M_5 = M_2 \times Q_1/Q_6 \times 1/25 \]

M_{5}: Amount (mg) of Betamethasone Valerate RS

Internal standard solution—Dissolve 20 mg of beclometasone dipropionate in 10 mL of methanol, and add the mixture of methanol and water (7:3) to make 200 mL.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 2.1 mm in inside diameter and 10 cm in length, packed with octadeceansilanized silica gel for liquid chromatography (3.5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of methanol and water (13:7).
Flow rate: Adjust the flow rate so that the retention time of betamethasone valerate is about 16 minutes.

System suitability—
System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

(2) Gentamicin sulfate—Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics 4.02 according to the following conditions.

(i) Test organism, agar media for base layer and seed layer, agar medium for transferring test organisms, and standard solutions—Proced as directed in the Assay under Gentamicin Sulfate.
(ii) Sample solutions—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to about 1 mg (potency) of Gentamicin Sulfate, transfer to a separator, add 50 mL of petroleum ether and exactly 100 mL of 0.1 mol/L phosphate buffer solution, pH 8.0, shake for 10 minutes, and allow to stand. Pipet a suitable amount of the water layer, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Betamipron

ベタミプロン

![Chemical Structure of Betamipron]

C_{10}H_{11}NO_3: 193.20
3-Benzoylaminopropionic acid

[3440-28-6]

Betamipron contains not less than 99.0% and not more than 101.0% of C_{10}H_{11}NO_3, calculated on the anhydrous basis.

Description Betamipron occurs as white crystals or crystalline powder.
It is freely soluble in methanol, soluble in ethanol (95.5), and slightly soluble in water.
It dissolves in sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Betamipron in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Betamipron as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 0.25 g of Betamipron in 100 mL of water by warming, and cool: the pH of this solution is 3.0 to 3.4.

Melting point <2.60> 132 ~ 135°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Betamipron in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Betamipron according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) β-Alanine—Dissolve 0.25 g of Betamipron in 10 mL of methanol, and use this solution as the sample solution. Separately, dissolve 50 mg of β-alanine in methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.06>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of methanol, ethyl acetate, ammonia solution (28) and water (200:200:63:37) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-butanol TS on the plate, and heat at 105°C for 5 minutes: the spot obtained from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

System suitability—
Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of betamipron obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: Dissolve 5 mg of Betamipron and 5 mg of benzoic acid in 200 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, benzoic acid and betamipron are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betamipron is not more than 2.0%.

Water <2.48> Not more than 0.5% (1 g, volumetric titration, direct titration).
Residue on ignition Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Betamipron, dissolve in 25 mL of ethanol (99.5), add 25 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction. Each mL of 0.1 mol/L sodium hydroxide VS = 19.32 mg of C_{10}H_{11}NO_3

Containers and storage Containers—Tight containers.

Betaxolol Hydrochloride

ベタキソロール塩酸塩

C_{18}H_{29}NO_3.HCl: 343.89

Betaxolol Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C_{18}H_{29}NO_3.HCl.

Description Betaxolol Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in water, and freely soluble in methanol, in ethanol (99.5) and in acetic acid (100).

Dissolve 1.0 g of Betaxolol Hydrochloride in 50 mL of water: the pH of the solution is between 4.5 and 6.5.

A solution of Betaxolol Hydrochloride (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Betaxolol Hydrochloride in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry 2.2.4, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Betaxolol Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry 2.2.5, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Betaxolol Hydrochloride (1 in 100) responds to the Qualitative Tests 1.09 (2) for chloride.

Melting point 2.60 114 – 117°C.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Betaxolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals 1.07—Proceed with 2.0 g of Betaxolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic 1.11—Prepare the test solution with 2.0 g of Betaxolol Hydrochloride according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substance I—Dissolve 0.10 g of Betaxolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.07. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100:10:3) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 1 hour: the number of the spots other than the principal spot obtained from the sample solution is not more than 3, and they are not more intense than the spot from the standard solution.

(5) Related substance II—Dissolve 0.10 g of Betaxolol Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography 2.07 according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than betaxolol obtained from the sample solution is not larger than the peak area of betaxolol from the standard solution, and the total area of the peaks other than the peak of betaxolol is not larger than 2 times the peak area of betaxolol from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octysilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted 0.05 mol/L potassium dihydrogen phosphate TS (1 in 2) with the pH adjusted to 3.0 with 1 mol/L hydrochloric acid TS, acetonitrile and methanol (26:7:7).

Flow rate: Adjust the flow rate so that the retention time of betaxolol is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of betaxolol beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 4 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of betaxolol obtained from 10 µL of this solution is equivalent to 14 to 26% of that from 10 µL of the standard solution.

System performance: Dissolve 50 mg of Betaxolol Hydrochloride and 5 mg of 2-naphthol in 200 mL of the mobile phase. When the procedure is run with 10 µL of this solution under the above operating conditions, betaxolol and 2-naphthol are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak...
area of betaxolol is not more than 2.0%.

(6) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Betaxolol Hydrochloride, previously dried, dissolve in 30 mL of acetic acid (100), add 30 mL of acetic anhydride, and titrate 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 34.39 mg of C₇H₁₇ClN₂O₂·HCl

Containers and storage Containers—Tight containers.

Bethanechol Chloride

ベタネコール塩化物

![Chemical Structure of Bethanechol Chloride](image)

C₇H₁₇ClN₂O₂: 196.68
(2RS)-2-Carbamoyloxy-\(N,\,N,\,N-\)trimethylpropylaminium chloride [590-63-6]

Bethanechol Chloride, when dried, contains not less than 98.0% and not more than 101.0% of C₇H₁₇ClN₂O₂.

Description Bethanechol Chloride occurs as colorless or white crystals or a white, crystalline powder. It is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

A solution of Bethanechol Chloride (1 in 10) shows no optical rotation.

It is hygroscopic.

Identification (1) To 2 mL of a solution of Bethanechol Chloride (1 in 40) add 0.1 mL of a solution of cobalt (II) chloride hexahydrate (1 in 100), then add 0.1 mL of potassium hexacyanoferrate (II) TS: A green color is produced, and almost entirely fades within 10 minutes.

(2) To 1 mL of a solution of Bethanechol Chloride (1 in 100) add 0.1 mL of iodine TS: a brown precipitate is produced, and the solution shows a brown color.

(3) Determine the infrared absorption spectrum of Bethanechol Chloride as directed in the paste method under Infrared Spectrophotometry <2.23>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Bethanechol Chloride (1 in 100) responds to the Qualitative Tests <1.09> for chlorides.

Melting point <2.60> 217 – 221°C (after drying).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Bethanechol Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 1.0 g of Bethanechol Chloride in 2.5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium acetate (1 in 100), acetone, 1-butanol and formic acid (20:20:20:1) to a distance of about 10 cm, and dry the plate at 105°C for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate, and allow to stand for 30 minutes: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Bethanechol Chloride, previously dried, dissolve in 2 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 19.67 mg of C₇H₁₇ClN₂O₂

Containers and storage Containers—Tight containers.

Bezafibrate

ベザフィブラート

![Chemical Structure of Bezafibrate](image)

C₁₉H₂₀ClNO₄: 361.82
2-(4-{[4-Chlorobenzoyl]amino}ethyl)phenoxy)-2-methylpropanoic acid [41859-67-0]

Bezafibrate, when dried, contains not less than 98.5% and not more than 101.0% of C₁₉H₂₀ClNO₄.

Description Bezafibrate occurs as a white crystalline powder.

It is freely soluble in N,N-dimethylformamide, soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Bezafibrate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of
Bezafibrate as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Bezafibrate as directed under Flame Coloration Test: a green color appears.

**Melting point**

$\text{181 – 186^\circ C}$

**Purity**

(1) Chloride: Dissolve 3.0 g of Bezafibrate in 15 mL of $N, N$-dimethylformamide, add water to make 60 mL, shake well, allow to stand for more than 12 hours, and filter. To 40 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.70 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of $N, N$-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.01%).

(2) Heavy metals: Proceed with 2.0 g of Bezafibrate according to Method $4$, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances: Dissolve 0.10 g of Bezafibrate in 35 mL of methanol, add diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 70 mL of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography, according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks having retention times of about 0.65 and 1.86 with respect to bezafibrate obtained from the sample solution are not larger than 1/2 times the peak area of bezafibrate from the standard solution, the area of the peak other than those and other than bezafibrate from the sample solution is not larger than 1/5 times the peak area of bezafibrate from the standard solution, and the total area of the peaks other than the peak of bezafibrate from the sample solution is not larger than 3/4 times the peak area of bezafibrate from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wave-length: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 100) (9:4).

Flow rate: Adjust the flow rate so that the retention time of bezafibrate is about 6 minutes.

Time span of measurement: About 2.5 times as long as the retention time of bezafibrate beginning after the solvent peak.

**System suitability—**

Test for required detectability: Measure exactly 5 mL of the standard solution, and add a mixture of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 50) (7:3) to make exactly 50 mL. Confirm that the peak area of bezafibrate obtained with 5 $\mu$L of this solution is equivalent to 7 to 13% of that with 5 $\mu$L of the standard solution.

System performance: Dissolve 20 mg of Bezafibrate and 10 mg of 4-chlorobenzoate in 70 mL of methanol, and add diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make 100 mL. When the procedure is run with 5 $\mu$L of this solution under the above operating conditions, 4-chlorobenzoate and bezafibrate are eluted in order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bezafibrate is not more than 2.0%.

**Loss on drying**

Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition**

Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.7 g of Bezafibrate, previously dried, dissolve in 50 mL of ethanol (99.5), and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS is equivalent to $36.18$ mg of $C_{19}H_{20}ClNO_4$.

**Containers and storage**

Tight containers.

**Bezafibrate Sustained Release Tablets**

ベースフィプラート徐放錠

Bezafibrate Sustained Release Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bezafibrate ($C_{19}H_{20}ClNO_4$: 361.82).

**Method of preparation**

Prepare as directed under Tablets, with Bezafibrate.

**Identification**

Mix well an amount of powdered Bezafibrate Sustained Release Tablets, equivalent to 0.1 g of Bezafibrate according to the labeled amount, with 100 mL of methanol, and filter. To 1 mL of the filtrate and add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry, and it exhibits a maximum between 227 nm and 231 nm.

**Uniformity of dosage units**

It meets the requirement of the Mass variation test.

**Dissolution**

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 7.2 as the dissolution medium, the dissolution rates of a 100-mg tablet in 1.5 hours, in 2.5 hours and in 8 hours are 15 – 45%, 35 – 65% and not less than 80%, respectively, and those of a 200-mg tablet in 1.5 hours, in 2.5 hours and in 8 hours are 15 – 45%, 30 – 60% and not less than 75%, respectively.

Start the test with 1 tablet of Bezafibrate Sustained Release Tablets, withdraw not less than 20 mL of the medi-
um at the specified minutes after starting the test, and immediately fill up the dissolution medium each time with exactly 20 mL of fresh dissolution medium, previously warmed to 37 ± 0.5°C. Filter these media through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the dissolution medium to make exactly V mL, so that each mL contains about 13 μg of bezafibrate (C₁₉H₂₀ClNO₄) according to the labeled amount, and use these solutions as the sample solutions. Separately, weigh accurately about 66 mg of bezafibrate for assay, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, \( A_{T00} \) (n = 1, 2, 3) and \( A_S \), of the sample solutions and standard solution at 228 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
M_S = \frac{M_S}{\sum_{i=1}^{n-1} \frac{A_{T00} \times 1}{A_S} + \frac{A_{T00} \times 1}{A_S} \times \frac{V}{V}} \times \frac{1}{C} \times 18
\]

\( M_S \): Amount (mg) of bezafibrate for assay
\( C \): Labeled amount (mg) of bezafibrate (C₁₉H₂₀ClNO₄) in 1 tablet

**Assay** Weigh accurately, and powder not less than 20 Bezafibrate Sustained Release Tablets. Weigh accurately a Assay Weigh accurately, and powder not less than 20 bezafibrate for assay, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, \( A_{T00} \) (n = 1, 2, 3) and \( A_S \), of the sample solutions and standard solution at 228 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) in each case of n with respect to the labeled amount of bezafibrate (C₁₉H₂₀ClNO₄)

\[
\frac{M_S}{\sum_{i=1}^{n-1} \frac{A_{T00} \times 1}{A_S} + \frac{A_{T00} \times 1}{A_S} \times \frac{V}{V}} \times \frac{1}{C} \times 18
\]

**Internal standard solution—**A solution of 4-nitrophenol in methanol (1 in 500).

**Operating conditions—**
- Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeckysilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of methanol and diluted acetic acid (100 mL) in 1000 mL exactly V mL so that each mL contains about 13 μg of bezafibrate (C₁₉H₂₀ClNO₄) according to the labeled amount, and use these solutions as the sample solutions. Separately, weigh accurately about 66 mg of bezafibrate for assay, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, \( A_{T00} \) (n = 1, 2, 3) and \( A_S \), of the sample solutions and standard solution at 228 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) in each case of n with respect to the labeled amount of bezafibrate (C₁₉H₂₀ClNO₄)

\[
M_S = \frac{M_S}{\sum_{i=1}^{n-1} \frac{A_{T00} \times 1}{A_S} + \frac{A_{T00} \times 1}{A_S} \times \frac{V}{V}} \times \frac{1}{C} \times 18
\]

**Bifonazole**

![Bifonazole Structure](image)

Bifonazole, when dried, contains not less than 98.5% of C₂₂H₁₈N₂. 1-(4RS)-(Biphenyl-4-yl)(phenyl)methyl]-1H-imidazole [60629-96-8]

**Description** Bifonazole occurs as a white to pale yellow powder. It is odorless and tasteless.

It is freely soluble in dichloromethane, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Bifonazole in methanol (1 in 100) does not show optical rotation.

**Identification** (1) Determine the absorption spectrum of a solution of Bifonazole in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bifonazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** 147 – 151°C

**Purity** (1) Chloride <1.03>—To 2.0 g of Bifonazole add 40 mL of water, warm for 5 minutes, and after cooling, filter. To 10 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(2) Sulfate <1.14>—To 10 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L of the standard solution under the above operating conditions, the internal standard and bezafibrate are eluted in this order with the resolution between these peaks being not less than 4.

**System repeatability:** When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bezafibrate to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
mol/L sulfuric acid VS (0.048%).

(3) Heavy metals

Proceed with 2.0 g of Bifonazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Bifonazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 25 mL and 5 mL of this solution, add methanol to make exactly 50 mL each, and use these solutions as the standard solutions (1) and (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 10 µL of each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ammonia solution (28) (49:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot with Rf value of about 0.20 from the sample solution is not more intense than the spot from the standard solution (1). And the spots other than the spot mentioned above and the principal spot from the sample solution are not more intense than the spot from the standard solution (2).

Loss on drying <2.47> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Bifonazole, previously dried, and dissolve in dichloromethane to make exactly 50 mL. Pipet 5 mL of this solution in a glass-stoppered conical flask, add 10 mL of water, 5 mL of dilute sulfuric acid and 25 mL of dichloromethane, and add 2 to 3 drops of a solution of methyl yellow in dichloromethane (1 in 500) as indicator, and titrate <2.30>, while shaking vigorously, with 0.01 mol/L sodium lauryl sulfate VS by a buret with 0.02-mL minimum graduation. The end point is reached when the color of the dichloromethane layer changes from yellow to orange-red after dropwise addition of 0.01 mol/L sodium lauryl sulfate VS, strong shaking, and standing for a while.

Each mL of 0.01 mol/L sodium lauryl sulfate VS = 3.104 mg of C22H18N2O3S.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

### Biotin

ビオチン

Biotin occurs as white crystals or a white crystalline powder.

It is very slightly soluble in water and in ethanol (99.5).

It dissolves in dilute sodium hydroxide TS.

Melting point: about 231°C (with decomposition).

Identification Determine the infrared absorption spectrum of Biotin as directed in the potassium bromide disc method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D: +89 – +93° (after drying, 0.4 g, dilute sodium hydroxide TS, 20 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Biotin in 10 mL of 0.5 mol/L sodium hydroxide TS: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Biotin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Place 0.7 g of Biotin in a Kjeldahl flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, place a small funnel on the mouth of the flask, and carefully heat until white fumes are evolved. After cooling, add 2 mL of nitric acid twice, heat, add 2 mL of hydrogen peroxide (30) several times, and heat until the color of the solution becomes colorless or pale yellow. After cooling, add 2 mL of saturated ammonium oxalate solution, and heat to concentrate until white fumes are evolved again. After cooling, add water to make 5 mL, and perform the test using this solution as the test solution (not more than 2.8 ppm).

(4) Related substances—Dissolve 0.10 g of Biotin in 10 mL of diluted ammonia solution (28) (7 in 100), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add diluted ammonia solution (28) (7 in 100) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted ammonia solution (28) (7 in 100) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (5:2:1) to a distance of about 10 cm, air-dry the plate, and then dry for 30 minutes at 105°C. Spray the plate evenly with a mixture of a
solution of 4-dimethylaminocinnamaldehyde in ethanol (99.5) (1 in 500) and a solution of sulfuric acid in ethanol (99.5) (1 in 50) (1:1); the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** 2.44 Not more than 0.5% (0.5 g, 105°C, 4 hours).

**Residue on ignition** 2.44 Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.25 g of Biotin, previously dried, dissolve by adding exactly 20 mL of 0.1 mol/L sodium hydroxide VS, and titrate 2.50 the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L sodium hydroxide VS = 24.43 mg of C_{10}H_{16}N_{2}O_{3}S

**Containers and storage** Containers—Tight containers.

**Biperiden Hydrochloride**

ビペリデン塩酸塩

C_{21}H_{29}NO.HCl: 347.92
1-(Bicyclo[2.2.1]hept-5-en-2-yl)-1-phenyl-3-(piperidin-1-yl)propan-1-ol monohydrochloride
[1235-82-1]

Biperiden Hydrochloride, when dried, contains not less than 99.0% of C_{21}H_{29}NO.HCl.

**Description** Biperiden Hydrochloride occurs as a white to brownish and yellowish white, crystalline powder.

It is freely soluble in formic acid, slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 270°C (with decomposition).

**Identification** (1) Dissolve 0.02 g of Biperiden Hydrochloride in 5 mL of phosphoric acid: a green color develops.
(2) Dissolve 0.01 g of Biperiden Hydrochloride in 5 mL of water by heating, cool, and add 5 to 6 drops of bromine TS: a green color develops.
(3) Determine the absorption spectrum of a solution of Biperiden Hydrochloride (1 in 2000) as directed under Ultra-violet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(4) Determine the infrared absorption spectrum of Biperiden Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
(5) Dissolve 0.02 g of Biperiden Hydrochloride in 10 mL of water by heating, and cool: the solution responds to the Qualitative Tests 1.09 for chloride.

**Purity** (1) Acidity or alkalinity—To 1.0 g of Biperiden Hydrochloride add 50 mL of water, shake vigorously, filter, and to 20 mL of the filtrate add 1 drop of methyl red TS: no red to yellow color develops.
(2) Heavy metals 1.07—Proceed with 1.0 g of Biperiden Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(3) Arsenic 1.11—Prepare the test solution with 1.0 g of Biperiden Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).
(4) Related substances—Dissolve 0.10 g of Biperiden Hydrochloride in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.05. Spot 50 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28:80:15:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** 2.44 Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** 2.44 Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Biperiden Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 60 mL of acetic anhydride, and titrate 2.50 with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 34.79 mg of C_{21}H_{29}NO.HCl

**Containers and storage** Containers—Tight containers.

**Storage**—Light-resistant.

**Bisacodyl**

ピサコジル

C_{22}H_{19}NO_{4}: 361.39
4,4′-(Pyridin-2-ylmethylene)bis(phenyl acetate)
[603-50-9]

Bisacodyl, when dried, contains not less than 98.5% of C_{22}H_{19}NO_{4}.
**Bisacodyl Suppositories**

Bisacodyl Suppositories contain not less than 90.0% and not more than 110.0% of the labeled amount of bisacodyl (C\(_{22}\)H\(_{19}\)NO\(_4\)) as C\(_{22}\)H\(_{19}\)NO\(_4\); 361.39.

**Method of preparation** Prepare as directed under Suppositories, with Bisacodyl.

**Identification (1)** To a quantity of Bisacodyl Suppositories, equivalent to 6 mg of Bisacodyl according to the labeled amount, add 20 mL of ethanol (95), warm on a water bath for 10 minutes, shake vigorously for 10 minutes, and allow to stand in ice water for 1 hour. Centrifuge the solution, filter the supernatant liquid, and to 2 mL of the filtrate add ethanol (95) to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \(\lambda_{	ext{max}}\): it exhibits a maximum between 261 nm and 265 nm.

**2. Use** the filtrate obtained in (1) as the sample solution. Separately, dissolve 6 mg of Bisacodyl RS in 20 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(\lambda_{	ext{max}}\). Spot 20 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-butane, chloroform, and xylene (1:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same \(Rf\) value.

**Uniformity of dosage units** \(\lambda_{0.02}\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 suppository of Bisacodyl Suppositories add tetrahydrofuran to make a solution containing about 0.2 mg of bisacodyl (C\(_{22}\)H\(_{19}\)NO\(_4\)) in each mL, warm to 40°C, and shake to dissolve. After cooling, add tetrahydrofuran to make exactly \(V\) mL so that each mL contains about 10 \(\mu\)g of bisacodyl (C\(_{22}\)H\(_{19}\)NO\(_4\)). Pipet 5 mL of this solution, and proceed as directed in the Assay.

\[
\text{Amount (mg) of bisacodyl (C}_{22}\text{H}_{19}\text{NO}_{4}) = M_s \times Q_r = V/50
\]

\(M_s\): Amount (mg) of Bisacodyl RS

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in acetonitrile (3 in 100,000).

**Assay** Weigh accurately not less than 20 Bisacodyl Suppositories, make them fine fragments carefully, and mix uniformly. Weigh accurately a portion of the fragments, equivalent to about 10 mg of bisacodyl (C\(_{22}\)H\(_{19}\)NO\(_4\)), add 40 mL of tetrahydrofuran, warm to 40°C, dissolve by shaking, cool, and add tetrahydrofuran to make exactly 50 mL. Pipet
5 mL of this solution, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 100 mL. Cool this solution in ice for 30 minutes, centrifuge, filter the supernatant liquid through a membrane filter with pore size of 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of Bisacodyl RS, previously dried at 105°C for 2 hours, and dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, proceed in the same manner as the sample solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, QT and QS, of the peak area of bisacodyl to that of the internal standard, respectively.

Amount (mg) of bisacodyl (C₂₂H₁₉NO₄) = M₅ × Q₅/Q₅
M₅: Amount (mg) of Bisacodyl RS

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (3 in 100,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of 0.01 mol/L citric acid TS, acetonitrile and methanol (2:1:1).
Flow rate: Adjust the flow rate so that the retention time of bisacodyl is about 8 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and bisacodyl are eluted in this order with the resolution between these peaks being not less than 2.0.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of bisacodyl to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

**Bismuth Subgallate**

**Dermatol**

次没食子酸ビスマス

Bismuth Subgallate, when dried, contains not less than 47.0% and not more than 51.0% of bismuth (Bi: 208.98).

**Description**
Bismuth Subgallate occurs as a yellow powder. It is odorless and tasteless. It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid, in dilute nitric acid and in dilute sulfuric acid on warming. It dissolves in sodium hydroxide TS, forming a clear, yellow solution, which turns red immediately.

It is affected by light.

**Identification (1)**
Ignite 0.5 g of Bismuth Subgallate: it chars at first, and leaves finally a yellow residue. The residue responds to the Qualitative Tests <1.09> for bismuth salt.

(2) To 0.5 g of Bismuth Subgallate add 25 mL of water and 20 mL of hydrogen sulfide TS, and shake well. Filter off the blackish brown precipitate, and add 1 drop of iron (III) chloride TS to the filtrate: a blue-black color is produced.

**Purity (1)**
Clarity of solution—Dissolve 1.0 g of Bismuth Subgallate in 40 mL of diluted sodium hydroxide TS (1 in 8): the solution is clear.

(2) Sulfate—Ignite 3.0 g of Bismuth Subgallate in a porcelain crucible, and cautiously dissolve the residue in 2.5 mL of nitric acid by warming. Pour the solution into 100 mL of water, shake, and filter. Evaporate 50 mL of the filtrate on a water bath to 15 mL. Add water to make 20 mL, filter again, and use the filtrate as the sample solution. To 5 mL of the sample solution add 2 to 3 drops of barium nitrate TS: no turbidity is produced.

(3) Nitrate—To 0.5 g of Bismuth Subgallate add 5 mL of dilute sulfuric acid and 25 mL of iron (II) sulfate TS, shake well, and filter. Superimpose carefully 5 mL of the filtrate on sulfuric acid: no red-brown color develops at the zone of contact.

(4) Ammonium—Dissolve 1.0 g of Bismuth Subgallate in 5 mL of sodium hydroxide TS, and heat: the gas evolved does not change moistened red litmus paper to blue.

(5) Copper—To 5 mL of the sample solution obtained in (2) add 1 mL of ammonia TS, and filter: no blue color develops in the filtrate.

(6) Lead—Ignite 1.0 g of Bismuth Subgallate at about 500°C in a porcelain crucible, dissolve the residue in a smallest possible amount of nitric acid added dropwise, evaporate over a low flame to dryness, and cool. Add 5 mL of a solution of potassium hydroxide (1 in 6) to the residue, boil carefully for 2 minutes, cool, and centrifuge. Take the supernatant liquid in a test tube, add 10 drops of potassium chromate TS, and acidify the solution by adding acetic acid (100 dropwise): neither turbidity nor a yellow precipitate is produced.

(7) Silver—To 5 mL of the sample solution obtained in (2) add 0.5 mL of nitric acid and 2 to 3 drops of dilute hydrochloric acid: no turbidity is produced.

(8) Alkaline earth metals and alkali metals—Boil 1.0 g of Bismuth Subgallate with 40 mL of dilute acetic acid (31) (1 in 2) for 2 minutes, cool, add water to make 40 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute hydrochloric acid, boil, immediately pass hydrogen sulfide thoroughly through the solution, filter the precipitate produced, and wash with water. Combine the filtrate and the washings, add 5 drops of sulfuric acid, and evaporate to dryness. Ignite as directed under Residue on Ignition <2.44>: the mass of the residue does not more than 5.0 mg.

(9) Arsenic <1.11>—Mix well 0.20 g of Bismuth Subgallate with 0.20 g of calcium hydroxide, and ignite the mixture. Dissolve the residue in 5 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 10 ppm).

(10) Gallic acid—To 1.0 g of Bismuth Subgallate add

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JP XVI
20 mL of ethanol (95), shake for 1 minute, and filter. Evaporate the filtrate on a water bath to dryness: the mass of the residue does not more than 5.0 mg.

**Loss on drying** <2.41> Not more than 6.0% (1 g, 105°C, 3 hours).

**Assay** Weigh accurately about 0.5 g of Bismuth Subgallate, previously dried, ignite at about 500°C for 30 minutes, and cool. Dissolve the residue in 5 mL of dilute nitric acid (2 in 5) by warming, and add water to make exactly 100 mL. Measure exactly 30 mL of this solution, add 200 mL of water, and titrate <2.50> with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to yellow (indicator: 2 to 3 drops of xylene orange TS).

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 4.180 mg of Bi

**Containers and storage** Containers—Well-closed containers.

**Description** Bismuth Subnitrate occurs as a white powder. It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It readily dissolves in hydrochloric acid and in nitric acid without effervescence.

It is slightly hygroscopic, and changes moistened blue litmus paper to red.

**Identification** Bismuth Subnitrate responds to the Qualitative Tests <1.09> for bismuth salt and nitrate.

**Purity (1)** Chloride <1.03>—Dissolve 0.7 g of Bismuth Subnitrate in 2 mL of water and 2 mL of nitric acid, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of nitric acid on a water bath to dryness, add 0.70 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.035%).

(2) Sulfate—Dissolve 3.0 g of Bismuth Subnitrate in 3.0 mL of warm nitric acid, pour this solution into 100 mL of water, shake, and filter. Concentrate the filtrate on a water bath to 30 mL, filter, and use this filtrate as the sample solution. To 5 mL of the sample solution add 2 to 3 drops of barium nitrate TS: no turbidity is produced.

(3) Ammonium—Boil 0.10 g of bismuth Subnitrate with 5 mL of sodium hydroxide TS: the gas evolved does not change moistened red litmus paper to blue.

(4) Copper—To 5 mL of the sample solution obtained in (2) add 2 mL of ammonia TS, and filter: no blue color develops.

(5) Lead—To 1.0 g of Bismuth Subnitrate add 5 mL of a solution of sodium hydroxide (1 in 6), boil carefully for 2 minutes, cool and centrifuge. Transfer the supernatant liquid to a test tube, add 10 drops of potassium chromate TS, and add dropwise acetic acid (31) to render the solution acid: no turbidity or yellow precipitate is produced.

(6) Silver—To 5 mL of the sample solution obtained in (2) add 0.5 mL of nitric acid and 2 to 3 drops of dilute hydrochloric acid: no turbidity is produced.

(7) Alkaline earth metals and alkali metals—Boil 2.0 g of Bismuth Subnitrate with 40 mL of diluted acetic acid (31) (1 in 2) for 2 minutes, cool, add water to make 40 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute hydrochloric acid, boil, immediately pass hydrogen sulfide thoroughly through the solution, filter, and wash the residue with water. Combine the filtrate and the washings, add 5 drops of sulfuric acid, evaporate to dryness, and ignite as directed under Residue on Ignition <2.44>: the residue does not exceed 5.0 mg.

(8) Arsenic <1.17>—To 0.20 g of Bismuth Subnitrate add 2 mL of sulfuric acid, heat until white fumes evolve, dilute cautiously with water to 5 mL, use this solution as the test solution, and perform the test (not more than 10 ppm).

**Loss on drying** <2.41> Not more than 3.0% (2 g, 105°C, 2 hours).

**Assay** Weigh accurately about 0.4 g of Bismuth Subnitrate, previously dried, dissolve in 5 mL of diluted nitric acid (2 in 5) by warming, and add water to make exactly 100 mL. Pipet 25 mL of the solution, add 200 mL of water and titrate <2.50> with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to yellow (indicator: 5 drops of xylene orange TS).

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 4.180 mg of Bi

**Containers and storage** Containers—Well-closed containers.

**Bisoprolol Fumarate**

**Description** Bisoprolol Fumarate occurs as white crystals

\[(C_{18}H_{31}NO_{4})_2\cdot C_4H_4O_4 \cdot 766.96\]

\[
\text{(2S,RS)-1-[(4-[2-(1-Methylethoxy)ethoxy]methyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol fumarate [104344-23-2]}
\]

Bisoprolol Fumarate, when dried, contains not less than 98.5% and not more than 101.0% of \((C_{18}H_{31}NO_{4})_2\cdot C_4H_4O_4\).

**Description** Bisoprolol Fumarate occurs as white crystals
or a white crystalline powder.

It is very soluble in water and in methanol, and freely soluble in ethanol (99.5) and in acetic acid (100).

A solution of Bisoprolol Fumarate (1 in 10) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Bisoprolol Fumarate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \( < 2.24 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bisoprolol Fumarate as directed in the potassium bromide disc method under Infrared Spectrophotometry \( < 2.25 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** \( < 2.60 \) 101 - 105°C

**Purity (1)** Heavy metals \( < 1.07 \)—Proceed with 2.0 g of Bisoprolol Fumarate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Bisoprolol Fumarate in 100 mL of a mixture of water and acetonitrile (4:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L of each of the sample solution and standard solution as directed under Liquid Chromatography \( < 2.01 \) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks other than bisoprolol obtained from the sample solution is not larger than 1/2 times the peak area of bisoprolol from the standard solution. Furthermore, the total of the areas of all peaks other than bisoprolol from the sample solution is not larger than the peak area of bisoprolol from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of bisoprolol is about 8 minutes.

Time span of measurement: About 2 times as long as the retention time of bisoprolol, beginning after the fumaric acid peak.

**System suitability**—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of water and acetonitrile (4:1) to make exactly 20 mL. Confirm that the peak area of bisoprolol obtained from 20 \( \mu \)L of this solution is equivalent to 7 to 13% of that from 20 \( \mu \)L of the standard solution.

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bisoprolol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bisoprolol is not more than 1.5%.

**Loss on drying** \( < 2.41 \) Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 5 hours).

**Residue on ignition** \( < 2.44 \) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.6 g of Bisoprolol Fumarate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate \( < 2.50 \) with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). The endpoint of titration is when the purple color of the solution turns blue and then blue-green. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 38.35 mg of \((C_{18}H_{31}NO_4)_2C_4H_4O_4\)

**Containers** Containers—Tight containers.

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**Bisoprolol Fumarate Tablets**

ビソプロロールフマル酸塩錠

Bisoprolol Fumarate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bisoprolol fumarate \([C_{18}H_{31}NO_4]_2C_4H_4O_4: 766.96\).

**Method of preparation** Prepare as directed under Tablets, with Bisoprolol Fumarate.

**Identification** To a quantity of powdered Bisoprolol Fumarate Tablets, equivalent to 10 mg of Bisoprolol Fumarate according to the labeled amount, add 60 mL of methanol, shake vigorously for 10 minutes, add methanol to make 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \)m. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry \( < 2.24 \); it exhibits a maximum between 271 nm and 275 nm.

**Uniformity of dosage units** \( < 6.02 \)—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Bisoprolol Fumarate Tablets, disintegrate by adding 8 mL of water, and add water to make exactly 10 mL, and then filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \)m. Discard the first 3 mL of the filtrate, pipet 1 mL of the subsequent filtrate, add water to make exactly 1 mL so that each mL contains about 0.1 mg of bisoprolol fumarate \([C_{18}H_{31}NO_4]_2C_4H_4O_4\), and use as the sample solution. Separately, weigh accurately about 20 mg of bisoprolol fumarate for assay, previously dried under reduced pressure at 80°C for 5 hours, using phosphorus (V) oxide as a dessicant, dissolve in water to make exactly 200 mL, and use as the standard solution. Determine the absor-
that each mL contains about 2.8 solution medium to make exactly 100 mL. Pipet 2 mL of this solution with exactly 50 mL, and use this solution as the standard solution. Perform the solution, add the dissolution medium to make exactly 100 mL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Qf and Qs, of the peak area of bisoprolol to that of the internal standard.

Amount (mg) of bisoprolol fumarate [(C18H31NO4)2.C4H4O4]i n1t a b l e t

\[ M_S = \frac{\text{Amount (mg) of bisoprolol fumarate for assay}}{\frac{A_T}{A_S} \times \frac{V}{V} \times \frac{1}{1/20}} \]

\[ M_S: \text{Amount (mg) of bisoprolol fumarate for assay} \]

\[ C: \text{Labeled amount (mg) of bisoprolol fumarate} \]

\[ [(C18H31NO4)2.C4H4O4], \text{in 1 tablet} \]

Dissolution rate (%) with respect to the labeled amount of bisoprolol fumarate [(C18H31NO4)2.C4H4O4]

\[ M_5 = \frac{A_S}{A_5} \times \frac{V}{V} \times \frac{1}{1/C} \times 18 \]

\[ M_5: \text{Amount (mg) of bisoprolol fumarate for assay} \]

\[ A_T, A_S: \text{bances of the sample solution and standard solution at 271.5 nm as directed under Ultraviolet-visible Spectrophotometry} <2.24>. \]

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in 800 mL of this solution, dissolve in the mixture of water and acetonitrile (3:1) to make 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of bisoprolol fumarate for assay, previously dried in vacuum at 80°C for 5 hours using phosphorus (V) oxide as the desiccant, add exactly 10 mL of the internal standard solution, dissolve in the mixture of water and acetonitrile (3:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Qf and Qs, of the peak area of bisoprolol to that of the internal standard.

Amount (mg) of bisoprolol fumarate [(C18H31NO4)2.C4H4O4] in 1 tablet

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mixture of water and acetonitrile (3:1) (1 in 250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of bisoprolol is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, fumaric acid, bisoprolol and the internal standard are eluted in this order with the resolution between the peaks of bisoprolol and the internal standard being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bisoprolol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Bleomycin Hydrochloride

Bleomycin Hydrochloride is the hydrochloride of a mixture of substances having antitumor activity produced by the growth of *Streptomyces verticillus*. It contains not less than 1400 μg (potency) and not more than 2000 μg (potency) per mg, calculated on the dried basis. The potency of Bleomycin Hydrochloride is expressed as mass (potency) of bleomycin A2 (C55H84ClN17O21S3: 1451.00).

**Description**
Bleomycin Hydrochloride occurs as a white to yellowish white powder. It is freely soluble in water, and slightly soluble in ethanol (95%). It is hygroscopic.

**Identification**
(1) To 4 mg of Bleomycin Hydrochloride add 5 mL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bleomycin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bleomycin Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

**pH**
The pH of a solution obtained by dissolving 0.10 g of Bleomycin Hydrochloride in 20 mL of water is between 4.5 and 6.0.

**Content ratio of the active principle**
Dissolve 10 mg of Bleomycin Hydrochloride in 20 mL of water, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of bleomycin A2 (the first principal peak) is between 55% and 70%, that of bleomycin B2 (the second principal peak) is between 25% and 32%, the total peak area of bleomycin A2 and bleomycin B2 is not less than 85%, the peak area of demethylbleomycin A2 (a peak having the relative retention time of 1.5 – 2.5 to bleomycin A2) is not more than 5.5%, and the total area of the rest peaks is not more than 9.5%.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of the mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of the mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following...
Purity (1) Clarity and color of solution—A solution obtained by dissolving 80 mg of Bleomycin Hydrochloride in 4 mL of water is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Bleomycin Hydrochloride in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to exactly 15 mL of Standard Copper Solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

- Gas: Combustible gas—Acetylene
- Supporting gas—Air
- Lamp: Copper hollow-cathode lamp
- Wavelength: 324.8 nm

Loss on drying <2.47> Not more than 5.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Take the sample to be tested while avoiding moisture absorption.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Mycobacterium smegmatis* ATCC 607

(ii) Agar medium for seed, base layer and transferring the test organism

| Glycerin   | 10.0 g |
| Peptone    | 10.0 g |
| Meat extract | 10.0 g |
| Sodium chloride | 3.0 g |
| Agar       | 15.0 g |
| Water      | 1000 mL |

Mix all the components, and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(iii) Liquid media for suspending the test organism

| Glycerin   | 10.0 g |
| Peptone    | 10.0 g |
| Meat extract | 10.0 g |

Sodium chloride 3.0 g
Water 1000 mL
Mix all the components, and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(iv) Preparation of seeded agar layer—Cultivate the test organism on the slant of the agar medium for transferring the test organism at 27°C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid media for suspending the test organism, cultivate with shaking at between 25°C and 27°C for 5 days, and use this as the suspension of test organism. Store the suspension of test organism at a temperature not exceeding 5°C, and use within 14 days. Add 0.5 mL of the suspension of test organism in 100 mL of the agar medium for seed previously kept at 48°C, mix thoroughly, and use as the seeded agar layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 1.7. Preparation of cylinder-agar plates under the Microbial Assay for Antibiotics, dispensing 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed into the Petri dish.

(vi) Standard solutions—Weigh accurately an amount of Bleomycin A2 Hydrochloride RS, previously dried under reduced pressure not exceeding 0.67 kPa at an ordinary temperature for 3 hours, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Bleomycin Hydrochloride, equivalent to about 15 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—tight containers.
Bleomycin Sulfate

Bleomycin Sulfate is the sulfate of a mixture of substances having antitumor activity produced by the growth of Streptomyces verticillus.

It contains not less than 1400 μg (potency) and not more than 2000 μg (potency) per mg, calculated on the dried basis. The potency of Bleomycin Sulfate is expressed as mass (potency) of bleomycin A₂ (C₅₅H₈₄ClN₁₇O₂₁S₃: 1451.00).

**Description**

Bleomycin Sulfate occurs as a white to yellowish white powder. It is freely soluble in water, and slightly soluble in ethanol (95).

It is hygroscopic.

**Identification**

(1) To 4 mg of Bleomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bleomycin Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bleomycin Sulfate (1 in 200) responds to the Qualitative Tests \(<1.09\rangle\) (1) and (2) for sulfate.

**pH** \(<2.54\rangle\)

The pH of a solution obtained by dissolving 10 mg of Bleomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

**Content ratio of the active principle**

Dissolve 10 mg of Bleomycin Sulfate in 20 mL of water, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography \(<2.01\rangle\) according to the following conditions, and determine each peak area by the automatic integration method: the peak area of bleomycin A₂ (the first principal peak) is between 55% and 70%, that of bleomycin B₂ (the second principal peak) is between 25% and 32%, the total peak area of bleomycin A₂ and bleomycin B₂ is not less than 85%, the peak area of demethylbleomycin A₂ (a peak having the relative retention time of 1.5 - 2.5 against bleomycin A₂) is not more than 5.5%, and the total area of the rest peaks is not more than 9.5%.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of the mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of the mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.
Flow rate: About 1.2 mL/min
Time span of measurement: Twenty minutes after elution of the peak of demethylbleomycin A₂ beginning after the solvent peak.
System suitability—
System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, bleomycin A₂ and bleomycin B₂ are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of bleomycin A₂ is not more than 2.0%.

**Purity (1)**
Clarity and color of solution—A solution obtained by dissolving 80 mg of Bleomycin Sulfate in 4 mL of water is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Bleomycin Sulfate in 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to exactly 15 mL of Standard Copper Solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions: the absorbance of the sample solution is not more than 2.41, and the absorbance of the standard solution is not more than 2.23, with the resolution between these peaks being not less than 5.

Wavelength: 324.8 nm
Lamp: Copper hollow-cathode lamp
Gas: Combustible gas—Acetylene
Supporting gas—Air

**Loss on drying <2.47>**
Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Take the sample to be tested while avoiding moisture absorption.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Mycobacterium smegmatis* ATCC 607
(ii) Agar medium for seed, base layer and transferring the test organism

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 60</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>60 – 75</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Mix all the components and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(iv) Preparation of seeded agar layer—Cultivate the test organism on the slant of the agar medium for transferring the test organism at 27°C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid medium for suspending the test organism, cultivate with shaking at between 25°C and 27°C for 5 days, and use this as the suspension of test organism. Store the suspension of test organism at a temperature not exceeding 5°C, and use within 14 days. Add 0.5 mL of the suspension of test organism in 100 mL of the agar medium for seed previously kept at 48°C, mix thoroughly, and use as the seeded agar layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 1.7. Preparation of cylinder-agar plates under the Microbial Assay for Antibiotics, dispensing 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed into the Petri dish.

(vi) Standard solutions—Weigh accurately an amount of Bleomycin A₂ Hydrochloride RS, previously dried under reduced pressure not exceeding 0.67 kPa at an ordinary temperature for 3 hours, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Bleomycin Sulfate, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Containers and storage**
Containers—Tight containers.

### Boric Acid

ホウ酸

**Identification**
Boric Acid, when dried, contains not less than 99.5% of H₃BO₃.

**Description**
Boric Acid occurs as colorless or white crystals or crystalline powder. It is odorless, and has a slight, characteristic taste.

It is freely soluble in warm water, in hot ethanol (95) and in glycerin, soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Boric Acid (1 in 20) is between 3.5 and 4.1.

**Identification** A solution of Boric Acid (1 in 20) responds...
to the Qualitative Tests <1.09> for borate.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Boric Acid in 25 mL of water or in 10 mL of hot ethanol (95): the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Boric Acid according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.1D>—Prepare the test solution with 0.40 g of Boric Acid according to Method 1, and perform the test (not more than 5 ppm).

**Loss on drying** <2.4I> Not more than 0.5% (2 g, silica gel, 5 hours).

**Assay** Weigh accurately about 1.5 g of Boric Acid, previously dried, add 15 g of D-sorbitol and 50 mL of water, and dissolve by warming. After cooling, titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS = 61.83 mg of H₃BO₃

**Containers and storage** Containers—Well-closed containers.

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**Freeze-dried Botulism Antitoxin, Equine**

【乾燥ボツリヌスウマ抗毒素】

Freeze-dried Botulism Antitoxin, Equine, is a preparation for injection which is dissolved before use.

It contains botulism antitoxin type A, botulism antitoxin type B, botulism antitoxin type E and botulism antitoxin type F in immunoglobulin of horse origin. It may contain one, two or three of these four antitoxins.

It conforms to the requirements of Freeze-dried Botulism Antitoxin, Equine, in the Minimum Requirements for Biological Products.

**Description** Freeze-dried Botulism Antitoxin, Equine, becomes a colorless or yellow-brown, clear liquid or a slightly white-turbid liquid on the addition of solvent.

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**Bromazepam**

【ブロマゼパム】

C₁₄H₁₀BrN₃O: 316.15
7-Bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one

[1812-30-2]

Bromazepam, when dried, contains not less than 99.0% and not more than 101.0% of C₁₄H₁₀BrN₃O.

**Description** Bromazepam occurs as white to light yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol, in ethanol (99.5) and in acetone, and practically insoluble in water.

Melting point: about 245°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Bromazepam in ethanol (99.5) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bromazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Bromazepam in a platinum crucible according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Bromazepam in 5 mL of a mixture of acetone and methanol (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mixture of acetone and methanol (3:2) to make exactly 50 mL. Pipet 5 mL of this solution, add the mixture of acetone and methanol (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28) and ethanol (99.5) (38:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution and the spot of the starting point are not more than 2, and not more intense than the spot from the standard solution.

**Loss on drying** <2.4I> Not more than 0.20% (1 g, 105°C, 4 hours).
Bromhexine Hydrochloride

**Description**  Bromhexine Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in formic acid, sparingly soluble in methanol, and slightly soluble in water and in ethanol (95%).

The pH of its saturated solution is between 3.0 and 5.0.

Melting point: about 239°C (with decomposition).

**Identification**

(1) Dissolve 3 mg of Bromhexine Hydrochloride in 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bromhexine Hydrochloride as directed in the potassium bromide disk method under Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Add 20 mL of water to 1 g of Bromhexine Hydrochloride. After thorough shaking, add 3 mL of sodium hydroxide TS, and extract with four 20-mL portions of diethyl ether. Neutralize the water layer with dilute nitric acid; the solution responds to the Qualitative Tests <1.09> (2) for chloride.

**Purity**

(1) Heavy metals <1.07>—Proceed with 2.0 g of Bromhexine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Bromhexine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than bromhexine is not larger than the peak area of bromhexine of the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 7.0 with 0.5 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 200 mL of this solution add 800 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of bromhexine is about 6 minutes.

Selection of column: To 0.05 g of bamethane sulfate add 0.5 mL of the sample solution, and add the mobile phase to make 10 mL. Proceed with 5 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of bamethane and bromhexine in this order with the resolution between these peaks being not less than 7.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of bromhexine from 5 μL of the standard solution is between 5 mm and 15 mm.

Time span of measurement: About 2 times as long as the retention time of bromhexine beginning after the solvent peak.

**Loss on drying** <2.41>—Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44>—Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.5 g of Bromhexine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and warm in a water bath at 50°C for 15 minutes. After cooling, titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to yellow-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 41.26 mg of \( \text{C}_{14}\text{H}_{10}\text{BrN}_{3}\text{O} \)

**Containers and storage**  Containers—Well-closed containers.

**Storage**—Light-resistant.
Bromocriptine Mesilate

Bromocriptine Mesilate occurs as a white to pale yellowish white or pale brownish white, crystalline powder. It is odorless, or has a faint characteristic odor. It is very soluble in acetic acid (100), freely soluble in methanol, sparingly soluble in ethanol (95), very slightly soluble in acetic anhydride, in dichloromethane and in chloroform, and practically insoluble in water and in diethyl ether.

It is gradually colored by light.

Identification (1) Dissolve 2 mg of Bromocriptine Mesilate in 1 mL of methanol, add 2 mL of a 4-dimethylaminobenzaldehyde-ferric chloride TS, and shake: a purplish blue color develops.

(2) Determine the absorption spectrum of a solution of Bromocriptine Mesilate in methanol (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bromocriptine Mesilate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Bromocriptine Mesilate as directed under Flame Coloration Test <1.04> (2): a green color appears.

Optical rotation <2.44> [α]D 20: +95° to +105° [0.1 g, calculated on the dried basis, a mixture of methanol and dichloromethane (1:1), 10 mL, 100 mm].

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Bromocriptine Mesilate in 10 mL of methanol: the solution is clear, and has no more color than the following control solution.

Control solution: To 2.5 mL of Cobalt (II) Chloride CS, 6.0 mL of Iron (III) Chloride CS and 1.0 mL of Copper (II) Sulfate CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

Bromovalerylurea

Bromovalerylurea occurs as colorless or white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.
It is soluble in ethanol (95%), sparingly soluble in diethyl ether, and very slightly soluble in water.

It dissolves in sulfuric acid, in nitric acid and in hydrochloric acid, and precipitates are produced on the addition of water.

It dissolves in sodium hydroxide TS.

Identification (1) Boil 0.2 g of Bromovalerylurea with 5 mL of a solution of sodium hydroxide (1 in 10): the gas evolved changes moistened red litmus paper to blue. Boil this solution with an excess of dilute sulfuric acid: the odor of valeric acid is perceptible.

(2) To 0.1 g of Bromovalerylurea add 0.5 g of anhydrous sodium carbonate, and decompose thoroughly by gentle heating. Dissolve the residue in 5 mL of hot water, cool, acidify with acetic acid (31), and filter: the filtrate responds to the Qualitative Tests (1.09) (2) for bromide.

Melting point 2.60° 151 – 155°C

Purity (1) Acidity or alkalinity—To 1.5 g of Bromovalerylurea add 30 mL of water, shake for 5 minutes, and filter: the filtrate is neutral.

(2) Chloride 1.03°—Perform the test with a 10-mL portion of the filtrate obtained in (1). Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Sulfate 1.14°—Perform the test with 10 mL of the filtrate obtained in (1). Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals 1.07°—Proceed with 2.0 g of Bromovalerylurea according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic 1.11°—Dissolve 0.5 g of Bromovalerylurea in 5 mL of sodium hydroxide TS, use this solution as the test solution, and perform the test (not more than 4 ppm).

(6) Readily carbonizable substances 1.15°—Perform the test with 0.5 g of Bromovalerylurea: the solution is not more colored than Matching Fluid A.

Loss on drying 2.4I Not more than 0.5% (1 g, 80°C, 2 hours).

Residue on ignition 2.4I Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Bromovalerylurea, previously dried, in a 300-mL conical flask, add 40 mL of sodium hydroxide TS, and boil gently for 20 minutes under a reflux condenser. Cool, wash the lower part of the reflux condenser and the mouth of the flask with 30 mL of water, and combine the washings with the solution in the conical flask. Add 5 mL of nitric acid and exactly 30 mL of 0.1 mol/L silver nitrate VS, and titrate 2.50° the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS = 22.31 mg of C₇H₁₃N₂O₃S₂

Containers and storage Containers—Well-closed containers.

Bucillamine

ブシラミン

C₇H₁₃NO₃S₂: 223.31

(2R)-2-(2-Methyl-2-sulfanylpropanoylamino)-3-sulfanylpropanoic acid [65002-17-7]

Bucillamine, when dried, contains not less than 98.5% and not more than 101.0% of C₇H₁₃NO₃S₂.

Description Bucillamine occurs as white, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and slightly soluble in water.

Identification (1) To 5 mL of a solution of Bucillamine (1 in 250) add 2 mL of sodium hydroxide TS and 2 drops of sodium pentacyanonitrosylferrate (III) TS: the solution reveals a red-purple color.

(2) Determine the infrared absorption spectrum of Bucillamine as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25°, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation 2.49° [α]D: +33.0° – +36.5° (after drying, 2 g, ethanol (95), 50 mL, 100 mm).

Melting point 2.60° 136 – 140°C

Purity (1) Heavy metals 1.07°—Proceed with 1.0 g of Bucillamine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic 1.11°—Prepare the test solution with 1.0 g of Bucillamine according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 60 mg of Bucillamine in 20 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mixture of water and methanol (1:1) to make exactly 200 mL, and use this solution as the standard solution. Immediately perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01° according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of related substances, having the relative retention time of about 2.3 and 3.1 with respect to the peak of bucillamine, obtained from the sample solution are not larger than 8/15 times and 2/5 times the peak area of bucillamine from the standard solution, respectively, and the area of the peak other than the peaks of bucillamine and two of the related substances mentioned above from the sample solution is not larger than 1/5 times the peak area of bucillamine from the standard solution. The total area of the peaks other than bucillamine from the sample solution is not larger than the peak area of bucil-
Bucillamine Tablets

Bucillamine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bucillamine (C7H13NO3S2: 223.31).

Method of preparation Prepare as directed under Tablets, with Bucillamine.

Identification (1) To a quantity of powdered Bucillamine Tablets, equivalent to 0.1 g of Bucillamine according to the labeled amount, add 0.1 g of sodium hydrogen carbonate and 10 mL of water, shake well, filter, and add 1 or 2 drops of ninhydrin TS to the filtrate: it exhibits a red-brown color.

(2) To a quantity of powdered Bucillamine Tablets, equivalent to 0.1 g of Bucillamine according to the labeled amount, add 25 mL of water, shake well, and filter. To 5 mL of the filtrate, add 2 mL of dilute sodium hydroxide TS and 1 or 2 drops of sodium pentacyanonitrosylferrate (III) TS: it exhibits a red-purple color.

Uniformity of dosage units <6.02>—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Store the sample solution and standard solution in a cold place until performing the measurements. Take 1 tablet of Bucillamine Tablets, add exactly 1 mL of the internal standard solution per 0.1 g of bucillamine (C7H13NO3S2), then add 3 mL of water and 6 mL of methanol per 0.1 g of bucillamine (C7H13NO3S2), and stir well until the tablet completely disintegrated. To 1 mL of this solution add the mobile phase to make 25 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Internal standard solution—A solution of 4-fluorobenzoic acid in methanol (1 in 100).

Dissolution <6.10>—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Bucillamine Tablets is not less than 80%.

Store the sample solution and standard solution in a cold place until performing the measurements. Start the test with 1 tablet of Bucillamine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, add 2 mL of dilute sodium hydroxide TS and 1 or 2 drops of sodium pentacyanonitrosylferrate (III) TS: it exhibits a red-purple color.
Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and methanol (11:9).
Flow rate: Adjust the flow rate so that the retention time of bucillamine is about 4 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bucillamine are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bucillamine is not more than 2.0%.

Assay—Store the sample solution and standard solution in a cold place until performing the measurements. Take 10 tablets of Bucillamine Tablets, add exactly 1 mL of the internal standard solution per 0.1 g of bucillamine (C7H13NO3S2), add 3 mL of water and 6 mL of methanol, and stir well until the tablets completely disintegrated. To 1 mL of this solution add the mobile phase to make 25 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use this solution as the sample solution. Separately, weigh accurately about 0.2 g of bucillamine for assay, previously dried in vacuum at 60°C to a constant weight, add exactly 2 mL of the internal standard solution as directed under Liquid Chromatography, and compare the ratio of the peak area of bucillamine to that of the internal standard.

\[
\text{Amount (mg) of bucillamine (C}_7\text{H}_{13}\text{NO}_3\text{S}_2) = M_5 \times \frac{Q_7}{Q_5} \times C \times \frac{1}{200}
\]

\(M_5\): Amount (mg) of bucillamine for assay
\(C\): Labeled amount (mg) of bucillamine (C7H13NO3S2) in 1 tablet

Internal standard solution—A solution of 4-fluorobenzoic acid in methanol (1 in 100).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and methanol (3:2).
Flow rate: Adjust the flow rate so that the retention time of bucillamine is about 4 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, bucillamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bucillamine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Bucumolol Hydrochloride

Bucumolol Hydrochloride, when dried, contains not less than 99.0% of C17H23NO4.HCl.

Description
Bucumolol Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in methanol and in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in diethyl ether.

Melting point: about 228°C (with decomposition).

Identification (1) Dissolve 0.01 g of Bucumolol Hydrochloride in 10 mL of diluted ethanol (95) (1 in 2), and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence. Render this solution alkaline by adding sodium hydroxide TS: the fluorescence disappears. Acidify the solution by adding dilute hydrochloric acid: the fluorescence reappears.

(2) Dissolve 0.1 g of Bucumolol Hydrochloride in 5 mL of water, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Bucumolol Hydrochloride (1 in 60,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Bucumolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Bucumolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Absorbance <2.24> \(E_{1 cm}^{10} = 330 \sim 360\) (after drying, 40 mg, water, 2500 mL).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Bucumolol Hydrochloride in 20 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of...
Bufetolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic (1.10)—Prepare the test solution with 1.0 g of Bufetolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Bufetolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia-ammonium chloride buffer solution, pH 10.7, (30:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** (2.41) Not more than 0.5% (1 g, 105°C, 5 hours).

**Residue on ignition** (2.44) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Bufetolol Hydrochloride, previously dried, add 45 mL of acetic acid (100), dissolve by warming at 60°C, and cool. Add 105 mL of acetic anhydride, and titrate (2.50) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 34.18 mg of C17H23NO4.HCl

**Containers and storage** Containers—Well-closed containers.

**Bufetolol Hydrochloride**

プフェトロール塩酸塩

C_{18}H_{29}NO_{4}.HCl: 359.89
1-(1,1-Dimethylethyl)amino-2-[5-(tetrahydrofuran-2-ylmethoxy)phenoxy]propan-2-ol monohydrochloride [35108-88-4]

Bufetolol Hydrochloride, when dried, contains not less than 98.5% of C_{18}H_{29}NO_{4}.HCl.

**Description** Bufetolol Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water and in methanol, soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Bufetolol Hydrochloride (1 in 10) is optically inactive.

**Identification** (1) To 5 mL of a solution of Bufetolol Hydrochloride (1 in 100) add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Bufetolol Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bufetolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Bufetolol Hydrochloride (1 in 50) responds to the Qualitative Tests (1.09) for chloride.

**Melting point** (2.60) 153 – 157°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Bufetolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate (1.14)—Perform the test with 0.5 g of Bufetolol Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals (1.07)—Proceed with 2.0 g of Bufetolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.20 g of Bufetolol Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone, ethanol (95) and ammonia solution (28) (40:20:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** (2.41) Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** (2.44) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Bufetolol Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate (2.50) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 35.99 mg of C_{18}H_{29}NO_{4}.HCl

**Containers and storage** Containers—Tight containers.
Buformin Hydrochloride

プホルミン塩酸塩

Buformin Hydrochloride occurs as a white crystalline powder. It is freely soluble in water and in ethanol (99.5).

Identification (1) To 5 mL of a solution of Buformin Hydrochloride (1 in 2000) add 1 mL of dilute sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS: a red-brown color develops.

(2) Determine the absorption spectrum of a solution of Buformin Hydrochloride (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Buformin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Buformin Hydrochloride (1 in 20) responds to the Qualitative Tests <1.00> for chlorides.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Buformin Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Buformin Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Buformin Hydrochloride in 200 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak other than buformin obtained from the sample solution is not larger than 1/5 times the peak area of buformin from the standard solution. Furthermore, the total of the areas of all peaks other than the buformin peak from the sample solution is not larger than 1/2 times the peak area of buformin from the standard solution.

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilsanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of a solution of sodium perchlorate monohydrate in diluted phosphoric acid (1 in 1000) (7 in 250) and acetonitrile (7:1).

Flow rate: Adjust the flow rate so that the retention time of buformin is about 6 minutes.

Time span of measurement: About 2 times as long as the retention time of buformin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of buformin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of buformin are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of buformin is not more than 1.0%.

Loss on drying <2.47> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Buformin Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100:7:3), and immediately titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 9.684 mg of C₆H₁₅N₅.HCl

Containers and storage Containers—Tight containers.

Buformin Hydrochloride Enteric-coated Tablets

プホルミン塩酸塩腸溶錠

Buformin Hydrochloride Enteric-coated Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of buformin hydrochloride (C₆H₁₅N₅.HCl: 193.68).

Method of preparation Prepare as directed under Tablets, with Buformin Hydrochloride.

Identification To a quantity of powdered Buformin Hydrochloride Enteric-coated Tablets, equivalent to 0.1 g of Buformin Hydrochloride according to the labeled amount, add 10 mL of water, shake well, and then filter. To 4 mL of the filtrate add 1 mL of a mixture of hydrogen peroxide TS,
sodium pentacyanonitrosylferrate (III) TS and a solution of sodium hydroxide (1 in 10) (2:1:1): the solution exhibits a red to red-purple color.

**Uniformity of dosage units** \(\leq 6.02\%\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Buformin Hydrochloride Enteric-coated Tablets add 5 mL of a mixture of ethanol (99.5) and acetone (1:1), disperse the pellicle to smaller using ultrasonic waves, add exactly 10 mL of the internal standard solution per 50 mg of buformin hydrochloride \((C_6H_{15}N_5.HCl)\), and then add diluted acetonitrile \((1 \text{ in } 2)\) to make 13\(V/20\) mL. Disintegrate the tablet using ultrasonic waves, then shake for 20 minutes, and add diluted acetonitrile \((1 \text{ in } 2)\) to make a solution, volume \(V\) mL, containing about 0.5 mg of buformin hydrochloride \((C_6H_{15}N_5.HCl)\) per mL. Centrifuge this solution, pipet 1 mL of the supernatant liquid, and add the mobile phase to make 50 mL. If necessary, filter this solution through a membrane filter with a pore size not exceeding 0.5 \(\mu\)m, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

**Internal standard solution**—A solution of \(p\)-acetasanisidine in diluted acetonitrile (1 in 2) (1 in 150).

**Dissolution** \(\leq 6.10\%\) When the tests are performed at 50 revolutions per minute according to the Paddle method, using 900 mL each of 1st fluid for dissolution test and 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 120 minutes of Buformin Hydrochloride Enteric-coated Tablets using the 1st fluid is not more than 5%, and that in 90 minutes of Buformin Hydrochloride Enteric-coated Tablets using the 2nd fluid is not less than 80%.

Start the test with 1 tablet of Buformin Hydrochloride Enteric-coated Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add the relevant dissolution medium to make exactly \(V\) mL so that each mL contains about 56 \(\mu\)g of buformin hydrochloride \((C_6H_{15}N_5.HCl)\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in the relevant dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the relevant dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\%\) according to the following conditions, and determine the buformin peak areas, \(A_T\) and \(A_S\), of both solutions.

**Operating conditions**—

**Detector:** An ultraviolet absorption photometer (wavelength: 230 nm).

**Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeceansilnized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

**Flow rate:** Adjust the flow rate so that the retention time of buformin is about 6 minutes.

**System suitability**—

**System performance:** When the procedure is run with 20 \(\mu\)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of buformin are not less than 3000 and not more than 2.0, respectively.

**System repeatability:** When the test is repeated 6 times with 20 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of buformin is not more than 2.0%.

**Assay** Add 20 mL of a mixture of ethanol (99.5) and acetone (1:1) to an amount of Buformin Hydrochloride Enteric-coated Tablets equivalent to 0.5 g of buformin hydrochloride \((C_6H_{15}N_5.HCl)\), disperse the pellicle to smaller using ultrasonic waves, and then add 100 mL of diluted acetonitrile (1 in 2). Disintegrate the tablets with the aid of ultrasonic waves, shake for 20 minutes, and then add diluted acetonitrile (1 in 2) to make exactly 200 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and then add diluted acetonitrile (1 in 2) to make 50 mL. Pipet 1 mL of this solution, and add the mobile phase to make 50 mL. If necessary, filter this solution through a membrane filter with a pore size not exceeding 0.5 \(\mu\)m, and use the filtrate as the sample solution.

Separately, weigh accurately about 25 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in an adequate amount of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution, and then add diluted acetonitrile (1 in 2) to make 50 mL. To 1 mL of this solution add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 \(\mu\)L of each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\%\) according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of buformin to that of the internal standard.

**Operating conditions**—

**Detector:** An ultraviolet absorption photometer (wavelength: 233 nm).

**Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeceansilnized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of a solution of sodium perchlorate (7 in 250) and acetonitrile (7:1).

Flow rate: Adjust the flow rate so that the retention time of buformin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, buformin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of buformin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Buformin Hydrochloride Tablets

ブホルミン塩酸塩

Buformin Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of buformin hydrochloride (C₆H₁₅N₅.HCl: 193.68).

Method of preparation Prepare as directed under Tablets, with Buformin Hydrochloride.

Identification To a quantity of powdered Buformin Hydrochloride Tablets, equivalent to 1 g of Buformin Hydrochloride according to the labeled amount, add 100 mL of water, shake well, and then filter. To 4 mL of the filtrate add 1 mL of dilute sodium pentacyanodisulfitoferrous (III)-potassium hexacyanoferrate (III) TS: the solution exhibits a red-brown color.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Buformin Hydrochloride Tablets, add water to make exactly 200 mL, and then treat with ultrasonic waves for 5 minutes. Take 40 mL of this solution and centrifuge. Pipet V mL of the supernatant liquid equivalent to about 0.5 mg of buformin hydrochloride (C₆H₁₅N₅.HCl), add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₅, of the sample solution and standard solution at 233 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A₅, at 233 nm.

Amount (mg) of buformin hydrochloride (C₆H₁₅N₅.HCl) = M₅ × A₅ / A₁ × V / V / 1 / C × 18

M₅: Amount (mg) of buformin hydrochloride for assay

C: Labeled amount (mg) of buformin hydrochloride (C₆H₁₅N₅.HCl) in 1 tablet

Assay Weigh accurately not less than 20 Buformin Hydrochloride Tablets, and weigh accurately a portion of the powder, equivalent to about 60 mg of buformin hydrochloride (C₆H₁₅N₅.HCl), add water to make exactly 200 mL, and treat with ultrasonic waves for 5 minutes. Take 40 mL of this solution, centrifuge, pipet 2 mL of the supernatant liquid, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A₅, at 233 nm.

Amount (mg) of buformin hydrochloride (C₆H₁₅N₅.HCl) = M₅ × A₅ / A₁ × V

M₅: Amount (mg) of buformin hydrochloride for assay

Containers and storage Containers—Well-closed containers.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Buformin Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Buformin Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V mL so that each mL contains about 5.6 μg of buformin hydrochloride (C₆H₁₅N₅.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A₅, at 233 nm.

Amount (mg) of buformin hydrochloride (C₆H₁₅N₅.HCl) = M₅ × A₅ / A₁ × V / V / 1 / C × 18

M₅: Amount (mg) of buformin hydrochloride for assay
Bumetanide

ブメタニド

C₁₇H₂₃N₂O₅S: 364.42
3-Butylamino-4-phenoxy-5-sulfamoylbenzoic acid
[28395-03-1]

Bumetanide, when dried, contains not less than 98.5% of C₁₇H₂₃N₂O₅S.

Description Bumetanide occurs as white crystals or crystal-line powder.

It is freely soluble in pyridine, soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in potassium hydroxide TS.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Bumetanide in 1 mL of pyridine, add 2 drops of copper (II) sulfate TS, shake, add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a light blue color develops in the chloroform layer.

(2) Dissolve 0.04 g of Bumetanide in 100 mL of phosphate buffer solution, pH 7.0, and dilute 10 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bumetanide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare with the spectrum of the Standard Solution: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 232 – 237°C

Purity (1) Clarity and color of solution—Dissolve 50 mg of Bumetanide in 2 mL of a solution of potassium hydroxide (1 in 30) and 8 mL of water: the solution is clear, and is not more colored than the following control solution.

Control solution: Pipet 0.5 mL each of Cobalt (II) Chloride CS, Iron (III) Chloride CS and Copper (II) Sulfate CS, mix them, and add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Chloride <1.05>—Mix well 0.5 g of Bumetanide with 0.7 g of potassium nitrate and 1.2 g of anhydrous sodium carbonate, transfer in small portions, to a red-hot platinum crucible, and heat to red-hot until the reaction is complete.

After cooling, to the residue add 14 mL of dilute sulfuric acid and 6 mL of water, boil for 5 minutes, filter, wash the residue with 10 mL of water, combine the filtrate and the washing, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Bumetanide according to Method 2, and perform the test.

Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Bumetanide according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Bumetanide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetic acid (100), cyclohexane and methanol (32:4:4:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.44> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Bumetanide, previously dried, dissolve in 50 mL of ethanol (95), and titrate <2.30> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 36.44 mg of C₁₇H₂₃N₂O₅S

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Bunazosin Hydrochloride

ブナゾシン塩酸塩

C₁₉H₂₇N₅O₃.HCl: 409.91
4-Amino-2-(4-butanoyl-1,4-diazepan-1-yl)-6,7-dimethoxyquinazoline monohydrochloride
[72712-76-2]

Bunazosin Hydrochloride, when dried, contains not less than 98.0% of C₁₉H₂₇N₅O₃.HCl.

Description Bunazosin Hydrochloride occurs as a white
crystalline powder.

It is very soluble in formic acid, slightly soluble in water and in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Melting point: About 273°C (with decomposition).

**Identification (1)** Dissolve 0.1 g of Bunazosin Hydrochloride in 10 mL of 0.2 mol/L hydrochloric acid TS, and boil for 3 minutes over a flame: butyric acid like odor is perceptible.

(2) Determine the infrared absorption spectrum of Bunazosin Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bunazosin Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Bunazosin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.05 g of Bunazosin Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. To exactly 1 mL of the sample solution add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of bunazosin from the sample solution is not larger than the peak area of bunazosin from the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecyldimethylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.44 g of sodium lauryl sulfate in a suitable amount of water, add 10 mL of acetic acid (100), 500 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of bunazosin is about 5 minutes.

Selection of column: Proceed with 20 μL of a mixture of the standard solution and a solution of procaine hydrochloride in the mobile phase (1 in 20,000) (1:1) under the above operating conditions, and calculate the resolution. Use a column giving elution of procaine and bunazosin in this order with the resolution between these peaks being not less than 3.0.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of bunazosin obtained from 20 μL of the standard solution is 20 to 60% of the full-scale.

Time span of measurement: About 6 times of the retention time of bunazosin.

**Loss on drying <2.41>** Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition <2.44>** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Bunazosin Hydrochloride, previously dried, dissolve in 6 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid, and heat for 20 minutes on a water bath. After cooling, add 20 mL of acetic acid (100), and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 40.99 mg of C₁₉H₂₇N₅O₃.HCl

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

## Bupranolol Hydrochloride

### ブプラノール塩酸塩

C₁₄H₂₃ClN₂O₂.HCl: 308.24
(2R,5S)-3-(2-Chloro-5-methylphenoxy)-1-(1,1-dimethylethyl)aminopropan-2-ol monohydrochloride [15148-80-8]

Bupranolol Hydrochloride, when dried, contains not less than 98.0% of C₁₄H₂₃ClN₂O₂.HCl.

**Description** Bupranolol Hydrochloride occurs as a white, crystalline powder.

It is sparingly soluble in methanol, slightly soluble in water, in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Bupranolol Hydrochloride (1 in 1000) is between 5.2 and 6.2.

**Identification (1)** Take 0.01 g of Bupranolol Hydrochloride in a test tube, mix with 25 mg of potassium iodide and 25 mg of oxalic acid dihydrate, cover the mouth of the test tube with filter paper moistened with a solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monooimine in ethanol (95) (1 in 100), and heat gently for several minutes. Expose the filter paper to ammonia gas: the filter paper acquires a blue color.

(2) Determine the absorption spectrum of a solution of Bupranolol Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bupranolol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Bupranolol Hydrochloride (1 in 200) responds to the Qualitative Tests <1.09> for chloride.
Absorbance $<2.24$  $E_{1\%}^{1\text{cm}}$ (275 nm): 57 – 60 (after drying, 50 mg, 0.1 mol/L hydrochloric acid TS, 500 mL).

**Purity** (1) Clarity and color of solution—Dissolve 0.1 g of Buprenolol Hydrochloride in 15 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.1 g of Buprenolol Hydrochloride in 15 mL of freshly boiled and cooled water, and add 1 drop of methyl red TS: a light red color develops. To this solution add 0.05 mL of 0.01 mol/L sodium hydroxide VS: the color changes to yellow.

(3) Sulfate $<1.14$—Perform the test with 0.1 g of Buprenolol Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.168%).

(4) Heavy metals $<1.07$—Proceed with 1.0 g of Buprenolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic $<1.15$—Prepare the test solution with 1.0 g of Buprenolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.30 g of Buprenolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 10 µL each of the sample solution and standard solution on a plate of polyamide with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and water (16:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** $<2.47$  Not more than 0.5% (0.5 g, 105°C, 4 hours).

**Residue on ignition** $<2.44$  Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.18 g of Buprenolol Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (2:1) by warming, cool, and titrate $<2.50$  with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 30.82 mg of C$_{14}$H$_{22}$ClNO$_2$.HCl

**Containers and storage** Containers—Well-closed containers.

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**Buprenorphine Hydrochloride**

ブプレノルフィン塩酸塩

C$_{29}$H$_{41}$NO$_4$.HCl: 504.10
(2S)-2-[(5R,6R,7R,14S)-17-(Cyclopropylmethyl)-4,5-epoxy-3-hydroxy-6-methoxy-6,14-ethanomorphinan-7-yl]-3,3-dimethylbutan-2-ol monohydrochloride [53152-21-9]

Buprenorphine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of C$_{29}$H$_{41}$NO$_4$.HCl.

**Description** Buprenorphine Hydrochloride occurs as white to yellowish white, crystals or a crystalline powder.

It is freely soluble in methanol and in acetic acid (100), and sparingly soluble in water and in ethanol (99.5). Melting point: about 268°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Buprenorphine Hydrochloride (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry $<2.24>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Buprenorphine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Buprenorphine Hydrochloride (1 in 100) responds to the Qualitative Tests $<1.09>$ for chloride.

**Optical rotation** $<2.49>$  [α]$_D^2$: $-92$ – $-98$° (after drying, 0.4 g, methanol, 20 mL, 100 mm).

**pH** $<2.54>$  The pH of a solution prepared by dissolving 1.0 g of Buprenorphine Hydrochloride in 200 mL of water is between 4.0 and 6.0.

**Purity** (1) Clarity and color of solution—A solution obtained by dissolving 0.1 g of Buprenorphine Hydrochloride in 10 mL of water is clear and colorless.

(2) Heavy metals $<1.07$—Proceed with 1.0 g of Buprenorphine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Buprenorphine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions. Determine each peak area of both solutions by the automatic integration...
Busulfan

Busulfan contains not less than 98.5% of C₆H₁₄O₆S₂, calculated on the dried basis.

**Description** Busulfan occurs as a white, crystalline powder. It is slightly soluble in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

**Identification** (1) To 0.1 g of Busulfan add 10 mL of water and 5 mL of sodium hydroxide TS, dissolve by heating, and use this solution as the sample solution.

(i) To 7 mL of the sample solution add 1 drop of potassium permanganate TS: the red-purple color of potassium permanganate TS changes from blue-purple through blue to green.

(ii) Acidify 7 mL of the sample solution with dilute sulfuric acid, and add 1 drop of potassium permanganate TS: the color of potassium permanganate TS remains.

(2) Determine the infrared absorption spectrum of Busulfan, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** 2.60 115 – 118°C

**Purity** (1) Sulfate 1.14 To 1.0 g of Busulfan add 40 mL of water, and dissolve by heating. Cool in ice for 15 minutes, and filter. Wash the residue with 5 mL of water, combine the washings with the filtrate, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(2) Heavy metals 1.07 Proceed with 1.0 g of Busulfan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** 2.41 Not more than 2.0% (1 g, 115°C, 3 hours).

**Residue on ignition** 2.44 Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Buprenorphine Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 50.41 mg of C₂₉H₄₁NO₄.HCl

**Containers and storage** Containers—Well-closed containers.

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**Busulfan**

C₆H₁₄O₆S₂: 246.30
Tetramethylenedimethanesulfonate [55-98-1]

Busulfan...
Butenafine Hydrochloride

Butenafine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C₂₃H₂₇N·HCl.

**Description** Butenafine Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in formic acid, freely soluble in methanol and in ethanol (99.5), and slightly soluble in water.

The pH of a solution dissolved 0.20 g of Butenafine Hydrochloride in 100 mL of water by warming and cooled is 3.0 to 4.0.

Melting point: about 214°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Butenafine Hydrochloride in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Butenafine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Butenafine Hydrochloride in dilute ethanol (1 in 200) responds to the Qualitative Tests <1.09> (1) for chloride.

**Purity** (1) Heavy metals <1.07>—Dissolve 2.0 g of Butenafine Hydrochloride in 20 mL of ethanol (99.5), add 2 mL of dilute acetic acid and ethanol (99.5) to make 50 mL, and perform the test using this solution as the test solution.

The control solution: To 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, and add ethanol (99.5) to make 50 mL (not more than 10 ppm).

(2) Related substances—Dissolve 30 mg of Butenafine Hydrochloride in 50 mL of a mixture of water and acetonitrile for liquid chromatography (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07>, according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.16 to butenafine, obtained from the sample solution is not larger than 3/10 times the peak area of butenafine from the standard solution, and the area of the peak other than butenafine and other than the peak mentioned above obtained from the sample solution is not larger than the peak area of butenafine from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 217 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Diluted 0.5 mol/L ammonium acetate TS (1 in 1000).

Mobile phase B: Acetonitrile for liquid chromatography. Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>60 → 20</td>
<td>40 → 80</td>
</tr>
<tr>
<td>10–60</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

Flow rate: 0.4 mL per minute.

Time span of measurement: For 60 minutes after injection, beginning after the solvent peak.

**System suitability**—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 10 mL. Confirm that the peak area of butenafine obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of butenafine are not less than 20,000 and 0.9 to 1.2, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of butenafine is not more than 2.0%.

(3) Residual solvents—Being specified separately.

**Loss on drying** <2.41> Not more than 0.1% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Butenafine Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 35.39 mg of C₂₃H₂₇N·HCl.

**Containers and storage** Containers—Tight containers.
Butenafine Hydrochloride Cream

Butenafine Hydrochloride Cream contains not less than 95.0% and not more than 105.0% of the labeled amount of butenafine hydrochloride (C$_{23}$H$_{27}$N.HCl: 353.93).

**Method of preparation** Prepare as directed under Creams, with Butenafine Hydrochloride.

**Identification** To an amount of Butenafine Hydrochloride Cream, equivalent to 20 mg of Butenafine Hydrochloride according to the labeled amount, add 20 mL of acetonitrile, and warm on a water bath to melt the bases. Shake thoroughly, add an appropriate amount of sodium chloride, and allow to stand for 30 minutes in an ice cold water keeping not exceeding 0°C to separate out the bases. Centrifuge, collect the supernatant liquid, add an appropriate amount of sodium chloride to the liquid, allow to stand for 1 hour in an ice cold water keeping not exceeding 0°C, and filter while cooling. To 1 mL of the filtrate add methanol to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 272 nm and 276 nm, between 281 nm and 285 nm, between 311 nm and 315 nm, and between 316 nm and 320 nm, and a shoulder between 289 nm and 299 nm.

**Assay** Weigh accurately a quantity of Butenafine Hydrochloride Cream, equivalent to about 5 mg of butenafine hydrochloride (C$_{23}$H$_{27}$N.HCl), add 20 mL of methanol, and add exactly 10 mL of the internal standard solution. Warm this in a water bath for 5 minutes, and shake vigorously for 20 minutes. Then, cool in an ice bath for 15 minutes, centrifuge, and filter the supernatant liquid with a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of butenafine hydrochloride for assay, previously dried in vacuum at 60°C for 3 hours using phosphorus (V) oxide as desiccant, and dissolve in methanol to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography: it exhibits maxima between 272 nm and 276 nm, between 281 nm and 285 nm, between 311 nm and 315 nm, and between 316 nm and 320 nm, and a shoulder between 289 nm and 299 nm.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 5 cm in length, packed with octyacylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and diluted 0.5 mol/L ammonium acetate TS (1 in 500) (4:1).

Flow rate: Adjust the flow rate so that the retention time of butenafine is about 2.5 minutes.

**System suitability**—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and butenafine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of butenafine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

Butenafine Hydrochloride Solution

Butenafine Hydrochloride Solution is a liquid for external use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of butenafine hydrochloride (C$_{23}$H$_{27}$N.HCl: 353.93).

**Method of preparation** Prepare as directed under Liquids and Solutions for Cutaneous Application, with Butenafine Hydrochloride.

**Identification** To an amount of Butenafine Hydrochloride Solution, equivalent to 10 mg of Butenafine Hydrochloride according to the labeled amount, add methanol to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 272 nm and 276 nm, between 281 nm and 285 nm, between 311 nm and 315 nm, and between 316 nm and 320 nm, and a shoulder between 289 nm and 299 nm.

**Assay** To an exact volume of Butenafine Hydrochloride Solution, equivalent to about 20 mg of butenafine hydrochloride (C$_{23}$H$_{27}$N.HCl), add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add methanol to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of butenafine hydrochloride for assay, previously dried in vacuum at 60°C for 3 hours using phosphorus (V) oxide as desiccant, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add methanol to make 25 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography: it exhibits maxima between 272 nm and 276 nm, between 281 nm and 285 nm, between 311 nm and 315 nm, and between 316 nm and 320 nm, and a shoulder between 289 nm and 299 nm.
Amount (mg) of butenafine hydrochloride (C\textsubscript{23}H\textsubscript{27}N.HCl) = \( M_s \times Q_T/Q_s \)

\( M_s \): Amount (mg) of butenafine hydrochloride for assay

**Internal standard solution**—A solution of diphenyl in methanol (3 in 2000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 5 cm in length, packed with octylsilanized silica gel for liquid chromatography (3 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and diluted 0.5 mol/L ammonium acetate TS (1 in 500) (4:1).

Flow rate: Adjust the flow rate so that the retention time of butenafine is about 2.5 minutes.

**System suitability**—

System performance: When the procedure is run with 5 \( \mu L \) of the standard solution under the above operating conditions, the internal standard and butenafine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of butenafine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

**Butenafine Hydrochloride Spray**

プテナフィン塩酸塩スプレー

Butenafine Hydrochloride Spray contains not less than 95.0% and not more than 105.0% of the labeled amount of butenafine hydrochloride (C\textsubscript{23}H\textsubscript{27}N.HCl: 353.93).

**Method of preparation** Prepare as directed under Pump Sprays for Cutaneous Application, with Butenafine Hydrochloride.

**Identification** To an amount of Butenafine Hydrochloride Spray, equivalent to 10 mg of Butenafine Hydrochloride according to the labeled amount, add methanol to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (<2.24>); it exhibits maxima between 272 nm and 276 nm, between 281 nm and 285 nm, between 311 nm and 315 nm, and between 316 nm and 320 nm, and a shoulder between 289 nm and 299 nm.

**Assay** To an exact volume of Butenafine Hydrochloride Spray, equivalent to about 20 mg of butenafine hydrochloride (C\textsubscript{23}H\textsubscript{27}N.HCl), add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add methanol to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of butenafine hydrochloride for assay, previously dried in vacuum at 60°C for 3 hours using phosphorus (V) oxide as desiccant, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add methanol to make 25 mL, and use this solution as the standard solution. Perform the test with 5 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography (<2.01> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_s \), of the peak area of butenafine to that of the internal standard.

Amount (mg) of butenafine hydrochloride (C\textsubscript{23}H\textsubscript{27}N.HCl) = \( M_s \times Q_T/Q_s \)

\( M_s \): Amount (mg) of butenafine hydrochloride for assay

**Internal standard solution**—A solution of diphenyl in methanol (3 in 2000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 5 cm in length, packed with octylsilanized silica gel for liquid chromatography (3 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and diluted 0.5 mol/L ammonium acetate TS (1 in 500) (4:1).

Flow rate: Adjust the flow rate so that the retention time of butenafine is about 2.5 minutes.

**System suitability**—

System performance: When the procedure is run with 5 \( \mu L \) of the standard solution under the above operating conditions, the internal standard and butenafine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of butenafine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

**Butropium Bromide**

ブトロピウム臭化物

Butropium Bromide occurs as white crystals or

\[
\text{Butropium Bromide (C}_{28}\text{H}_{38}\text{BrNO}_4 : 532.51}
\]

(1R,3r,5S)-8-(4-Butoxybenzyl)-3-[(25S)-hydroxy-2-phenylpropanoyloxy]-8-methyl-8-azoniabicyclo[3.2.1]octane bromide
[29025-14-7]

Butropium Bromide, when dried, contains not less than 98.0% of C\textsubscript{28}H\textsubscript{38}BrNO\textsubscript{4}.

**Description** Butropium Bromide occurs as white crystals or
Butropium Bromide in methanol (1 in 100,000) as directed.

**Identification (1)** To 1 mg of Butropium Bromide add 3 drops of fuming nitric acid, and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of N,N-dimethylformamide, and add 5 to 6 drops of tetracylammonium hydroxide TS: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Butropium Bromide in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Butropium Bromide in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Butropium Bromide in methanol (1 in 20) responds to the Qualitative Tests <1.09> (1) for bromide.

**Purity (1)** Heavy metals <1.07>—Dissolve 1.0 g of Butropium Bromide in 40 mL of ethanol (95), add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test, using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Butropium Bromide in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the peak area having a ratio of the retention time about 0.5 to butropium from the sample solution is not larger than 1/4 times the peak area from the standard solution, and the total area of all peaks other than the peak eluted first, the peak having a ratio of the retention time to butropium about 0.5 and butropium peak from the sample solution is not larger than the peak area from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.15 g of sodium lauryl sulfate in 1000 mL of a mixture of acetonitrile and 0.005 mol/L sulfuric acid (3:2).

Flow rate: Adjust the flow rate so that the retention time of butropium is about 5 minutes.

Selection of column: Dissolve 0.50 g of Butropium Bromide in 9 mL of ethanol (99.5) and 1 mL of 0.1 mol/L potassium hydroxide-ethanol TS, and heat at 70°C for 15 minutes. After cooling, to 1 mL of this solution add the mobile phase to make 100 mL. Proceed with 5 µL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the peak of butropium and the peak having a ratio of the retention time about 0.7 to butropium with the resolution between these peaks being not less than 2.5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of the butropium obtained from 5 µL of the standard solution is between 10 mm and 30 mm.

Time span of measurement: About twice as long as the retention time of butropium.

**Loss on drying <2.49>** Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition <2.44>** Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.8 g of Butropium Bromide, previously dried, dissolve in 5 mL of formic acid, add 100 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid-dioxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS = 53.25 mg of C11H14O3Br.

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

**Butyl Parahydroxybenzoate**

パラオキシ安息香酸ブチル

Butyl 4-hydroxybenzoate

C11H14O3: 194.23
Butyl 4-hydroxybenzoate [94-26-8]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (**). Butyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of C11H14O3.

**Description** Butyl Parahydroxybenzoate occurs as colorless crystals or white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and practically insoluble in water.

**Identification (1)** The melting point <2.60> of Butyl Parahydroxybenzoate is between 68°C and 71°C.

(2) Determine the infrared absorption spectrum of Butyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at...
the same wave numbers.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Butyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Cupper (II) Sulfate CS add water to make 1000 mL.

(2) Acidity—Dissolve 0.20 g of Butyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromoresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

*Heavy metals (3)*—Dissolve 1.0 g of Butyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).

(4) Related substances—Dissolve 0.10 g of Butyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.07>*. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot is not more intense than the spot obtained with the standard solution.

**Residue on ignition** *<2.44>—*Not more than 0.1% (1 g).

**Assay** Weigh accurately about 1 g of Butyl Parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate *<2.50>—*the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

\[
each \text{mL of 1 mol/L sodium hydroxide VS} = 194.2 \text{ mg of C}_{12}\text{H}_{22}\text{O}_{3}
\]

*Containers and storage* Containers—Well-closed containers.

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**Cadralazine**

カドララジン

\[\text{C}_{12}\text{H}_{21}\text{N}_{5}\text{O}_{3}: 283.33}\]

Ethyl 3-(6-ethyl[2RS]-2-hydroxypropyl)amino)pyridazin-3-yl)carbazate

[64241-34-5]

Cadralazine, when dried, contains not less than 98.5% and not more than 101.0% of \(\text{C}_{12}\text{H}_{22}\text{N}_{5}\text{O}_{3}\).

**Description** Cadralazine occurs as a pale yellow to light yellow crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in ethanol (99.5), and slightly soluble in water.

It dissolves in 0.05 mol/L sulfuric acid TS.

A solution of Cadralazine in methanol (1 in 40) shows no optical rotation.

Melting point: about 165°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Cadralazine in 0.05 mol/L sulfuric acid TS (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cadralazine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)** Chloride *<1.03>—*Dissolve 0.40 g of Cadralazine in 15 mL of methanol, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS by adding 15 mL of methanol, 6 mL of dilute nitric acid, and water to make 50 mL (not more than 0.036%).

(2) Heavy metals *<1.07>—*Proceed with 1.0 g of Cadrala-
Cadralazine Tablets / Official Monographs

Cadralazine Tablets

Cadralazine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cadralazine (C\(_{12}\)H\(_{21}\)N\(_{5}\)O\(_{3}\): 283.33).

**Method of preparation**
Prepare as directed under Tablets, with Cadralazine.

**Identification**
To a quantity of powdered Cadralazine Tablets, equivalent to 20 mg of Cadralazine according to the labeled amount, add 50 mL of 0.05 mol/L sulfuric acid TS, shake well, and centrifuge. To 1 mL of the supernatant liquid add 0.05 mol/L sulfuric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 247 nm and 251 nm.

**Uniformity of dosage units**
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cadralazine Tablets add 30 mL of 0.05 mol/L sulfuric acid TS, shake well to disintegrate, and add 0.05 mol/L sulfuric acid TS to make exactly 50 mL. Centrifuge this solution, pipet 3 mL of the supernatant liquid, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL contains about 6 \(\mu\)g of cadralazine (C\(_{12}\)H\(_{21}\)N\(_{5}\)O\(_{3}\)), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of cadralazine for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL. Pipet 3 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_1\) and \(A_2\), of the sample solution and standard solution at 249 nm as directed under Ultraviolet-visible Spectrophotometry:

\[
\text{Amount (mg) of cadralazine (C}_{12}\text{H}_{21}\text{N}_{5}\text{O}_{3}) = M_S \times A_1 / A_2 \times V / 200
\]

\(M_S\): Amount (mg) of cadralazine for assay

**Dissolution**
When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Cadralazine Tablets is not less than 80%.

Start the test with 1 tablet of Cadralazine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains
about 5.6 μg of cadralazine (C₁₂H₂₁N₅O₃) to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of cadralazine for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 254 nm as directed under Ultraviolet-visible Spectrophotometry <2.20>.

Dissolution rate (%) with respect to the labeled amount of cadralazine (C₁₂H₂₁N₅O₃)  
\[
\frac{M_S \times A_T}{M_S \times A_T + V/V \times 1/C \times 18}
\]

\( M_S \): Amount (mg) of cadralazine for assay
\( C \): Labeled amount (mg) of cadralazine (C₁₂H₂₁N₅O₃) in 1 tablet

**Assay** To 10 Cadralazine Tablets add 70 mL of 0.05 mol/L sulfuric acid TS, shake well to disintegrate, add 0.05 mol/L sulfuric acid TS to make exactly 200 mL. Centrifuge this solution, pipet a volume of the supernatant liquid, equivalent to exactly 5 mL of the internal standard solution, add water to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of cadralazine for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L of sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add water to make 25 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of cadralazine to that of the internal standard.

\[
\text{Amount (mg) of cadralazine (C₁₂H₂₁N₅O₃)} = M_S \times \frac{Q_T}{Q_S} \times \frac{1}{10}
\]

\( M_S \): Amount (mg) of cadralazine for assay

**Internal standard solution**—A solution of \( p \)-toluenesulfonylamine in acetonitrile (1 in 50).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 250 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 13.6 g of sodium acetate trihydrate in 800 mL of water, adjust the pH to 5.8 with dilute acetic acid, and add water to make 1000 mL. To 860 mL of this solution add 140 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of cadralazine is about 10 minutes.

**System suitability**—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cadralazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cadralazine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

### Anhydrous Caffeine

C₆H₁₀N₄O₂: 194.19
1,3,7-Trimethyl-1H-purine-2,6-(3H,7H)-dione [58-08-2]

**Anhydrous Caffeine**, when dried, contains not less than 98.5% of C₆H₁₀N₄O₂.

**Description** Anhydrous Caffeine occurs as white crystals or powder. It is odorless, and has a bitter taste.

It is freely soluble in chloroform, sparingly soluble in water, in acetic anhydride and in acetic acid (100), and slightly soluble in ethanol (95) and in diethyl ether. The pH of a solution of Anhydrous Caffeine (1 in 100) is between 5.5 and 6.5.

**Identification** (1) To 2 mL of a solution of Anhydrous Caffeine (1 in 500) add tannic acid TS dropwise: a white precipitate, which dissolves upon the dropwise addition of tannic acid TS, is produced.

(2) To 0.01 g of Anhydrous Caffeine add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple, and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(3) Dissolve 0.01 g of Anhydrous Caffeine in water to make 50 mL. To 5 mL of this solution add 3 mL of diluted acetic acid (31) (3 in 100) and 5 mL of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5), and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

**Melting point** <2.60> 235 – 238°C

**Purity** (1) Chloride <1.02>—Dissolve 2.0 g of Anhydrous Caffeine in 80 mL of hot water, cool rapidly to 20°C, add water to make 100 mL, and use this solution as the sample solution. To 40 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(2) Sulfate <1.14>—To 40 mL of the sample solution obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).
(3) Heavy metals \(<1.07\)—Proceed with 2.0 g of Anhydrous Caffeine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Anhydrous Caffeine in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(2.03\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(5) Readily carbonizable substances \(<1.15\)—Perform the test using 0.5 g of Anhydrous Caffeine: the solution is not more colored than Matching Fluid D.

**Loss on drying** \(<2.41\) Not more than 0.5% (1 g, 80°C, 4 hours).

**Residue on ignition** \(<2.44\) Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 0.4 g of Anhydrous Caffeine, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate \(<2.50\) with 0.1 mol/L perchloric acid VS until the solution changes from purple through green to yellow (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

\[=\text{19.42 mg of C}_7\text{H}_8\text{O}_2\text{N}_4\text{C}_6\]

**Containers and storage** Containers—Tight containers.

**Caffeine Hydrate**

カフェイン水和物

\[
\text{C}_9\text{H}_8\text{N}_4\text{O}_2\cdot\text{H}_2\text{O}: 212.21
\]

1,3,7-Trimethyl-1H-purine-2,6-(3H,7H)-dione monohydrate [5743-12-4]

Caffeine Hydrate, when dried, contains not less than 98.5% of caffeine (C\(_9\)H\(_8\)O\(_4\)N\(_4\)): 194.19.

**Description** Caffeine Hydrate occurs as white, soft crystals or powder. It is odorless, and has a slightly bitter taste. It is freely soluble in chloroform, sparingly soluble in water, in acetic acid (100) and in acetic anhydride, slightly soluble in ethanol (95), and very slightly soluble in diethyl ether.

The pH of a solution of Caffeine Hydrate (1 in 100) is between 5.5 and 6.5. It effloresces in dry air.

**Identification** (1) To 2 mL of a solution of Caffeine Hydrate (1 in 500) add tannic acid TS dropwise: a white precipitate, which dissolves upon the dropwise addition of tannic acid TS, is produced.

(2) To 0.01 g of Caffeine Hydrate add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate to dryness on a water bath: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple, and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(3) Dissolve 0.01 g of Caffeine Hydrate in water to make 50 mL. To 5 mL of this solution add 3 mL of diluted acetic acid (31) (3 in 100) and 5 mL of a solution of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5), and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

**Melting point** \(<2.60\) 235 – 238°C (after drying).

**Purity** (1) Chloride \(<1.03\)—Dissolve 2.0 g of Caffeine Hydrate in 80 mL of hot water, cool rapidly to 20°C, add water to make 100 mL, and use this solution as the sample solution. To 40 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(2) Sulfate \(<1.14\)—To 40 mL of the sample solution obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals \(<1.07\)—Proceed with 2.0 g of Caffeine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Caffeine Hydrate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography \(<2.03\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(5) Readily carbonizable substances \(<1.15\)—Perform the test using 0.5 g of Caffeine Hydrate: the solution is not more colored than Matching Fluid D.

**Loss on drying** \(<2.41\) 0.5 – 8.5% (1 g, 80°C, 4 hours).

**Residue on ignition** \(<2.44\) Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 0.4 g of Caffeine Hydrate,
Caffeine and Sodium Benzoate

Description

Caffeine and Sodium Benzoate occurs as a white powder. It is odorless, and has a slightly bitter taste. It is freely soluble in water, soluble in acetic acid (100) and in acetic anhydride, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Dissolve 1 g of Caffeine and Sodium Benzoate in 10 mL of water in a separator, add 1 drop of phenolphthalein TS, and add carefully 0.01 mol/L sodium hydroxide VS dropwise until a faint red color develops. Extract with three 20-mL portions of chloroform by thorough shaking, and separate the chloroform layer from the water layer. Use the water layer for test (2). Filter the combined chloroform extracts, evaporate the filtrate to dryness on a water bath, and proceed the following tests with the residue:

(i) To 2 mL of a solution of the residue (1 in 500) add tannic acid TS dropwise: a white precipitate, which dissolves upon the dropwise addition of tannic acid TS, is produced.

(ii) To 0.01 g of the residue add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, evaporate to dryness on a water bath: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple, and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(iii) Dissolve 0.01 g of the residue in water to make 50 mL. To 5 mL of this solution add 3 mL of dilute acetic acid (31) (3 in 100) and 5 mL of a solution of pyridine (1 in 10), mix, add 2 mL of dilute sodium hypochlorite TS (1 in 5), and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

(2) To 5 mL of the water layer obtained in (1) add 5 mL of water: the solution responds to the Qualitative Tests <1.09> (2) for benzoate.

(3) Heat Caffeine and Sodium Benzoate: white fumes are evolved. Ignite furthermore, and to the residue add hydrochloric acid: bubbles are produced, and the solution responds to the Qualitative Tests <1.09> (1) for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Caffeine and Sodium Benzoate in 5 mL of water: the solution is clear and colorless.

Assay (1) Sodium benzoate—Weigh accurately about

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Calcitonin (Salmon)

Calcitonin (Salmon) occurs as a white powder. It is freely soluble in water and may dissolve in dilute acetic acid. It also dissolves in 1 mL of a mixture of acetic anhydride and acetic acid for nonaqueous titration (6:1), cool, and titrate $\leq 2.5\%$ with 0.1 mol/L perchloric acid-dioxane VS to the first equivalence point (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS $= 14.41$ mg of $\text{C}_2\text{H}_3\text{NaO}_2$.

(2) Caffeine—Continue the titration $\leq 2.5\%$ in (1) with 0.1 mol/L perchloric acid-dioxane VS from the first equivalence point to the second equivalence point (potentiometric titration).

Each mL of 0.1 mol/L perchloric acid-dioxane VS $= 19.42$ mg of $\text{C}_2\text{H}_3\text{NaO}_2$.

Containers and storage Containers—Well-closed containers.

Calcitonin (Salmon)

Calcitonin (Salmon) is a synthetic polypeptide consisting of 32 amino acid residues. It is a hormone with a blood calcium lowering effect.

It contains not less than 4000 Units of calcitonin (salmon) per 1 mg of peptide.

Description Calcitonin (Salmon) occurs as a white powder. It is freely soluble in water. It dissolves in dilute acetic acid.

Dissolve $20$ mg of Calcitonin (Salmon) in $2$ mL of water: the pH of the solution is between 5.0 and 7.0.

It is hygroscopic.

Identification Dissolve $1$ mg of Calcitonin (Salmon) in $1$ mL of dilute acetic acid. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\leq 2.5\%$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance $\leq 2.24\%$ $E_{1\%}^{1\text{cm}}$ (275 nm): 3.3 - 4.0 (1 mg, dilute acetic acid, 1 mL).

Optical rotation $\leq 2.49\%$ $[\alpha]_D$: $-24 - -32\%$ (25 mg, dilute acetic acid (100) (1 in 2), 10 mL, 100 mm).

Constituent amino acids Weigh accurately about $1$ mg of Calcitonin (Salmon), put in a test tube for hydrolysis, dissolve in 0.5 mL of diluted hydrochloric acid (1 in 2), freeze in a dry ice-acetone bath, seal the tube under reduced pressure, and heat at $110 \pm 2\%$C for 24 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, dissolve the residue in exactly 5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 27 mg of L-aspartic acid, about 24 mg of L-threonine, about 21 mg of L-serine, about 29 mg of L-glutamic acid, about 23 mg of L-proline, about 15 mg of glycine, about 18 mg of L-alanine, about 23 mg of L-valine, about 48 mg of L-cystine, about 30 mg of methionine, about 26 mg of L-isoleucine, about 26 mg of L-leucine, about 36 mg of L-tyrosine, about 33 mg of phenylalanine, about 37 mg of L-lysine hydrochloride, about 42 mg of L-histidine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride, dissolve them in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 mL each of the sample solution and standard solution as directed under Liquid Chromatography $\leq 2.01\%$ according to the following conditions: 13 peaks of amino acids appear on the chromatogram obtained from the sample solution, and their respective molar ratios with respect to leucine ($= 5$) are 1.9 - 2.3 for lysine, 0.8 - 1.1 for histidine, 0.9 - 1.1 for arginine, 1.9 - 2.1 for aspartic acid, 4.5 - 4.9 for threonine, 3.2 - 3.8 for serine, 2.8 - 3.1 for glutamic acid, 1.9 - 2.4 for proline, 2.7 - 3.3 for glycine, 1.5 - 2.5 for 1/2 cystine, 0.9 - 1.0 for valine, and 0.8 - 1.0 for tyrosine.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 6 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (Na type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table.

<table>
<thead>
<tr>
<th></th>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
<th>Mobile phase C</th>
<th>Mobile phase D</th>
<th>Mobile phase E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>12.80 g</td>
<td>6.10 g</td>
<td>—</td>
</tr>
<tr>
<td>Monohydrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trisodium citrate</td>
<td>6.19 g</td>
<td>7.74 g</td>
<td>13.31 g</td>
<td>26.67 g</td>
<td>—</td>
</tr>
<tr>
<td>Dihydrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>8.00 g</td>
<td>—</td>
</tr>
<tr>
<td>Chloride</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>3.74 g</td>
<td>54.35 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol (99.5%)</td>
<td>130.0 mL</td>
<td>20.0 mL</td>
<td>4.0 mL</td>
<td>100.0 mL</td>
<td>—</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.0 mL</td>
<td>—</td>
</tr>
<tr>
<td>Thiodiglycol</td>
<td>5.0 mL</td>
<td>5.0 mL</td>
<td>5.0 mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lauromacrogol</td>
<td>4.0 mL</td>
<td>4.0 mL</td>
<td>4.0 mL</td>
<td>4.0 mL</td>
<td>4.0 mL</td>
</tr>
<tr>
<td>Solution (1 in 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caprylic acid</td>
<td>0.1 mL</td>
<td>0.1 mL</td>
<td>0.1 mL</td>
<td>0.1 mL</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Water</td>
<td>a sufficient</td>
<td>a sufficient</td>
<td>a sufficient</td>
<td>a sufficient</td>
<td>a sufficient</td>
</tr>
<tr>
<td>amount</td>
<td>amount</td>
<td>amount</td>
<td>amount</td>
<td>amount</td>
<td>amount</td>
</tr>
</tbody>
</table>

Total volume 1000 mL 1000 mL 1000 mL 1000 mL 1000 mL

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B, C, D and E as directed in the following table.
Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for 10 minutes while passing nitrogen, and use this solution as solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for 30 minutes while passing nitrogen, and use this solution as solution B. Mix solution A and solution B before use.

Flow rate of mobile phase: About 0.4 mL per minute.
Flow rate of reaction reagent: About 0.35 mL per minute.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine are eluted in this order with the resolutions between the peaks of threonine and serine, glycine and alanine, and isoleucine and leucine being not less than 1.2, 1.0 and 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of aspartic acid, proline, valine and arginine are not more than 2.0%, respectively.

Peptide content Calculate the peptide content in Calcitonin (Salmon) by the following equation using amino acid analysis values (μmol/mL) obtained in the Constituent amino acids: its is not less than 80.0%.

\[
P \text{Peptide content} = \frac{343.185 \times 5/A 	imes A/11 \times 100}{M \times \text{Amount (μmol/mL)}}
\]

A: Total (μmol/mL) of the amino acid analysis values of valine, leucine, glycine and proline
M: Amount (μg) of sample
11: Total of the theoretical residue numbers of valine, leucine, glycine and proline per one mole of calcitonin (salmon)

Purity (1) Acetic acid—Weigh accurately about 10 mg of Calcitonin (Salmon), dissolve in water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of acetic acid (100), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.0) according to the following conditions. Determine the peak areas, A₁ and A₅, of acetic acid in each solution, and calculate the amount of acetic acid by the following equation: the amount of acetic acid is not more than 7.0%.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase A: To 0.7 mL of phosphoric acid add 900 mL of water, adjust the pH to 3.0 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL.
Mobile phase B: Methanol.
Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 1.5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1.5 – 4</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>4 – 12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12 – 26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>26 – 30</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 4 minutes.

System suitability—
System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acetic acid are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetic acid is not more than 2.0%.

(2) Related substances—Dissolve 2 mg of Calcitonin (Salmon) in 2 mL of dilute acetic acid, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography (2.0) according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the total amount of the peaks other than calcitonin (salmon) is not more than 3%.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of 1% trimethylamine-phosphate buffer solution, pH 3.0, and acetonitrile (27:13).
Flow rate: Adjust the flow rate so that the retention time
of calcitonin (salmon) is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of calcitonin (salmon) beginning after the solvent peak.

System suitability—
Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of calcitonin (salmon) obtained from 20 μL of this solution is equivalent to 5 to 15% of that from 20 μL of the solution for system suitability test.

System performance: Dissolve 5 mg of methyl parahydroxybenzoate and 7 mg of ethyl parahydroxybenzoate in 100 mL of acetonitrile. When the procedure is run with 20 μL of this solution under the above operating conditions methyl parahydroxybenzoate and ethyl parahydroxybenzate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of calcitonin (salmon) is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water <2.48> Not more than 10.0% (5 mg, coulometric titration).

Assay (i) Test animals: Select healthy albino rats weighing between 55 and 180 g, fasted for 24 hours before the test but allowed to drink water ad libitum.

(ii) Standard solutions: Dissolve a quantity of Calcitonin (Salmon) RS in acetic acid buffer solution containing 0.1% bovine serum albumin, and prepare a high-dose standard solution SH and a low-dose standard solution SL containing exactly 0.050 and 0.025 Units per mL, respectively.

(iii) Sample solutions: According to the labeled units, weigh accurately a suitable amount of Calcitonin (Salmon), and dissolve in acetic acid buffer solution containing 0.1% bovine serum albumin, and prepare a high-dose sample solution TH and the low-dose sample solution TL having Units equal to the standard solutions in equal volumes, respectively.

(iv) Dose for injection: Inject 0.3 mL per animal.

(v) Procedure: Divide the test animals at random into 4 groups, A, B, C and D, with not less than 8 animals and equal numbers in each group. Inject SH, SL, TH and TL into the tail vein or subcutaneously into the neck of each animal of the respective groups. At 1 hour after the injection, collect blood from the abdominal aorta in a way that minimizes the suffering of the animals, allow the blood samples to stand at room temperature for about 30 minutes, and centrifuge at 3000 revolutions per minute for 10 minutes to separate serum.

(vi) Serum calcium determination: Pipet 0.1 mL of the serum, add exactly 6.9 mL of strontium TS, mix well, and use this solution as the sample solution for calcium determination. Separately, pipet a suitable volume of Standard Calcium Solution for Atomic Absorption Spectrophotometry, dissolve in strontium TS to make a solution so that each mL contains 0.2 to 3 μg of calcium (Ca: 40.08), and use this solution as the standard solution for calcium determination. Perform the test as directed under Atomic Absorption Spectrometry <2.23> according to the following conditions, and calculate the calcium content of the sample solution for calcium determination from the calibration curve obtained from the absorbance of the standard solution for calcium determination.

Amount (mg) of Calcium (Ca) in 100 mL of the serum
= Calcium content (ppm) in the sample solution for calcium determination × 7

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Calcium hollow-cathode lamp.
Wavelength: 422.7 nm.

(vii) Calculation: Amounts of calcium in the serum obtained with SH, SL, TH and TL are symbolized as y1, y2, y3 and y4, respectively. Sum up y1, y2, y3 and y4 on each set to Y1, Y2, Y3 and Y4, respectively.

Units per mg of peptide = antilog M × b/a × 1/c × 5

M = 0.3010 × (Y1/Y5)
Y5 = Y1 – Y2 + Y3 + Y4
Y6 = Y1 – Y2 + Y3 – Y4
a: Amount (mg) of sample
b: Total volume (mL) of the high-dose sample solution prepared by dissolving Calcitonin (Salmon) in acetic acid buffer solution containing 0.1% bovine serum albumin.
c: Peptide content (%)

F' computed by the following equation should be smaller than F1 shown in the table against n with which s2 is calculated. Calculate L (P = 0.95) by use of the following equation:
L should be not more than 0.20. If F' exceeds F1, or if L exceeds 0.20, repeat the test, increasing the number of animals or arranging the assay conditions so that F' is not more than F1 and L is not more than 0.20.

F' = (−Y1 + Y2 + Y3 − Y4)/4f^2
f: Number of the test animals of each group.
s2 = (Σy^2 − (Y/f))/n
Σy^2: The sum of squares of y1, y2, y3 and y4 in each group.
Y = Y1^2 + Y2^2 + Y3^2 + Y4^2
n = 4(f − 1)
L = 2L(SC − 1)(CM^2 + 0.09062)
C = Y1^2/Y3^4 − 4f^2t^2

t^2: Value shown in the following table against n used to calculate s^2.

<table>
<thead>
<tr>
<th>n</th>
<th>t^2 = F1</th>
<th>n</th>
<th>t^2 = F1</th>
<th>n</th>
<th>t^2 = F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>161.45</td>
<td>13</td>
<td>4.667</td>
<td>25</td>
<td>4.242</td>
</tr>
<tr>
<td>2</td>
<td>18.51</td>
<td>14</td>
<td>4.600</td>
<td>26</td>
<td>4.225</td>
</tr>
<tr>
<td>3</td>
<td>10.129</td>
<td>15</td>
<td>4.543</td>
<td>13</td>
<td>4.210</td>
</tr>
<tr>
<td>4</td>
<td>7.709</td>
<td>16</td>
<td>4.494</td>
<td>13</td>
<td>4.210</td>
</tr>
<tr>
<td>5</td>
<td>6.608</td>
<td>17</td>
<td>4.451</td>
<td>13</td>
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</tr>
<tr>
<td>6</td>
<td>5.987</td>
<td>18</td>
<td>4.414</td>
<td>14</td>
<td>4.171</td>
</tr>
<tr>
<td>7</td>
<td>5.591</td>
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<td>14</td>
<td>4.085</td>
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<tr>
<td>8</td>
<td>5.318</td>
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<tr>
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</tr>
<tr>
<td>10</td>
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<tr>
<td>11</td>
<td>4.844</td>
<td>23</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>4.747</td>
<td>24</td>
<td>4.260</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Precipitated Calcium Carbonate

CaCO₃: 100.09

Precipitated Calcium Carbonate, when dried, contains not less than 98.5% of calcium carbonate (CaCO₃).

Description Precipitated Calcium Carbonate occurs as a white, fine crystalline powder. It is odorless and tasteless. It is practically insoluble in water, but its solubility in water is increased in the presence of carbon dioxide. It is practically insoluble in ethanol (95%) and in diethyl ether.

It dissolves with effervescence in dilute acetic acid, in dilute hydrochloric acid and in dilute nitric acid.

Identification (1) Dissolve 0.5 g of Precipitated Calcium Carbonate in 10 mL of dilute hydrochloric acid, boil, then cool, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> for calcium salt.

(2) Precipitated Calcium Carbonate responds to the Qualitative Tests <1.09> (1) for carbonate.

Purity (1) Acid-insoluble substances—To 5.0 g of Precipitated calcium Carbonate add 50 mL of water, then add 20 mL of hydrochloric acid dropwise with stirring, boil for 5 minutes, cool, add water to make 200 mL, and filter through filter paper for quantitative analysis. Wash the residue until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the residue is not more than 10.0 mg.

(2) Heavy metals <1.07>—Mix 2.0 g of Precipitated Calcium Carbonate with 5 mL of water, add slowly 6 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of water, and filter. To 25 mL of the filtrate add 2 mL of dilute acetic acid, 1 drop of ammonia TS and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 3 mL of hydrochloric acid on a water bath to dryness, and add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(3) Barium—Mix 1.0 g of Precipitated Calcium Carbonate with 10 mL of water, add dropwise 4 mL of hydrochloric acid with stirring, boil for 5 minutes, cool, add water to make 40 mL, and filter. With the filtrate, perform the test as directed under Flame Coloration Test <1.06> (1): no green color appears.

(4) Magnesium and alkali metals—Dissolve 1.0 g of Precipitated Calcium Carbonate in a mixture of 20 mL of water and 10 mL of dilute hydrochloric acid, boil, neutralize with ammonia TS, and add ammonium oxalate TS until precipitation of calcium oxalate is completed. Heat the mixture on a water bath for 1 hour, cool, dilute with water to 100 mL, shake well, and filter. To 50 mL of the filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite at 600°C to constant mass: the mass of the residue is not more than 5.0 mg.

(5) Arsenic <1.11>—Moisten 0.40 g of Precipitated Calcium Carbonate with 1 mL of water, then dissolve in 4 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 5 ppm).

Loss on drying <2.4I> Not more than 1.0% (1 g, 180°C, 4 hours).

Assay Weigh accurately about 0.12 g of Precipitated Calcium Carbonate, previously dried, and dissolve in 20 mL of water and 3 mL of dilute hydrochloric acid. Add 80 mL of water, 15 mL of a solution of potassium hydroxide (1 in 10) and 0.05 g of NN indicator, and titrate <2.5D> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 5.005 mg of CaCO₃

Containers and storage Containers—Hermetic containers.

Precipitated Calcium Carbonate Fine Granules

Precipitated Calcium Carbonate Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of calcium carbonate (CaCO₃: 100.09).

Method of preparation Prepare as directed under Granules, with Precipitated Calcium Carbonate.

Identification (1) To a quantity of powdered Precipitated Calcium Carbonate Fine Granules, equivalent to 0.5 g of Precipitated Calcium Carbonate according to the labeled amount, add 10 mL of dilute hydrochloric acid, shake thoroughly, and filter. Boil the filtrate, then cool, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> (1), (2) and (3) for calcium salt.

(2) Powdered Precipitated Calcium Carbonate Fine Granules responds to the Qualitative Tests <1.09> (1) for carbonate.

Uniformity of dosage units <6.02> The granules in single-unit containers meet the requirement of the Mass variation test.

Dissolution <6.1D> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 10 minutes of Precipitated Calcium Carbonate Fine Granules is not less than 80%.

Start the test with an accurately weighed amount of Precipitated Calcium Carbonate Fine Granules, equivalent to about 0.5 g of calcium carbonate (CaCO₃) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL
Precipitated Calcium Carbonate Tablets

Precipitated Calcium Carbonate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of calcium carbonate (CaCO₃: 100.09).

**Method of preparation** Prepare as directed under Tablets, with Precipitated Calcium Carbonate.

**Identification** (1) To a quantity of powdered Precipitated Calcium Carbonate Tablets, equivalent to 0.5 g of Precipitated Calcium Carbonate, add 10 mL of dilute hydrochloric acid, shake throughly, and filter, if necessary. Boil, then cool, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> (1), (2) and (3) for calcium salt.

(2) Powdered Precipitated Calcium Carbonate Tablets responds to the Qualitative Tests <1.09> (1) for carbonate.

**Uniformity of dosage units** <6.02> It meets the requirement of the Mass variation test.

**Disintegration** <6.09> Apply to the preparation intended to be used as antacid.

Perform the test using the disk: it meets the requirement.

**Dissolution** <6.10> Apply to the preparation intended to be used as hyperphosphatemia.

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 10 minutes of Precipitated Calcium Carbonate Tablets is not less than 80%.

Start the test with 1 tablet of Precipitated Calcium Carbonate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V mL so that each mL contains about 56 μg of calcium carbonate (CaCO₃), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of calcium carbonate for assay, previously dried at 180°C for 4 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution.

Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of calcium of both solutions.

Dissolution rate (%) with respect to the labeled amount of calcium carbonate (CaCO₃) = \( \frac{M₃}{M₁} \times \frac{A₁}{A₅} \times \frac{1}{C} \times 1800 \)

Where:
- \( M₃ \): Amount (mg) of calcium carbonate for assay
- \( M₁ \): Amount (mg) of Precipitated Calcium Carbonate Fine Granules
- \( C \): Labeled amount (mg) of calcium carbonate (CaCO₃) in 1 g

**System suitability**

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, sodium and calcium are eluted in this order with the resolution between these peaks being not less than 4.5. System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of calcium is not more than 2.0%.

**Particle size** <6.09> It meets the requirements of Fine granules.

**Assay** Weigh accurately a quantity of powdered Precipitated Calcium Carbonate Fine Granules, equivalent to about 0.12 g of calcium carbonate (CaCO₃), add 20 mL of water and 3 mL of dilute hydrochloric acid, and agitate for 15 minutes with the aid of ultrasonic waves. Then, add 80 mL of water, 15 mL of a solution of potassium hydroxide (1 in 10) and 50 mg of NN indicator, and titrate <2.50> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetaacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetaacetate VS = 5.005 mg of CaCO₃

**Containers and storage** Containers—Well-closed containers.

of the subsequent filtrate, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of calcium carbonate for assay, previously dried at 180°C for 4 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of calcium of both solutions.
Operation conditions—
Detector: An electric conductivity detector.
Column: A polyether ether ketone tube 4.6 mm in inside diameter and 10 cm in length, packed with slightly acidic ion-exchange silica gel for liquid chromatography (7 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of a solution of tartaric acid (3 in 2000) and a solution of dipicolinic acid (1 in 3000) (1:1).
Flow rate: Adjust the flow rate so that the retention time of calcium is about 8 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, sodium and calcium are eluted in this order with the resolution between these peaks being not less than 4.5.
System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of calcium is not more than 2.0%.

Acid-neutralizing capacity ≤6.0mg
Apply to the preparation intended to be used as antacid.

Weigh accurately and powder not less than 40 Precipitated Calcium Carbonate Tablets. Perform the test with an accurately weighed amount of the powder, equivalent to about 0.25 g of Calcium Carbonate according to the labeled amount: the amount of 0.1 mol/L hydrochloric acid VS consumed per 1 g of Precipitated Calcium Carbonate is not less than 190 mL.

Assay Weigh accurately and powder not less than 20 Precipitated Calcium Carbonate Tablets. To an accurately weighed portion of the powder, equivalent to about 0.12 g of calcium carbonate (CaCO₃), add 20 mL of water, 3 mL of dilute hydrochloric acid, and agitate, if necessary, for 15 minutes with the aid of ultrasonic waves. Then, add 80 mL of water, 15 mL of a solution of potassium hydroxide (1 in 10) and 50 mg of NN indicator, and titrate ≤2.50 mL immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 5.005 mg of CaCO₃

Containers and storage Containers—Tight containers.

Calcium Chloride Hydrate
塩化カルシウム水和物

CaCl₂·2H₂O: 147.01

Calcium Chloride Hydrate contains not less than 96.7% and not more than 103.3% of CaCl₂·2H₂O.

Description Calcium Chloride Hydrate occurs as white granules or masses. It is odorless.
It is very soluble in water, and soluble in ethanol (95), and practically insoluble in diethyl ether.
It is deliquescent.

Identification A solution of Calcium Chloride Hydrate (1 in 10) responds to the Qualitative Tests ≤1.09% for calcium salt and for chloride.

pH ≤2.54
The pH of a solution of 1.0 g of Calcium Chloride Hydrate in 20 mL of freshly boiled and cooled water is between 4.5 and 9.2.

Purity (1) Clarity and color of solution—A solution of 1.0 g of Calcium Chloride Hydrate in 20 mL of water is clear and colorless.

(2) Sulfate ≤1.14—Take 1.0 g of Calcium Chloride Hydrate, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Hypochlorite—Dissolve 0.5 g of Calcium Chloride Hydrate in 5 mL of water, add 2 to 3 drops of dilute hydrochloric acid and 2 to 3 drops of zinc iodide-starch TS: no blue color develops immediately.

(4) Heavy metals ≤0.07—Proceed with 2.0 g of Calcium Chloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Iron, aluminum or phosphate—Dissolve, in a Nessler tube, 1.0 g of Calcium Chloride Hydrate in 20 mL of water and 1 drop of dilute hydrochloric acid, boil, then cool, add 3 drops of ammonia TS, and heat the solution to boil: no turbidity or precipitate is produced.

(6) Barium—Dissolve 0.5 g of Calcium Chloride Hydrate in 5 mL of water, add 2 drops of dilute hydrochloric acid and 2 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

(7) Arsenic ≤0.11—Prepare the test solution with 1.0 g of Calcium Chloride Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Assay Weigh accurately about 0.4 g of Calcium Chloride Hydrate, and dissolve in water to make exactly 200 mL. Measure exactly 20 mL of this solution, add 40 mL of water, 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator, and titrate ≤2.50 mL immediately with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.940 mg of CaCl₂·2H₂O

Containers and storage Containers—Tight containers.

Calcium Chloride Injection
塩化カルシウム注射液

Calcium Chloride Injection is an aqueous solution for injection.
It contains not less than 95.0% and not more than 105.0% of the labeled amount of calcium chloride (CaCl₂: 110.98).
The concentration of Calcium Chloride Injection is expressed as the quantity of calcium chloride (CaCl₂).

Method of preparation Prepare as directed under Injection, with Calcium Chloride Hydrate.
Calcium Folate

Calcium Folinate / Official Monographs

Description Calcium Chloride Injection is a clear, colorless liquid.

Identification Calcium Chloride Injection responds to the Qualitative Tests \(<1.00\) for calcium salt and for chloride.

pH \(<2.54\) 4.5 – 7.5

Bacterial endotoxins \(<4.01\) Less than 0.30 EU/mg.

Extractable volume \(<6.05\) It meets the requirement.

Foreign insoluble matter \(<6.06\) Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter \(<6.07\) It meets the requirement.

Sterility \(<4.06\) Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Calcium Chloride Injection, equivalent to about 0.4 g of calcium chloride (CaCl₂), and proceed as directed in the Assay under Calcium Chloride Hydrate.

Containers and storage Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

Calcium Folate

Calcium Leucovorin

ホリナートカルシウム

Calcium Folate contains not less than 95.0% and not more than 102.0% of \(\text{C}_{20}\text{H}_{21}\text{CaN}_{7}\text{O}_{7}\), calculated on the anhydrous basis.

Description Calcium Folate occurs as a white to light yellow, crystalline powder.

It is sparingly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Calcium Folate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.25\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelength.

(2) A solution of Calcium Folate (1 in 100) responds to the Qualitative Tests \(<1.09\) (2) and (3) for calcium salt.

Optical rotation \(<2.49\) \([\alpha]_D^\text{20} +14− +19^\circ\) (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH \(<2.54\) To 1.25 g of Calcium Folate add 50 mL of freshly boiled and cooled water, and warm to 40°C, if necessary, to dissolve: the pH of this solution is between 6.8 and 8.0.

Purity (1) Clarity and color of solution—To 1.25 g of Calcium Folate add 50 mL of freshly boiled and cooled water, and warm to 40°C, if necessary, to dissolve: the solution is clear, and the absorbance at 420 nm of it, determined as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), is not more than 0.25.

(2) Heavy metals \(<1.07\)—Proceed with 0.40 g of Calcium Folate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 50 ppm).

(3) Related substances—Dissolve 10 mg of Calcium Folate in 25 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak obtained from the sample solution is not larger than the peak area obtained from the standard solution, and the total area of the peaks other than the peak of folinate is not larger than 5 times the peak area of folinate from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of folinate, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of folinate obtained from 20 \(\mu\)L of this solution is equivalent to 7 to 13% of that from 20 \(\mu\)L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of folinate is not more than 2.0%.

Water \(<2.48\) Not less than 7.0% and not more than 17.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 10 mg each of Calcium Folate and Calcium Folate RS (separately determine the water \(<2.48\) in the same manner as Calcium Folate), dis-
solve in water to make them exactly 25 mL. Pipet 5 mL each of these solutions, add the mobile phase to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.03> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of folinate of both solutions.

\[
M_S: \text{Amount (mg) of Calcium Folinate RS, calculated on the anhydrous basis}
\]

\[
M_S = \frac{A_T}{A_S} \times \frac{A_T}{A_S}
\]

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of disodium hydrogen phosphate dodecahydrate solution (287 in 100,000), methanol and tetrabutylammonium hydroxide TS (385:110:4), adjusted to pH 7.5 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of folinate is about 10 minutes.

**System suitability**

System performance: Dissolve 10 mg each of Calcium Folinate and folic acid in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, folinate and folic acid are eluted at 15.4 minutes and 45.7 minutes, respectively. Perform the test with exactly 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.03>.

Flow rate: Adjust the flow rate so that the retention time of folinate is about 10 minutes. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 g of Calcium Gluconate Hydrate and 1.0 g of Calcium Folinate Hydrate in 20 mL of water by warming: the pH of the solution is between 6.0 and 8.0.

**Purity (1)**

Clarity and color of solution—Dissolve 1.0 g of Calcium Gluconate Hydrate in 50 mL of water by warming: the solution is clear and colorless.

(2) Chloride

Take 0.40 g of Calcium Gluconate Hydrate, and perform the test. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.071%).

(3) Sulfate

Take 1.0 g of Calcium Gluconate Hydrate, and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(4) Heavy metals

Dissolve 1.0 g of Calcium Gluconate Hydrate in 30 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).

(5) Arsenic

Dissolve 0.6 g of Calcium Gluconate Hydrate in 5 mL of water by warming, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, and concentrate on a water bath to 5 mL. Perform the test with this solution as the test solution (not more than 3.3 ppm).

(6) Sucrose and reducing sugars—To 0.5 g of Calcium Gluconate Hydrate add 10 mL of water and 2 mL of dilute hydrochloric acid, and boil the solution for 2 minutes. After cooling, add 5 mL of sodium carbonate TS, allow to stand for 5 minutes, add water to make 20 mL, and filter. To 5 mL of the filtrate add 2 mL of Fehling’s TS, and boil for 1 minute: no orange-yellow to red precipitate is formed immediately.

**Loss on drying**

Not more than 1.0% (1 g, 80°C, 2 hours).

**Assay**

Weigh accurately about 0.4 g of Calcium Gluconate Hydrate, previously dried, dissolve in 100 mL of water, add 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator, and titrate immediately with 0.05 mol/L
Calcium Hydroxide / Official Monographs

Calcium Hydroxide

Slaked Lime

Calcium Hydroxide contains not less than 90.0% of Ca(OH)₂.

Description Calcium Hydroxide occurs as a white powder. It has a slightly bitter taste. It is slightly soluble in water, very slightly soluble in boiling water, and practically insoluble in ethanol (95) and in diethyl ether. It dissolves in dilute acetic acid, in dilute hydrochloric acid and in dilute nitric acid. It absorbs carbon dioxide from air.

Identification (1) Mix Calcium Hydroxide with 3 to 4 times its mass of water: the mixture is slushy and is alkaline.

(2) Dissolve 1 g of Calcium Hydroxide in 30 mL of dilute acetic acid, and boil. After cooling, neutralize with ammonia TS: the solution responds to the Qualitative tests <1.09> (2) and (3) for calcium salt.

Purity (1) Acid-insoluble substances—To 5 g of Calcium Hydroxide add 100 mL of water, add hydrochloric acid dropwise with stirring until the solution becomes acidic, and further add 1 mL of hydrochloric acid. Boil this solution for 5 minutes, cool, and filter through a tared glass filter (G4). Wash the residue with boiling water until the last washing is clear.

(2) Heavy metals <1.07>—Dissolve 1.0 g of Calcium Hydroxide in 10 mL of dilute hydrochloric acid, evaporate on a water bath to dryness, dissolve the residue in 40 mL of water, and filter. To 20 mL of the filtrate add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 5 mL of dilute hydrochloric acid on a water bath to dryness, and add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 40 ppm).

(3) Magnesium and alkali metals—Dissolve 1.0 g of Calcium Hydroxide in a mixture of 20 mL of water and 10 mL of dilute hydrochloric acid, boil, neutralize with ammonium TS, and precipitate calcium oxalate completely by adding dropwise ammonium oxalate TS. Heat the mixture on a water bath for 1 hour, cool, dilute with water to 100 mL, shake, and filter. To 50 mL of the filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite at 600°C to constant mass: the mass of the residue does not exceed 24 mg.

(4) Arsenic <1.17>—Dissolve 0.5 g of Calcium Hydroxide in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 4 ppm).

Assay Weigh accurately about 1 g of Calcium Hydroxide, dissolve by adding 10 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Measure exactly 10 mL of this solution, add 90 mL of water and 1.5 mL of 8 mol/L potassium hydroxide TS, shake, allow to stand for 3 to 5 minutes, and then add 0.1 g of NN indicator. Titrate <2.5D> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, until the red-purple color of the solution changes to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

= 3.705 mg of Ca(OH)₂

Containers and storage Containers—Well-closed containers.

Calcium Lactate Hydrate

Monocalcium disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

= 22.42 mg of C₁₂H₂₂CaO₁₄.H₂O

Containers—Well-closed containers.

Calcium Lactate Hydrate occurs as a white powder. It exhibits no turbidity upon addition of silver nitrate TS, and precipitates calcium oxalate completely by adding dropwise ammonium oxalate TS. Heat the mixture on a water bath for 1 hour, cool, dilute with water to 100 mL, and perform the test using this solution as the test solution. Prepare the control solution from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 20 ppm).

Calcium Lactate Hydrate, when dried, contains not less than 97.0% of calcium lactate (C₆H₁₀CaO₆: 218.22).

Description Calcium Lactate Hydrate occurs as white powder or granules. It is odorless, and has a slightly acid taste. A 1 g portion of it dissolves gradually in 20 mL of water, and it is slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is partly efflorescent at ordinary temperature, and yields the anhydride at 120°C.

Identification A solution of Calcium Lactate Hydrate (1 in 20) responds to the Qualitative tests <1.09> for calcium salt and for lactate.

Purity (1) Clarity of solution—Dissolve 1.0 g of Calcium Lactate Hydrate in 20 mL of water by warming: the solution is clear.

(2) Acidity or alkalinity—To the solution obtained in (1) add 2 drops of phenolphthalein TS: no red color is produced. Then add 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Calcium Lactate Hydrate in 30 mL of water and 5 mL of dilute acetic acid by warming, cool, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 20 ppm).

(4) Magnesium or alkali metals—Dissolve 1.0 g of Calcium Lactate Hydrate in 40 mL of water, add 0.5 g of am-
Calcium Pantothenate occurs as a white powder. It is odorless, and has a bitter taste. Calcium Pantothenate, when dried, contains not less than 5.7% and not more than 6.0% of nitrogen (N: 14.01), and not less than 8.2% and not more than 8.6% of calcium (Ca: 40.08).

**Description**
Calcium Pantothenate occurs as a white powder. It is odorless, and has a bitter taste. It is freely soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Calcium Pantothenate (1 in 20) is 7.50–8.60.

**Containers and storage**
Containers—Tight containers.

### Calcium Oxide

**Quick Lime**

酸化カルシウム

CaO: 56.08

Calcium Oxide, when incinerated, contains not less than 98.0% of CaO.

**Description**
Calcium Oxide occurs as hard, white masses, containing a powder. It is odorless.

It is very slightly soluble in boiling water, and practically insoluble in ethanol (95).

One gram of Calcium Oxide dissolves almost completely in 2500 mL of water.

It slowly absorbs moisture and carbon dioxide from air.

**Identification**
(1) Moisten Calcium Oxide with water: heat is generated and a white powder is obtained. Mix the powder with about 5 times its mass of water; the mixture is alkaline.

(2) Dissolve 1 g of Calcium Oxide in 20 mL of water by adding a few drops of acetic acid (31): the solution responds to the Qualitative Tests <1.09> for calcium salt.

**Purity**
(1) Acid-insoluble substances—Disintegrate 5.0 g of Calcium Oxide with a small amount of water, add 100 mL of water, add dropwise hydrochloric acid with stirring until the solution becomes acidic, and further add 1 mL of hydrochloric acid. Boil the solution for 5 minutes, cool, filter through a glass filter (G4), wash the residue with boiling water until no turbidity is produced when silver nitrate TS is added to the last washing, and dry at 105°C to constant mass: the mass of the residue is not more than 15 mg.

(2) Carbonate—Disintegrate 1.0 g of Calcium Oxide with a small amount of water, mix thoroughly with 50 mL of water, allow to stand for a while, remove most of the supernatant milky liquid by decantation, and add an excess of dilute hydrochloric acid to the residue: no vigorous effervescence is produced.

(3) Magnesium and alkali metals—Dissolve 1.0 g of Calcium Oxide in 75 mL of water by adding dropwise hydrochloric acid, and further add 1 mL of hydrochloric acid. Boil for 1 to 2 minutes, neutralize with ammonia TS, add dropwise an excess of hot ammonium oxalate TS, heat the mixture on a water bath for 2 hours, cool, add water to make 200 mL, mix thoroughly, and filter. Evaporate 50 mL of the filtrate with 0.5 mL of sulfuric acid to dryness, and heat the residue strongly at 600°C to constant mass: the mass of the residue is not more than 15 mg.

**Loss on ignition** <2.43> Not more than 10.0% (1 g, 900°C, constant mass).

**Assay**
Weigh accurately about 0.7 g of Calcium Oxide, previously incinerated at 900°C to constant mass and cooled in a desiccator (silica gel), and dissolve in 50 mL of water and 8 mL of diluted hydrochloric acid (1 in 3) by heating. Cool, and add water to make exactly 250 mL. Pipet 10 mL of the solution, add 50 mL of water, 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator, and titrate <2.50> immediately with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red-purple color of the solution changes to blue.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.122 mg of CaO

### Containment and Storage
Containers—Tight containers.
Calcium Paraaminosalicylate Hydrate

**Pas-calcium Hydrate**

\[
\text{C}_7\text{H}_5\text{CaNO}_3 \cdot 3 \frac{1}{2}\text{H}_2\text{O}: 254.25
\]

Monocalcium 4-amino-2-oxidobenzoate hemiheptahydrate (133-15-3, anhydride)

Calcium Paraaminosalicylate Hydrate contains not less than 97.0% and not more than 103.0% of calcium para-aminosalicylic acid (C₇H₅CaNO₃: 191.20), calculated on the anhydrous basis.

**Description**
Calcium Paraaminosalicylate Hydrate occurs as a white to slightly colored powder. It has a slightly bitter taste.

It is very slightly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

It is gradually colored to brown by light.

**Identification (1)** To 50 mg of Calcium Paraaminosalicylate Hydrate add 100 mL of water, shake well, and filter. To 10 mL of the filtrate add 1 mL of 1 mol/L hydrochloric acid TS, shake, and add 1 drop of iron (III) chloride TS: a red-purple color develops.

(2) Determine the infrared absorption spectrum of Calcium Paraaminosalicylate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 3 g of Calcium Paraaminosalicylate Hydrate add 15 mL of ammonium chloride TS and 15 mL of water, heat on a water bath until almost dissolved, and filter after cooling: the filtrate responds to the Qualitative Tests <1.09> (1), (2) and (3) for calcium salt.

**Purity (1)** Chloride <1.03>—Dissolve 1.0 g of Calcium Paraaminosalicylate Hydrate in 15 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.025%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Calcium Paraaminosalicylate Hydrate according to method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Dissolve 0.40 g of Calcium Paraaminosalicylate Hydrate in 20 mL of 0.1 mol/L hydrochloric acid TS by warming on a water bath, use this solution as the test solution, and perform the test (not more than 5 ppm).

(4) 3-Aminophenol—To 0.10 g of Calcium Paraaminosalicylate Hydrate add 5 mL of 0.1 mol/L disodium ethylenediamine tetraacetate VS, then 10 mL of the filtrate add 1 mL of 1 mol/L hydrochloric acid VS (not more than 0.025%).

**Containers and storage**
Containers—Tight containers.
Calcium Paraaminosalicylate Granules

Pass-calcium Granules

パラアミノサリチル酸カルシウム顆粒

Calcium Paraaminosalicylate Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of calcium paraaminosalicylate hydrate (C₇H₅CaNO₃.3½H₂O: 254.25).

Method of preparation Prepare as directed under Granules, with Calcium Paraaminosalicylate Hydrate.

Identification Powder Calcium Paraaminosalicylate Granules, weigh a portion of the powder, equivalent to 50 mg of Calcium Paraaminosalicylate Hydrate according to the labeled amount, add 100 mL of water, shake, and filter. To 10 mL of the filtrate add 1 mL of 1 mol/L hydrochloric acid TS, shake, and add 1 drop of iron (III) chloride TS: a red-purple color develops.

Dissolution <6.10 When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Calcium Paraaminosalicylate Granules is not less than 75%.

Start the test with an accurately weighed amount of Calcium Paraaminosalicylate Granules, equivalent to about 0.25 g of calcium paraaminosalicylate hydrate (C₇H₅CaNO₃.3½H₂O) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the standard solution. Separately, weigh accurately about 28 mg of calcium paraaminosalicylate hydrate for assay (separately determine the water <2.40 in the same manner as Calcium Paraaminosalicylate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₂ and A₈, at 300 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24.

Dissolution rate (%) with respect to the labeled amount of calcium paraaminosalicylate hydrate (C₇H₅CaNO₃.3½H₂O)

\[ \frac{M_S}{M_T} \times \frac{A_T}{A_S} \times \frac{1}{C} \times 900 \times 1.330 \]

Mₕ: Amount (mg) of calcium paraaminosalicylate hydrate for assay, calculated on the anhydrous basis
Mₜ: Amount (g) of sample
C: Labeled amount (mg) of calcium paraaminosalicylate hydrate (C₇H₅CaNO₃.3½H₂O) in 1 g

Assay Powder Calcium Paraaminosalicylate Granules, weigh accurately a portion of the powder, equivalent to about 0.2 g of calcium paraaminosalicylate hydrate (C₇H₅CaNO₃.3 1/2H₂O), add 60 mL of water and 0.75 mL of dilute hydrochloric acid, and dissolve by heating on a water bath. After cooling, add water to make exactly 100 mL, and filter. Pipet 30 mL of the filtrate, transfer to an iodine flask, and proceed as directed in the Assay under Calcium Para-aminosalicylate Hydrate.

Each mL of 0.05 mol/L bromine VS

\[ = 3.187 \text{ mg of } C₇H₅CaNO₃ \]

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Calcium Paraaminosalicylate

Powder Calcium Paraaminosalicylate Granules, with Calcium Paraaminosalicylate Hydrate.

Identification Powder Calcium Paraaminosalicylate Granules, weigh a portion of the powder, equivalent to 50 mg of Calcium Paraaminosalicylate Hydrate according to the labeled amount, add 100 mL of water, shake, and filter. To 10 mL of the filtrate add 1 mL of 1 mol/L hydrochloric acid TS, shake, and add 1 drop of iron (III) chloride TS: a red-purple color develops.
Anhydrous Dibasic Calcium Phosphate

無水リン酸水素カルシウム

CaHPO$_4$: 136.06
[7757-93-9]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols ($\ast$ $\bullet$).

Anhydrous Dibasic Calcium Phosphate contains not less than 98.0% and not more than 103.0% of CaHPO$_4$.

*Description*  Anhydrous Dibasic Calcium Phosphate occurs as white, crystalline powder or granules.

It is practically insoluble in water and in ethanol (99.5). It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Identification (1)  Dissolve 0.1 g of Anhydrous Dibasic Calcium Phosphate in 10 mL of 2 mol/L hydrochloric acid TS by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2)  Dissolve 0.1 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of dilute nitric acid, and add 2 mL of hexammonium heptamolybdate TS after warming at 70°C for 1 to 2 minutes: a yellow precipitate is produced.

Purity (1)  Acid-insoluble substances—Dissolve 5.0 g of Anhydrous Dibasic Calcium Phosphate in 40 mL of water and 10 mL of hydrochloric acid, and boil gently for 5 minutes. After cooling, collect the insoluble substance using a filter paper for assay. Wash with water until no more turbidity forms. It dissolves in dilute hydrochloric acid.

(2)  Chloride $<1.00$—Dissolve 0.20 g of Anhydrous Dibasic Calcium Phosphate in 20 mL of water and 13 mL of dilute nitric acid, add water to make 100 mL, and filter, if necessary. Perform the test using a 50-mL portion of this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.248%).

(3)  Sulfate $<1.14$—Dissolve 0.5 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of water and 5 mL of dilute hydrochloric acid, add water to make 100 mL, and filter, if necessary. Take 20 mL of this solution, add 1 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.48%).

(4)  Carbonate—Mix 1.0 g of Anhydrous Dibasic Calcium Phosphate with 5 mL of freshly boiled and cooled water, and add immediately 2 mL of hydrochloric acid: no effervescence occurs.

*5* Heavy metals $<0.07$—Dissolve 0.65 g of Anhydrous Dibasic Calcium Phosphate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).

(6)  Barium—Heat 0.5 g of Anhydrous Dibasic Calcium Phosphate with 10 mL of water, add 1 mL of hydrochloric acid dropwise with stirring, and filter after cooling, if necessary. Add 2 mL of potassium sulfate TS to this solution, and allow to stand for 10 minutes: no turbidity forms.

*7* Arsenic $<1.10$—Dissolve 1.0 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

**8** Loss on ignition $<2.43$  Not less than 6.6% and not more than 8.5% (1 g, 800 – 825°C, constant mass).

Assay  Weigh accurately about 0.4 g of Anhydrous Dibasic Calcium Phosphate, dissolve in 12 mL of dilute hydrochloric acid, and add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate $2.50$ the excess of disodium dihydrogen ethylenediaminetetraacetate with 0.02 mol/L zinc sulfate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination in the same manner.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate VS = 2.721 mg of CaHPO$_4$.

*Containers and storage*  Containers—Well-closed containers.

Dibasic Calcium Phosphate Hydrate

リン酸水素カルシウム水和物

CaHPO$_4$.2H$_2$O: 172.09
[7789-77-7]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols ($\ast$ $\bullet$).

Dibasic Calcium Phosphate Hydrate contains not less than 98.0% and not more than 105.0% of CaHPO$_4$.2H$_2$O.

*Description*  Dibasic Calcium Phosphate Hydrate occurs as a white, crystalline powder.

It is practically insoluble in water and in ethanol (99.5). It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Identification (1)  Dissolve 0.1 g of Dibasic Calcium
Phosphate Hydrate in 10 mL of 2 mol/L hydrochloric acid TS by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Dibasic Calcium Phosphate Hydrate in 5 mL of dilute nitric acid, and add 2 mL of hexammonium heptamolybdate TS after warming at 70°C for 1 to 2 minutes: a yellow precipitate is produced.

**Purity (1)** Acid-insoluble substance—Dissolve 5.0 g of Dibasic Calcium Phosphate Hydrate in 40 mL of water and 10 mL of hydrochloric acid, and boil gently for 5 minutes. After cooling, collect the insoluble substance using a filter paper for assay. Wash with water until no more turbidity of the washing is produced when silver nitrate TS is added. Ignite to incinerate the residue and filter paper at 600°C: the mass is not more than 10 mg (not more than 0.2%).

(2) Chloride (1.07)—Dissolve 0.20 g of Dibasic Calcium Phosphate Hydrate, in 20 mL of water and 13 mL of dilute nitric acid, add water to make 100 mL, and filter, if necessary. Perform the test using a 50-mL portion of this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.24%).

(3) Sulfate (1.14)—Dissolve 0.5 g of Dibasic Calcium Phosphate Hydrate in 5 mL of water and 5 mL of dilute hydrochloric acid, add water to make 100 mL, and filter, if necessary. Take 20 mL of this solution, add 1 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.48%).

(4) Carbonate—Mix 1.0 g of Dibasic Calcium Phosphate Hydrate with 5 mL of freshly boiled and cooled water, and add immediately 2 mL of hydrochloric acid: no effervescence occurs.

*5* Heavy metals (1.07)—Dissolve 0.65 g of Dibasic Calcium Phosphate Hydrate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).

(6) Barium—Heat 0.5 g of Dibasic Calcium Phosphate Hydrate with 10 mL of water, add 1 mL of hydrochloric acid dropwise with stirring, and filter after cooling, if necessary. Add 2 mL of potassium sulfate TS to this solution, and allow to stand for 10 minutes: no turbidity forms.

*7* Arsenic (1.17)—Dissolve 1.0 g of Dibasic Calcium Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

**Loss on ignition (2.43)** Not less than 24.5% and not more than 26.5% (1 g, 800–825°C, constant mass).

**Assay** Weigh accurately about 0.4 g of Dibasic Calcium Phosphate Hydrate, dissolve in 12 mL of dilute hydrochloric acid, and add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonium-ammonium chloride buffer solution, pH 10.7, and titrate 0.02 mol/L zinc sulfate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination in the same manner.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

\[
= 3.442 \text{ mg of CaHPO}_4\cdot2\text{H}_2\text{O}
\]

**Containers and storage** Containers—Well-closed containers.

### Monobasic Calcium Phosphate Hydrate

リン酸二水素カルシウム水和物

\[
\text{Ca(H}_2\text{PO}_4)_2\cdot\text{H}_2\text{O}: 252.07
\]

Monobasic Calcium Phosphate Hydrate, when dried, contains not less than 90.0% of \(\text{Ca(H}_2\text{PO}_4)_2\cdot\text{H}_2\text{O}\).

**Description** Monobasic Calcium Phosphate Hydrate occurs as white crystals or crystalline powder. It is odorless and has an acid taste.

It is sparingly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

It is slightly deliquescent.

**Identification (1)** Dissolve 0.1 g of Monobasic Calcium Phosphate Hydrate in 10 mL of diluted hydrochloric acid (1 in 6) by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Monobasic Calcium Phosphate Hydrate in 5 mL of dilute nitric acid, and add 2 mL of hexammonium heptamolybdate TS after warming for 1 to 2 minutes at 70°C: a yellow precipitate is produced.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 19 mL of water and 2 mL of diluted hydrochloric acid (3 in 4), and heat on a water bath for 5 minutes with occasional shaking: the solution is clear and colorless.

(2) Dibasic phosphate and acid—Triturate 1.0 g of Monobasic Calcium Phosphate Hydrate with 3 mL of water, and add 100 mL of water and 1 drop of methyl orange TS: a red color develops. Then add 1.0 mL of 1 mol/L sodium hydroxide VS: the color changes to yellow.

(3) Chloride (1.03)—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 20 mL of water and 12 mL of dilute nitric acid, add water to make exactly 100 mL, and filter, if necessary. Perform the test using 50 mL of this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than...
0.018%).

(4) Sulfate <1.14>—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 20 mL of water and 1 mL of hydrochloric acid, add water to make 100 mL, and filter, if necessary. Perform the test using 50 mL of this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals <1.07>—Dissolve 0.65 g of Monobasic Calcium Phosphate Hydrate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).

(6) Arsenic <1.17>—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

Loss on drying <2.41> Not more than 3.0% (1 g, silica gel, 24 hours).

Assay Weigh accurately about 0.4 g of Monobasic Calcium Phosphate Hydrate, previously dried, dissolve in 3 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.02 mol/L zinc acetate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 5.041 mg of Ca(H2PO4)2 H2O

Containers and storage Containers—Tight containers.

Calcium Polystyrene Sulfonate

ポリスチレンスルホン酸カルシウム

Calcium Polystyrene Sulfonate is a cation exchange resin prepared as the calcium form of the sulfonated styrene divinylbenzene copolymer.

When dried, it contains not less than 7.0% and not more than 9.0% of calcium (Ca: 40.08).

Each g of Calcium Polystyrene Sulfonate, when dried, exchanges with 53 to 71 mg of potassium (K: 39.10).

Description Calcium Polystyrene Sulfonate occurs as a pale yellowish white to light yellow powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Determine the infrared absorption spectrum of Calcium Polystyrene Sulfonate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Mix 0.5 g of Calcium Polystyrene Sulfonate with 10 mL of dilute hydrochloric acid, filter, and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests <1.09> for calcium salt.

Purity (1) Ammonium—Place 1.0 g of Calcium Polystyrene Sulfonate in a flask, add 5 mL of sodium hydroxide TS, cover the flask with a watch glass having a moistened strip of red litmus paper on the underside, and boil for 15 minutes: the gas evolved does not change the red litmus paper to blue (not less than 5 ppm).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Calcium Polystyrene Sulfonate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.17>—Prepare the test solution with 1.0 g of Calcium Polystyrene Sulfonate according to Method 3, and perform the test (not more than 2 ppm).

(4) Styrene—To 10.0 g of Calcium Polystyrene Sulfonate...
add 10 mL of acetone, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of styrene in acetone to make exactly 100 mL. Pipet 1 mL of this solution, dilute with acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Gas Chromatography ≤0.2% according to the following conditions.

Determine the peak heights, H_T and H_S, of styrene in each solution: H_T is not larger than H_S.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A stainless steel column 3 mm in inside diameter and 2 m in length, having polyethylene glycol 20 M coated at the ratio of 15% on siliceous earth for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 90°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of styrene is about 9 minutes.

System suitability—

System performance: Mix 10 mg of styrene with 1000 mL of acetone. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of styrene are not less than 800 and 0.8 to 1.2, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of styrene is not more than 3%.

(5) Sodium—Pipet 2 mL of the 50-mL solution obtained in the Assay (1), add 0.02 mol/L hydrochloric acid TS to make exactly 500 mL, and use this solution as the sample solution. Separately, weigh accurately 0.2542 g of sodium chloride, previously dried at 130°C for 2 hours, and dissolve in 0.02 mol/L hydrochloric acid TS to make exactly 1000 mL. Pipet a suitable volume of this solution, and dilute with 0.02 mol/L hydrochloric acid TS to make a solution containing 1 to 3 μg of sodium (Na: 22.99) per mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions according to the Atomic Absorption Spectrophotometry ≤0.2% under the following conditions, and determine the amount of sodium in the sample solution using the calibration curve obtained from the standard solutions: the amount of sodium is not more than 1%.

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: A sodium hollow-cathode lamp.

Wavelength: 589.0 nm.

Loss on drying ≤0.4% Not more than 10.0% (1 g, in vacuum, 80°C, 5 hours).

Microparticles (i) Apparatus: Use an apparatus as shown in the illustration.

(ii) Procedure: Weigh accurately about 5.5 g of Calcium Polystyrene Sulfonate, previously dried, add 300 mL of water of 25°C, and mix for 5 minutes. Transfer this turbid solution to the sedimentation tube J, keeping a temperature at 25°C, add water of 25°C to 2 mm below the mark F of 20 cm of the sedimentation tube J, and then insert the pipet. Open the two-way stopcock C, exhaust air, add exactly water from the vent-hole D to the mark F of 20 cm, and close the two-way stopcock C. Shake the apparatus vertically and horizontally, disperse Calcium Polystyrene Sulfonate in water, and then open the two-way stopcock, and allow to stand at 25 ± 1°C for 5 hours and 15 minutes.

Then, draw exactly the meniscus of the turbid solution in the sedimentation tube J up to the mark of pipet bulb A by suction, open the two-way stopcock C to the outlet of pipet H, and transfer exactly measured 20 mL of the turbid solution to a weighing bottle. Repeat the procedure, and combine exactly measured 20 mL of the turbid solution. Evaporate 20 mL of this turbid solution on a water bath to dryness, dry to constant mass at 105°C, and weigh the residue as M_S (g).

Pipet 20 mL of used water, and make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 200 mL, and adjust with ammonia TS to a pH of 2.2 to 2.4. Pipet 5 mL of the supernatant liquid as the sample solution, using the calibration curve obtained from the following equation: the amount of microparticles is not more than 0.1%.

\[
S (\%) = \frac{(mi \times V)}{(20 \times m_T) \times 100}
\]

M_T: Amount (g) of Calcium Polystyrene Sulfonate

V: Actual volume (mL) to the mark of 20 cm at which the suction part of pipet is inserted.

Assay (1) Calcium—Weigh accurately about 1 g of Calcium Polystyrene Sulfonate, previously dried, and disperse in 5 mL of 3 mol/L hydrochloric acid TS. Transfer this mixture, and wash out completely with the aid of a small quantity of 3 mol/L hydrochloric acid TS to a column 12 mm in inside diameter and 70 mm in length, packed with a pledged of fine glass wool in the bottom of it, placing a 50-mL volumetric flask as a receiver under the column. Then collect about 45 mL of eluate, adding 3 mol/L hydrochloric acid TS to the column, and add water to make exactly 50 mL. Pipet 20 mL of this solution, adjust with ammonia TS to a pH of exactly 10. Titrate ≤0.50% immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red-purple color of the solution disappears, and a blue color develops (indicator: 0.04 g eriochrome black T-sodium dihydrogen ethylenediamine tetraacetate VS) until the purple color of the solution develops, and a blue color develops (indicator: 0.04 g eriochrome black T-sodium dihydrogen ethylenediamine tetraacetate VS) until the purple color of the solution disappears, and a blue color develops (indicator: 0.04 g eriochrome black T-sodium dihydrogen ethylenediamine tetraacetate VS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.004 mg of Ca

(2) Potassium exchange capacity—Pipet 50 mL of Standard Potassium Stock Solution into a glass-stoppered flask containing about 1 g of dried Calcium Polystyrene Sulfonate, accurately weighed, stir for 120 minutes, filter, and discard the first 20 mL of the filtrate. Pipet 5 mL of the subsequent filtrate, and add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the sample solution. Separately, measure exactly a suitable volume of Standard Potassium Stock Solution, dilute with 0.02 mol/L hydrochloric acid TS to make solutions containing 0.5 to 2.5 μg of potassium (K: 39.10) per mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry ≤0.2% according to the following conditions, and determine the amount, Y (mg), of potassium in 1000 mL of the sample solution, using the calibration curve obtained from
the standard solutions. The exchange quantity for potassium per g of dried Calcium Polystyrene Sulfonate is 53 to 71 mg, calculating by the following equation.

Exchange quantity (mg) for potassium (K) per g of dried Calcium Polystyrene Sulfonate

\[ \frac{X}{M} = \frac{(X - 100) Y}{M} \]

Where:
- \( X \): The amount (mg) of potassium in 50 mL of Standard Potassium Stock Solution before exchange.
- \( M \): The amount (g) of dried Calcium Polystyrene Sulfonate.
- \( Y \): The amount (mg) of Standard Lead Solution.

Gas: Combustible gas—Acetylene
Supporting gas—Air
Lamp: A potassium hollow-cathode lamp
Wavelength: 766.5 nm

Containers and storage  Containers—Tight containers.

Calcium Stearate

Calcium Stearate mainly consists of calcium salts of stearic acid (C\textsubscript{17}H\textsubscript{35}O\textsubscript{2}: 284.48) and palmitic acid (C\textsubscript{16}H\textsubscript{32}O\textsubscript{2}: 256.42).

Calcium Stearate, when dried, contains not less than 6.4% and not more than 7.1% of calcium (Ca: 40.08).

Description  Calcium Stearate occurs as a white, light, bulky powder. It feels smooth when touched, and is adhesive to the skin. It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification

(1) Shake vigorously 3 g of Calcium Stearate with 20 mL of diluted hydrochloric acid (1 in 2) and 30 mL of diethyl ether for 3 minutes, and allow to stand: the separated aqueous layer responds to the Qualitative Tests for calcium salt.

(2) Wash the diethyl ether layer obtained in (1) with 20 mL and 10 mL of dilute hydrochloric acid and 20 mL of water successively, and evaporate the diethyl ether on a water bath: the residue melts at a temperature not below 54°C.

Purity

(1) Heavy metals  Not more than 0.1 mg of Calcium Stearate with caution at the beginning, and heat further, gradually raising the temperature, to incineration. After cooling, add 2 mL of hydrochloric acid, evaporate on a water bath to dryness, warm the residue with 20 mL of water and 2 mL of dilute acetic acid for 2 minutes, cool, filter, and wash the residue with 15 mL of water. Combine the filtrate and the washings, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution by evaporating 2 mL of hydrochloric acid on a water bath to dryness and by adding 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Arsenic  To 1.0 g of Calcium Stearate add 5 mL of diluted hydrochloric acid (1 in 2) and 20 mL of chloroform, shake vigorously for 3 minutes, allow to stand, and separate the water layer. Perform the test with the water layer as the test solution (not more than 2 ppm).

Loss on drying  Not more than 4.0% (1 g, 105°C, 3 hours).

Assay  Weigh accurately about 0.5 g of Calcium Stearate, previously dried, heat gently with caution at first, and then ignite gradually to ash. Cool, add 10 mL of dilute hydrochloric acid to the residue, warm for 10 minutes on a water bath, and transfer the contents to a flask with the aid of 10-mL, 10-mL, and 5-mL portions of hot water. Add sodium hydroxide TS until the solution becomes slightly turbid, and then add 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 10 mL of ammonium-ammonium chloride buffer solution, pH 10.7, 4 drops of eriochrome black T TS and 5 drops of methyl yellow TS, and titrate rapidly the excess disodium dihydrogen ethylenediamine tetraacetate with 0.05 mol/L magnesium chloride VS, until the green color of the solution disappears and a red color develops. Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

\[ \frac{2.004}{\text{mg}} = \frac{X}{\text{mg}} \]

Containers and storage  Containers—Well-closed containers.

Camellia Oil

Oleum Camelliae

ツバキ油

Camellia Oil is the fixed oil obtained from the peeled seeds of Camellia japonica Linné (Theaceae).

Description  Camellia Oil is a colorless or pale yellow, clear oil. It is nearly odorless and tasteless.

It is miscible with diethyl ether and with petroleum ether. It is miscible with diethyl ether and with petroleum ether. It is miscible with diethyl ether and with petroleum ether.

It is miscible with diethyl ether and with petroleum ether. It is miscible with diethyl ether and with petroleum ether. It is miscible with diethyl ether.

Identification  To 2 mL of Camellia Oil add dropwise 10 mL of a mixture of fuming nitric acid, sulfuric acid, and water (1:1:1), previously cooled to room temperature: a bluish green color develops at the zone of contact.

Acid value  Not more than 2.8.

Saponification value  188 – 194

Unsaponifiable matters  Not more than 1.0%.

Iodine value  78 – 83

Containers and storage  Containers—Tight containers.
Camostat Mesilate

Description
Camostat Mesilate occurs as white crystals or crystalline powder. It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification
(1) To 4 mL of a solution of Camostat Mesilate (1 in 1000) add 2 mL of 1-naphthol TS and 1 mL of diacetyl TS, and allow to stand for 10 minutes: a red color develops.

(2) Determine the absorption spectrum of a solution of Camostat Mesilate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Camostat Mesilate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A 0.1 g portion of Camostat Mesilate responds to the Qualitative Tests 1.00 (1) for mesilate.

Melting point
194 - 198°C

Purity
(1) Heavy metals 1.07—Dissolve 1.0 g of Camostat Mesilate in 40 mL of water by warming, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid (not more than 20 ppm).

(2) Arsenic 1.11—Dissolve 2.0 g of Camostat Mesilate in 20 mL of 2 mol/L hydrochloric acid TS by heating in a water bath, and continue to heat for 20 minutes. After cooling, centrifuge, take 10 mL of the supernatant liquid, and use this solution as the test solution. Perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 30 mg of Camostat Mesilate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.01. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100:200:100:50:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand overnight in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying 2.41—Not more than 1.0% (1 g, silica gel, 105°C, 3 hours).

Residue on ignition 2.44—Not more than 0.2% (1 g).

Assay
Weigh accurately about 50 mg each of Camostat Mesilate and Camostat Mesilate RS, previously dried, and dissolve each in water to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Qt and Qs, of the peak area of camostat to that of the internal standard.

\[
\text{Amount (mg)} = M_S \times \frac{Q_t}{Q_s}
\]

Mₜ: Amount (mg) of C₂₀H₂₂N₄O₅.CH₄O₃S

Residue on ignition 2.44—Not more than 0.2% (1 g).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 265 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of methanol, a solution of sodium 1-heptane sulfonate (1 in 500), a solution of sodium laureyl sulfate (1 in 1000) and acetic acid (100) (200:100:50:1).
Flow rate: Adjust the flow rate so that the retention time of camostat is about 10 minutes.

System suitability—
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, camostat and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of camostat to that of the internal standard is not more than 1.0%.

Containers and storage
Containers—Tight containers.
**d-Camphor**

\[ C_{10}H_{16}O: 152.23 \]

(1R,4R)-1,7,7-Trimethylbicyclo[2.2.1]-heptan-2-one [464-49-3]

*d*-Camphor contains not less than 96.0% of 
\[ C_{10}H_{16}O. \]

**Description**  
*d*-Camphor occurs as colorless or white, translucent crystals, crystalline powder or masses. It has a characteristic, agreeable odor, and a slightly bitter taste, followed by a pleasant, cooling sensation. It is freely soluble in ethanol (95), in diethyl ether and in carbon disulfide, and slightly soluble in water. It slowly volatilizes at room temperature.

**Identification**  
Dissolve 0.1 g of *d*-Camphor in 2 mL of methanol, add 1 mL of 2,4-dinitrophenylhydrazine TS, and heat for 5 minutes on a water bath: an orange-red precipitate is formed.

**Optical rotation** &lt;2.49&gt;  
[α]D₂₀: +41.0 – +43.0° (5 g, ethanol (95), 50 mL, 100 mm).

**Melting point** &lt;2.60&gt;  
177 – 182°C

**Purity**  
(1) Water—Shake 1.0 g of *d*-Camphor with 10 mL of carbon disulfide: the solution is clear.

(2) Chlorinated compounds—Mix 0.2 g of finely powdered *d*-Camphor with 0.4 g of sodium peroxide in a dried porcelain crucible. Heat the crucible gently by the open flame until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid, and filter the solution into a Nessler tube. Wash the filter paper with three 5-mL portions of hot water, adding acid until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid, and filter the solution into a Nessler tube. Wash the filter paper with three 5-mL portions of hot water, adding acid until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid, and filter the solution into a Nessler tube.

(3) Non-volatile residue—Heat 2.0 g of *d*-Camphor on a water bath until sublimation is complete, then dry the residue at 105°C for 3 hours: the mass of the residue does not exceed 1.0 mg.

**Assay**  
Weigh accurately about 0.1 g each of *d*-Camphor and *d*-Camphor RS, add exactly 5 mL each of the internal standard solution, dissolve in ethanol (99.5) to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 2 μL each of these solutions as directed under Gas Chromatography &lt;2.02&gt; according to the following conditions, and calculate the ratios, \( Q_d \) and \( Q_s \), of the peak area of *d*-camphor to that of the internal standard.

\[ \text{Amount (mg) of } C_{10}H_{16}O = M_s \times Q_s/Q_d \]

\( M_s \): Amount (mg) of *d*-Camphor RS

**Internal standard solution**—A solution of methyl salicylate in ethanol (99.5) (1 in 25).

**Operating conditions**—  
Detector: A hydrogen flame-ionization detector.  
Column: A glass column 3 mm in inside diameter and 3 m in length, which is packed with 10% of polyethylene glycol 20 M for gas chromatography supported on 180 to 250 μm mesh silanized siliceous earth for gas chromatography.

**Column temperature**: A constant temperature of about 160°C.

**Carrier gas**: Nitrogen

**Flow rate**: Adjust the flow rate so that the retention time of *d*-camphor is about 6 minutes.

**System suitability**—  
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, *d*-camphor and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of *d*-camphor to that of the internal standard is not more than 1.0%.

**Containers and storage**  
Containers—Tight containers.

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**dl-Camphor**

**Synthetic Camphor**

\[ C_{10}H_{16}O: 152.23 \]

(1RS,4RS)-1,7,7-Trimethylbicyclo[2.2.1]-heptan-2-one [76-22-2]

*dl*-Camphor contains not less than 96.0% of 
\[ C_{10}H_{16}O. \]

**Description**  
*dl*-Camphor occurs as colorless or white, translucent crystals, crystalline powder or masses. It has a characteristic, agreeable odor, and has a slightly bitter taste followed by a pleasant, cooling sensation. It is freely soluble in ethanol (95), in diethyl ether and in carbon disulfide, and slightly soluble in water. It slowly volatilizes at room temperature.

**Identification**  
Dissolve 0.1 g of *dl*-Camphor in 2 mL of methanol, add 1 mL of 2,4-dinitrophenylhydrazine TS, and heat for 5 minutes on a water bath: an orange-red precipitate is formed.

**Optical rotation** &lt;2.49&gt;  
[α]D₂₀: −1.5 – +1.5° (5 g, ethanol (95), 50 mL, 100 mm).

**Melting point** &lt;2.60&gt;  
175 – 180°C
Candesartan Cilexetil

**C₁₃H₁₂N₂O₆: 610.66**
(1RS)-1-(Cyclohexyloxycarbonyloxy)ethyl-2-ethoxy-1-[[2’-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-1H-benzo[δ]imidazole-7-carboxylate

Candesartan Cilexetil contains not less than 99.0% and not more than 101.0% of C₁₃H₁₂N₂O₆, calculated on the anhydrous basis.

**Description**
Candesartan Cilexetil occurs as white crystals or a white crystalline powder.

It is soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Candesartan Cilexetil in methanol (1 in 100) shows no optical rotation.

**Identification**
(1) Determine the absorption spectrum of a solution of Candesartan Cilexetil in methanol (1 in 50,000) as directed under Infrared Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Candesartan Cilexetil as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

If any difference appears between the spectra, recrystallize the sample and the RS according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

**Purity**
(1) Heavy metals <1.07>—Proceed with 1.0 g of Candesartan Cilexetil according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Candesartan Cilexetil in 50 mL of a mixture of acetonitrile and water (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.21 according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks, having the relative retention time of about 0.4 and about 2.0 to Candesartan Cilexetil, obtained from the sample solution is not
larger than 1/5 times the peak area of candesartan cilexetil from the standard solution, the area of the peak, having the relative retention time of about 0.5, from the sample solution is not larger than 3/10 times the peak area of candesartan cilexetil from the standard solution, the area of the peak other than candesartan cilexetil and the peaks mentioned above is not smaller than 1/10 times the peak area of candesartan cilexetil from the standard solution, and the total area of the peaks other than candesartan cilexetil from the sample solution is not larger than 3/5 times the peak area of candesartan cilexetil from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of acetonitrile, water and acetic acid (100) (57:43:1).

Mobile phase B: A mixture of acetonitrile, water and acetic acid (100) (90:10:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
</tbody>
</table>

Flow rate: 0.8 mL per minute.

Time span of measurement: For 30 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of acetonitrile and water (3:2) to make exactly 20 mL. Confirm that the peak area of candesartan cilexetil obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 12,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water <2.48> Not more than 0.3% (0.5 g, coulometric titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Candesartan Cilexetil, dissolve in 60 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 61.07 mg of C33H34N6O6

Containers and storage Containers—Well-closed containers.

Candesartan Cilexetil Tablets

Candesartan Cilexetil Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of candesartan cilexetil (C33H34N6O6: 610.66).

Method of preparation Prepare as directed under Tablets, with Candesartan Cilexetil.

Identification Pulverize Candesartan Cilexetil Tablets. To a portion of the powder, equivalent to 1 mg of Candesartan Cilexetil according to the labeled amount, add 50 mL of methanol, shake vigorously for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits its absorption maxima between 252 nm and 256 nm and between 302 nm and 307 nm.

Purity Related substances—Weigh the mass of not less than 10 Candesartan Cilexetil Tablets, and powder. To a portion of the powder, equivalent to 6 mg of Candesartan Cilexetil according to the labeled amount, add 15 mL of a mixture of acetonitrile and water (3:2), shake vigorously for 10 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 0.5 to candesartan cilexetil obtained from the sample solution is not larger than 1.5 times the peak area of candesartan cilexetil from the standard solution, the area of the peak having the relative retention time of about 0.8, about 1.1 and about 1.5 to candesartan cilexetil is not larger than 1/2 times the peak area of candesartan cilexetil from the standard solution, the area of the peak having the relative retention time of about 2.0 from the sample solution is not larger than the peak area of candesartan cilexetil from the standard solution, and the total area of the peaks other than candesartan cilexetil from the sample solution is not larger than 4 times the peak area of candesartan cilexetil from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.
25°C.

Mobile phase A: A mixture of acetonitrile, water and acetic acid (100) (57:43:1).

Mobile phase B: A mixture of acetonitrile, water and acetic acid (100) (90:10:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

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</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
</tbody>
</table>

Flow rate: 0.8 mL per minute.

Time span of measurement: For 30 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of acetonitrile and water (3:2) to make exactly 20 mL. Confirm that the peak area of candesartan cilexetil with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 12,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Candesartan Cilexetil Tablets add 30 mL of a mixture of acetonitrile and water (3:2), shake vigorously for 20 minutes, then add a mixture of acetonitrile and water (3:2) to make exactly V mL so that each mL contains about 40 μg of candesartan cilexetil (C23H34N6O6), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of candesartan cilexetil for assay (separately determine the water <2.48> in the same manner as Candesartan Cilexetil), and dissolve in acetonitrile to make exactly 50 mL. Pipet 4 mL of this solution, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A5, of the sample solution and standard solution at 305 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Amount (mg) of candesartan cilexetil (C23H34N6O6)} = M_S \times A_1/A_5 \times V/1250
\]

\[
M_S: \text{Amount (mg) of candesartan cilexetil for assay, calculated on the anhydrous basis}
\]

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution of polysorbate 20 (1 in 100) as the dissolution medium, the dissolution rate in 45 minutes of Candesartan Cilexetil Tablets is not less than 75%.

Start the test with 1 tablet of Candesartan Cilexetil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V’ mL so that each mL contains about 2.2 μg of candesartan cilexetil (C23H34N6O6) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of candesartan cilexetil for assay (separately determine the water <2.48> in the same manner as Candesartan Cilexetil), and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add acetonitrile to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07>, and determine the peak areas, A1 and A5, of candesartan cilexetil of both solutions.

\[
\text{Dissolution rate (％) with respect to the labeled amount of candesartan cilexetil (C23H34N6O6)} = M_S \times A_1/A_5 \times V'/V \times 1/C \times 18/5
\]

\[
M_S: \text{Amount (mg) of candesartan cilexetil for assay, calculated on the anhydrous basis}
\]

C: Labeled amount (mg) of candesartan cilexetil (C23H34N6O6) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Candesartan Cilexetil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 6 mg of candesartan cilexetil (C23H34N6O6), add exactly 15 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 150 mL, shake vigorously for 10 minutes, and allow to stand. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of candesartan cilexetil for assay (separately determine the water <2.48> in the same manner as Candesartan Cilexetil), dissolve in acetonitrile to make exactly 50 mL. Pipet 4 mL of this solution, add exactly 10 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Qo and Qs, of the peak area of candesartan cilexetil to that of the internal standard.
Amount (mg) of candesartan cilexetil \((C_{33}H_{34}N_6O_6)\)

\[ M_s = \frac{M_s \times Q_T \times Q_s}{3/25} \]

Candesartan cilexetil for assay, calculated on the anhydrous basis

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 800).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (57:43:1).

Flow rate: Adjust the flow rate so that the retention time of candesartan cilexetil is about 13 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and candesartan cilexetil are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of candesartan cilexetil to that of the internal standard is not more than 1.0%.

Containers and storage—Tight containers.

Candiesartan cilexetil

Candiesartan cilexetil for assay, calculated on the anhydrous basis.

Candesartan cilexetil for assay, calculated on the anhydrous basis.

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Candesartan cilexetil for assay, calculated on the anhydrous basis.
Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography \(<2.0D\rangle\) according to the following conditions, and determine the peak area, \(A_F\) and \(A_S\), of 1,1’-[3,3’-dithiobis(2-methyl-1-oxopropyl)]-L-diproline of these solutions: \(A_F\) is not larger than \(A_S\).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Flow rate: Adjust the flow rate so that the retention time of 1,1’-[3,3’-dithiobis(2-methyl-1-oxopropyl)]-L-diproline is about 10 minutes.

System performance—Dissolve 25 mg each of Captopril and 1,1’-[3,3’-dithiobis(2-methyl-1-oxopropyl)]-L-diproline in 200 mL of methanol. When the procedure is run with 20 μL of this solution under the above operating conditions, captopril and 1,1’-[3,3’-dithiobis(2-methyl-1-oxopropyl)]-L-diproline are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of 1,1’-[3,3’-dithiobis(2-methyl-1-oxopropyl)]-L-diproline is not more than 2.0%.

**Loss on drying <2.4I>** Not more than 1.0% (1 g, in vacuum, 80°C, 3 hours).

**Residue on ignition <2.4I>** Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.3 g of Captopril, dissolve in 100 mL of water, add 20 mL of dilute sulfuric acid and 1 g of potassium iodide, and shake. Titrate \(<2.50\rangle\) with 1/60 mol/L potassium iodate VS (indicator: 2 mL of starch TS). Perform a blank determination in the same manner, and determine the peak area, and leaves a slightly bitter aftertaste.

It is freely soluble in chloroform, sparingly soluble in ethanol (95) and in acetone, and very slightly soluble in water and in diethyl ether.

**Identification (1)** To 0.1 g of Carbamazepine add 2 mL of nitric acid, and heat on a water bath for 3 minutes: an orange-red color is produced.

(2) To 0.1 g of Carbamazepine add 2 mL of sulfuric acid, and heat on a water bath for 3 minutes: a yellow color is produced with a green fluorescence.

(3) Examine Carbamazepine under ultraviolet light: the solution shows an intense blue fluorescence.

(4) Determine the absorption spectrum of the solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point <2.60>** 189 – 193°C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Carbamazepine in 10 mL of chloroform: the solution is clear and colorless to pale yellow.

(2) Acidity—To 2.0 g of Carbamazepine add exactly 40 mL of water, stir well for 15 minutes, and filter through a glass filter (G3). To 10 mL of this filtrate add 1 drop of phe- nolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color is produced.

(3) Alkalinity—To 10 mL of the filtrate obtained in (2) add 1 drop of methyl red TS and 0.50 mL of 0.01 mol/L hydrochloric acid VS: a red color is produced.

(4) Chloride \(<1.03\rangle—\)Dissolve 0.25 g of Carbamazepine in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.20 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(5) Heavy metals \(<1.07\rangle—\)Proceed with 2.0 g of Carbamazepine according to Method 2, and perform the test. Prepared the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Related substances—Dissolve 0.25 g of Carbamazepine in 10 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 5.0 mg of iminodibenzyln in chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.0D\rangle\). Spot 10 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the plate: the spots other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying <2.4I>** Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition <2.4I>** Not more than 0.1% (1 g).

**Assay** Dissolve about 50 mg of Carbamazepine, previously
dried and accurately weighed, in ethanol (95) to make exactly 250 mL. Pipet 5 mL of this solution and add ethanol (95) to make exactly 100 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.4 \), and determine the absorbance \( A \) of this solution at the wavelength of maximum absorption at about 285 nm.

\[
\text{Amount (mg) of } C_{16}H_{14}N_2O_5 = \frac{A}{490} \times 50,000
\]

Containers and storage  Containers—Tight containers.

### Carbazochrome Sodium Sulfonate Hydrate

カルバゾクロムスルホン酸ナトリウム水和物

\[
\text{C}_{10}\text{H}_{11}\text{N}_{4}\text{NaO}_{5}\text{S} \cdot 3\text{H}_{2}\text{O}: 376.32
\]

Monosodium (2RS)-1-methyl-6-oxo-5-semicarbazono-2,3,5,6-tetrahydroindole-2-sulfonate trihydrate

[51460-26-5, anhydride]

Carbazochrome Sodium Sulfonate Hydrate contains not less than 98.0% and not more than 102.0% of carbazochrome sodium sulfonate \( (C_{16}H_{14}N_2O_5S) : 322.27 \), calculated on the anhydrous basis.

**Description**  Carbazochrome Sodium Sulfonate Hydrate occurs as orange-yellow, crystals or crystalline powder. It is sparingly soluble in water, very slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Carbazochrome Sodium Sulfonate (1 in 100) shows no optical rotation.

Melting point: about 210°C (with decomposition).

**Identification (1)**  Determine the absorption spectrum of a solution of Carbazochrome Sodium Sulfonate Hydrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.24 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)  Determine the infrared absorption spectrum of Carbazochrome Sodium Sulfonate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry \( \leq 2.25 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3)  A solution of Carbazochrome Sodium Sulfonate Hydrate (1 in 100) responds to the Qualitative Tests \( \leq 1.09 \) (1) for sodium salt.

**pH \( \leq 2.54 \)**  Dissolve 0.8 g of Carbazochrome Sodium Sulfonate Hydrate in 50 mL of water by warming, and cool: the pH of this solution is between 5.0 and 6.0.

**Purity (1)**  Clarity of solution—Dissolve 1.0 g of Carbazochrome Sodium Sulfonate Hydrate in 50 mL of water by warming, and allow to cool: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.4 \): the absorbance at 590 nm is not more than 0.070.

(2)  Heavy metals \( \leq 1.07 \)—Proceed with 1.0 g of Carbazochrome Sodium Sulfonate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3)  Related substances—Dissolve 50 mg of Carbazochrome Sodium Sulfonate Hydrate in 100 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( \leq 2.01 \) according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than the peak of carbazochrome sulfonate from the sample solution is not larger than the peak area of carbazochrome sulfonate from the standard solution.

**Operating conditions—**  
   - Detector: An ultraviolet absorption photometer (wavelength: 360 nm).
   - Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 \( \mu \)m in particle diameter).
   - Column temperature: A constant temperature of about 40°C.
   - Mobile phase: Dissolve 1.2 g of ammonium dihydrogen phosphate in 1000 mL of water, and filter through a membrane filter (0.4 \( \mu \)m in pore size) if necessary. To 925 mL of this solution add 75 mL of ethanol (95), shake, and adjust the pH to 3 with phosphoric acid.
   - Flow rate: Adjust the flow rate so that the retention time of carbazochrome sulfonate is about 7 minutes.
   - Time span of measurement: About 3 times as long as the retention time of carbazochrome sulfonate beginning after the solvent peak.

**System suitability—**  
   - Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of carbazochrome sulfonate obtained from 10 \( \mu \)L of this solution is equivalent to 7 to 13% of that of carbazochrome sulfonate obtained from 10 \( \mu \)L of the standard solution.
   - System performance: Dissolve 10 mg each of Carbazochrome Sodium Sulfonate and carbazochrome in 100 mL of water by warming. When the procedure is run with 10 \( \mu \)L of this solution under the above operating conditions, carbazochrome sulfonate and carbazochrome are eluted in this order with the resolution between these peaks being not less than 3.

**System repeatability:**  When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carbazochrome sulfonate is not more than 2.0%.

**Water \( \leq 2.48 \) 13.0 – 16.0% (0.3 g, volumetric titration, direct titration).

**Assay**  Weigh accurately about 0.25 g of Carbazochrome Sodium Sulfonate Hydrate, dissolve in 50 mL of water, apply to a chromatographic column, 10 mm in diameter, previously prepared with 20 mL of strongly acidic ion ex-
change resin for column chromatography (type H), and allow to flow at a rate of 4 mL per minute. Wash the column with 150 mL of water, combine the washing and the former effluent solution, and titrate \(<2.5\times\) with 0.05 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L sodium hydroxide VS = 16.11 mg of \(\text{C}_6\text{H}_4\text{N}_2\text{NaO}_5\text{S}\)

Containers and storage Containers—Well-closed containers.

**Carbidopa Hydrate**

![Chemical structure of Carbidopa Hydrate](image)

\(\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_4\cdot\text{H}_2\text{O}: 244.24\)

(2S)-2-(3,4-Dihydroxybenzyl)-2-hydrazinopropanoic acid monohydrate

[38821-49-7]

Carbidopa Hydrate contains not less than 98.0% of \(\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_4\cdot\text{H}_2\text{O}\).

**Description** Carbidopa Hydrate occurs as a white to yellowish white powder.

It is sparingly soluble in methanol, slightly soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: About 197°C (with decomposition).

**Identification (1)** Dissolve 0.01 g of Carbidopa Hydrate in 250 mL of a solution of hydrochloric acid in methanol (9 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \(<2.2\times\) at the wavelengths between 240 nm and 300 nm, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Carbidopa RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carbidopa Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.2\times\), and compare the spectrum with the reference spectrum of the spectrum of Carbidopa RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \(<2.4\times\) \([\alpha]_D^{20}\): \(-21.0\) to \(-23.5\)° (1 g, aluminum (III) chloride TS, 100 mL, 100°C).

**Purity (1)** Heavy metals \(<1.07\)—Proceed with 2.0 g of Carbidopa Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Carbidopa Hydrate in 70 mL of the mobile phase, by warming and using ultrasonication, if necessary. After cooling, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.0\times\) according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the total area of all peaks other than the peak of carbidopa from the sample solution is not larger than the peak area of carbidopa from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of carbidopa.

**System suitability**—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of carbidopa obtained from 20 \(\mu\)L of this solution is equivalent to 7 to 13% of that of carbidopa obtained from 20 \(\mu\)L of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

**Loss on drying** \(<2.4\times\) 6.9 – 7.9% (1 g, in vacuum not exceeding 0.67 kPa, 100°C, 6 hours).

**Residue on ignition** \(<2.4\times\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 50 mg each of Carbidopa Hydrate and Carbidopa RS (determine separately the loss on drying \(<2.4\times\) in the same manner as Carbidopa Hydrate), and dissolve each in 70 mL of the mobile phase, by warming and using ultrasonication if necessary. After cooling, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.0\times\) according to the following conditions, and determine the peak areas, \(A_1\) and \(A_5\), of carbidopa in each solution.

Amount (mg) of \(\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_4\cdot\text{H}_2\text{O}\) = \(M_5\times \frac{A_1}{A_5}\times 1.080\)

\(M_5\): Amount (mg) of Carbidopa RS, calculated on the dried basis

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 950 mL of 0.05 mol/L sodium dihydrogen phosphate TS add 50 mL of ethanol (95), and adjust the pH to 2.7 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of carbidopa is about 6 minutes.

**System suitability**—

System performance: Dissolve 50 mg each of Carbidopa and methyldopa in 100 mL of the mobile phase. When the procedure is run with 20 \(\mu\)L of this solution under the above operating conditions, methyldopa and carbidopa are eluted
in this order with the resolution between these peaks being not less than 0.9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carbodiopa is not more than 1.0%.

Containers and storage  Containers—Tight containers.

Storage—Light-resistant.

### L-Carbocisteine

L-カルボシステイン

\[
C_5H_9NO_4S: 179.19
\]

(2R)-2-Amino-3-carboxymethylsulfanylpropanoic acid

[C5H9NO4S]-2-Amino-3-carboxymethylsulfanylpropanoic acid

L-Carbocisteine, when dried, contains not less than 98.5% of C5H9NO4S.

Description  L-Carbocisteine occurs as a white crystalline powder. It is odorless, and has a slightly acid taste.

It is very slightly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid or in sodium hydroxide TS.

Melting point: about 186°C (with decomposition).

Identification (1)  To 0.2 g of L-Carbocisteine add 1 mL of lead acetate TS and 3 mL of water, shake, add 0.2 g of sodium hydroxide, and heat over a flame for 1 minute: a dark brown to black precipitate is formed.

(2)  Determine the infrared absorption spectrum of L-Carbocisteine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49>  \([\alpha]_D^{20} = -33.5 \sim -36.5^\circ\) Weigh accurately about 5 g of L-Carbocisteine, previously dried, dissolve in 20 mL of water and a suitable amount of a solution of sodium hydroxide (13 in 100), and adjust the pH with 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS to 6.0, and add water to make exactly 50 mL. Determine the optical rotation of this solution in a 100-mm cell.

Purity (1)  Clarity and color of solution—Dissolve 1.0 g of L-Carbocisteine in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2)  Chloride <1.03>—Dissolve 0.20 g of L-Carbocisteine in 10 mL of water and 20 mL of nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.40 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of nitric acid and water to make 50 mL (not more than 0.071%).

(3)  Ammonium <1.02>—Perform the test with 0.25 g of L-Carbocisteine using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4)  Heavy metals <1.07>—Proceed with 1.0 g of L-Carbocisteine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5)  Arsenic <1.17>—Prepare the test solution with 1.0 g of L-Carbocisteine according to Method 3, and perform the test (not more than 2 ppm).

(6)  Related substances—Dissolve 0.30 g of L-Carbocisteine in 10 mL of 0.2 mol/L sodium hydroxide TS, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add 0.2 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 1 mL of this solution, and add 0.2 mol/L sodium hydroxide TS to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution, in 15 mm length along the starting line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.47>  Not more than 0.30% (1 g, 105°C, 2 hours).

Residue on ignition <2.44>  Not more than 0.1% (1 g).

Assay  Weigh accurately about 0.25 g of L-Carbocisteine, previously dried, dissolve in exactly 20 mL of 0.1 mol/L perchloric acid VS and 50 mL of acetic acid (100), and titrate <2.30> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 17.92 mg of C5H9NO4S

Containers and storage  Containers—Tight containers.

## Carbon Dioxide

二酸化炭素

CO₂: 44.01

[124-38-9]

Carbon Dioxide contains not less than 99.5 vol% of CO₂.

Description  Carbon Dioxide is a colorless gas at room temperature and under atmospheric pressure. It is odorless.

A 1 mL volume of Carbon Dioxide dissolves in 1 mL of water, and the solution is slightly acid.

1000 mL of Carbon Dioxide at 0°C and under a pressure of 101.3 kPa weighs about 1.978 g.

Identification (1)  Pass 100 mL of Carbon Dioxide through a carbon dioxide measuring detector tube: the detector tube is changed to a stipulated color tone by each detector tube, provided that the detector tube with a upper limit of measurement of not less than 10% is used.
(2) Pass Carbon Dioxide into calcium hydroxide TS: a white precipitate is produced. Collect the precipitate, and add acetic acid (31): it dissolves with effervescence.

**Purity (1)** Acidity—Place 50 mL of freshly boiled and cooled water in a Nessler tube, and pass 1000 mL of Carbon Dioxide into it for 15 minutes through an introducing tube about 1 mm in diameter extending to 2 mm from the bottom of the Nessler tube, then add 0.10 mL of methyl orange TS: the solution is not more colored than the following control solution.

Control solution: To 50 mL of freshly boiled and cooled water in a Nessler tube add 0.10 mL of methyl orange TS and 1.0 mL of 0.01 mol/L hydrochloric acid VS.

(1) Hydrogen phosphate, hydrogen sulfide or reducing organic substances—Place 25 mL of silver nitrate-ammonia TS and 3 mL of ammonia TS in each of two Nessler tubes A and B, and designate the solution in each tube as solution A and solution B, respectively. Pass 1000 mL of Carbon Dioxide into solution A in the same manner as directed in (1): the turbidity and color of this solution are the same as that of solution B.

(3) Carbon monoxide—Pass a specified amount of Carbon Dioxide through a carbon monoxide measuring detector tube: the concentration of carbon monoxide is less than 15 ppm, provided that the passing amount (mL) of Carbon Dioxide is stipulated according to each detector tube.

**Assay** Place 125 mL of a solution of potassium hydroxide (1 in 2) in a gas pipet of suitable capacity. Measure exactly about 100 mL of Carbon Dioxide in a 100-mL gas buret filled with water. Force the entire volume of gas into the gas pipet, and shake for 5 minutes. Draw some of the unabsorbed gas into the gas buret, measure the volume, force the residual back upon the surface of the liquid in the gas pipet, and repeat this procedure until a constant volume of the residual reading is obtained. Determine the volume $V_1$ (mL) of the residual gas. Calculate the volume of the sample and $V_2$ on the basis of the gas volume at 20°C and at 101.3 kPa.

$$\text{Volume (mL) of CO}_2 = \text{volume (mL) of the sample} - V_1 (\text{mL})$$

**Containers and storage** Containers—Cylinders. Storage—Not exceeding 40°C.

## Carmellose

### Carboxymethylcellulose

カルメロース

[9000-11-7]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (•).°

Carmellose is a carboxymethyl ether of cellulose.

•**Description** Carmellose occurs as a white powder. It is practically insoluble in ethanol (95). It swells with water to form suspension. It becomes viscid in sodium hydroxide TS. It is hygroscopic.

**Identification (1)** Determine the infrared absorption spectrum of Carmellose as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) The pH <2.54> of a suspension, obtained by shaking 1 g of Carmellose with 100 mL of water, is between 3.5 and 5.0.

**Purity (1)** Chloride <1.03>—Shake well 0.8 g of Carmellose with 50 mL of water, dissolve in 10 mL of sodium hydroxide TS, and add water to make 100 mL. Heat 20 mL of this solution with 10 mL of dilute nitric acid on a water bath until a flocculent precipitate is produced, cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant liquid and the washings, and add water to make 100 mL. Take 25 mL of this solution, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.36%).

(2) Sulfate <1.14>—Shake well 0.40 g of Carmellose with 25 mL of water, dissolve in 5 mL of sodium hydroxide TS, and add 20 mL of water. Heat this solution with 2.5 mL of hydrochloric acid in a water bath until a flocculent precipitate is produced. Cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant liquid and the washings, and add water to make 100 mL. Filter this solution, discard 5 mL of the first filtrate, take 25 mL of the subsequent filtrate, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.5 mL of 0.005 mol/L sulfuric acid VS (not more than 0.72%).

•**Heavy metals** <1.07>—Proceed with 1.0 g of Carmellose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <2.41> Not more than 8.0% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 1.5% (after drying, 1 g).

•**Containers and storage** Containers—Tight containers.

## Carmellose Calcium

### Carboxymethylcellulose Calcium

カルメロースカルシウム

[9050-04-8]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (•).°
Carmellose Calcium is the calcium salt of a polycarboxymethyl ether of cellulose.

**Description** Carmellose Calcium occurs as a white to yellowish white powder. It is practically insoluble in ethanol (95%) and in diethyl ether. It swells with water to form a suspension. The pH of a suspension, obtained by shaking 1.0 g of Carmellose Calcium with 100 mL of water, is between 4.5 and 6.0.

It is hygroscopic.

**Identification (1)** Shake thoroughly 0.1 g of Carmellose Calcium with 10 mL of water, followed by 2 mL of sodium hydroxide TS, allow to stand for 10 minutes, and use this solution as the sample solution. To 1 mL of the sample solution add water to make 5 mL. To 1 drop of this solution add 0.5 mL of chromotropic acid TS, and heat in a water bath for 10 minutes: a red-purple color develops.

(2) Shake 5 mL of the sample solution obtained in (1) with 10 mL of acetone: a white, flocculent precipitate is produced.

(3) Shake 5 mL of the sample solution obtained in (1) with 1 mL of iron (III) chloride TS: a brown, flocculent precipitate is produced.

(4) Ignite 1 g of Carmellose Calcium to ash, dissolve the residue in 10 mL of water and 6 mL of acetic acid (31), and filter, if necessary. Boil the filtrate, cool, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> (1) and (3) for calcium salt.

**Purity (1)** Alkalinity—Shake thoroughly 1.0 g of Carmellose Calcium with 50 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS: no red color develops.

(2) Chloride <1.03>—Shake thoroughly 0.80 g of Carmellose Calcium with 50 mL of water, add 10 mL of sodium hydroxide TS to dissolved, add water to make 100 mL, and use this solution as the sample solution. Heat 20 mL of the sample solution with 10 mL of 2 mol/L nitric acid TS on a water bath until a flocculent precipitate is produced. After cooling, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant and the washings, and add water to make 100 mL. Take 25 mL of this solution, and add 1 mL of nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.36%).

(3) Sulfate <1.14>—Heat 10 mL of the sample solution obtained in (2) with 1 mL of hydrochloric acid in a water bath until a flocculent precipitate is produced. Cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant and the washings, and add water to make 100 mL. Perform the test with 25 mL this solution as the test solution. Prepare the control solution with 0.42 mL of 0.005 mol/L sulfuric acid VS. To the test solution and the control solution add 1 mL of 3 mol/L hydrochloric acid TS and 3 mL of barium chloride TS, then add water to make 50 mL, and mix. Allow to stand for 10 minutes, and compare the turbidity of these solutions: the turbidity obtained with the test solution is not more than that obtained with the control solution (not more than 1.0%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Carmellose Calcium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying <2.41>** Not more than 10.0% (1 g, 105°C, 4 hours).

**Residue on ignition <2.44>** 10 - 20% (after drying 1 g).

**Containers and storage** Containers—Tight containers.

**Carmellose Sodium**

**Carboxymethylcellulose Sodium**

カルメロースナトリウム

**[9004-32-4]**

Carmellose Sodium is the sodium salt of a polycarboxymethyl ether of cellulose.

It, when dried, contains not less than 6.5% and not more than 8.5% of sodium (Na: 22.99).

**Description** Carmellose Sodium occurs as a white to yellowish white powder or granules. It has no taste. It is practically insoluble in methanol, in ethanol (95), in acetic acid (100) and in diethyl ether. It forms a viscous solution in water and in warm water.

It is hygroscopic.

**Identification (1)** Dissolve 0.2 g of Carmellose Sodium in 20 mL of warm water with stirring, cool, and use this solution as the sample solution. To 1 mL of the sample solution add water to make 5 mL. To 1 drop of this solution add 0.5 mL of concentrated chromotropic acid TS, and heat in a water bath for 10 minutes: a red-purple color develops.

(2) To 10 mL of the sample solution obtained in test (1) add 1 mL of copper (II) sulfate TS: a blue flocculent precipitate is produced.

(3) To 3 g of Carmellose Sodium add 20 mL of methanol and 2 mL of dilute hydrochloric acid, boil gently on a water bath for 5 minutes, and filter. Evaporate the filtrate to dryness, and add 20 mL of water to the residue: the solution responds to the Qualitative Tests <1.09> for sodium salt.

**pH <2.54>** Add 1.0 g of Carmellose Sodium in small portions to 100 mL of warm water with stirring, dissolve, and cool: the pH of this solution is between 6.0 and 8.0.

**Purity (1)** Clarity and color of solution—Firmly attach a glass plate of good quality 2 mm in thickness, to the bottom of a glass column 250 mm in height, 25 mm in inner diameter and 2 mm in thickness. This is used as an outer tube. Similarly prepare an inner tube by attaching a glass plate of good quality 2 mm in thickness to the bottom of a glass column 300 mm in height, 15 mm in inner diameter and 2 mm in thickness. Dissolve 1.0 g of Carmellose Sodium in 100 mL of water, pour this solution into the outer tube, and place on a piece of white paper on which 15 parallel black lines 1 mm in width and 1 mm in interval are drawn. Moving the inner tube up and down and observing from the upper part, deter-
mine the height of the solution up to the lower edge of the inner tube when the distinction of the lines becomes impossible. Repeat the operation 3 times, and calculate the mean value: it is larger than that calculated from the similar operation, using the following control solution.

Control solution: To 5.50 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid, 5 mL of ethanol (95) and water to make 50 mL. Add 2 mL of barium chloride TS, mix well, and allow to stand for 10 minutes. SHAKE WELL this solution before use.

(2) Chloride <1.0%>—Dissolve 0.5 g of Carmellose Sodium in 50 mL of water, and use this solution as the sample solution. SHAKE 10 mL of the sample solution with 10 mL of dilute nitric acid, heat to produce a flocculent precipitate in a water bath, cool, and centrifuge. Separate the supernatant liquid, wash the precipitate with three 10-mL portions of water, centrifuging each time, combine the supernatant liquid and washings, and dilute with water to 200 mL. PERFORM THE TEST using 50 mL of this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.640%).

(3) Sulfate <1.14>—Add 1 mL of hydrochloric acid to 10 mL of the sample solution obtained in (2), shake well, heat to produce a flocculent precipitate in a water bath, cool, and centrifuge. Separate the supernatant liquid, wash the precipitate with three 10-mL portions of water, centrifuging each time, combine the washings with the supernatant liquid mentioned above, and dilute to 50 mL with water. TAKE 10 mL of this solution, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.960%).

(4) Silicate—WEIGH accurately about 1 g of Carmellose Sodium, ignite in a platinum dish, add 20 mL of dilute hydrochloric acid, cover with a watch glass, and boil gently for 30 minutes. Remove the watch glass, and evaporate on a water bath to dryness with the aid of a current of air. Continue heating for further 1 hour, add 10 mL of hot water, stir well, and filter through a filter paper for qualitative analysis. Wash the residue with hot water, dry together with the filter paper after no turbidity is produced on the addition of silver nitrate with three 10-mL portions of water, centrifuging each time, combine the supernatant liquid and washings, and filter through a filter paper for quantitative analysis. Prepare the test solution (not more than 10 ppm).

(5) Heavy metals <1.07>—Proceed with 1.0 g of Carmellose Sodium according to Method 2, and perform the test. PREPARE THE CONTROL SOLUTION with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.17>—To 1.0 g of Carmellose Sodium add 20 mL of nitric acid, heat gently until it becomes fluid, cool, add 5 mL of sulfuric acid, and heat until white fumes are evolved. ADD, if necessary, 5 mL of nitric acid after cooling, and heat again. Repeat this operation until the solution becomes colorless or slightly yellow. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved again, cool, and dilute with water to 25 mL. TAKE 5 mL of this solution as the test solution, and perform the test. The solution has no more color than the following color standard.

Color standard: Without using Carmellose Sodium, proceed in the same manner, then transfer 5 mL of this solution to a generator bottle, add exactly 2 mL of Standard Arsenic Solution, and proceed as directed for the test with the test solution (not more than 10 ppm).

(7) Starch—Add 2 drops of iodine TS to 10 mL of the sample solution obtained in (2): no blue color develops.

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.5 g of Carmellose Sodium, previously dried, add 80 mL of acetic acid (100), connect with a reflux condenser, and heat in an oil bath maintained at 130°C for 2 hours. Cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). PERFORM A BLANK DETERMINATION, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 2.299 mg of Na.

Containers and storage Containers—Tight containers.

Carmofur

カルモフール

C_{11}H_{16}FN_{3}O_{3}: 257.26
5-Fluoro-1-(hexylaminocarbonyl)uracil
[61422-45-5]

Carmofur, when dried, contains not less than 98.0% of C_{11}H_{16}FN_{3}O_{3}.

Description Carmofur occurs as a white crystalline powder.

It is very soluble in N,N-dimethylformamide, freely soluble in acetic acid (100), soluble in diethyl ether, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point: about 111°C (with decomposition).

Identification (1) Proceed with 5 mg of Carmofur as directed under Oxygen Flask Combustion Method <1.09>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid, and prepare the test solution: the test solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Carmofur in a mixture of methanol and phosphoric acid-acetic acid-boric acid buffer solution, pH 2.0, (9:1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Carmofur, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of
Carmofur according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.02 g of Carmofur in 10 mL of a mixture of methanol and acetic acid (100:99:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and acetic acid (100:99:1) to make exactly 500 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 15 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene and acetone (5:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. After exposure of the plate to bromine vapor for 30 second, spray evenly a solution of fluorescein in ethanol (95) (1 in 2500): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 50°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Carmofur, previously dried, dissolve in 20 mL of N,N-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide-methanol VS until the color of the solution changes from yellow through blue-green to blue (indicator: 3 drops of thymol blue-dimethylformamide TS).

Each mL of 0.1 mol/L tetramethylammonium hydroxide-methanol VS = 25.73 mg of C11H16FN3O3

Containers and storage Containers—Well-closed containers.

Carnauba Wax

Cera Carnauba

カルナウバロウ

Carnauba Wax is the wax obtained from the leaves of Copernicia cerifera Mart (Palmae).

Description Carnauba Wax occurs as light yellow to light brown, hard and brittle masses or white to light yellow powder. It has a slight, characteristic odor. It is tasteless.

It is practically insoluble in water, in ethanol (95), in diethyl ether and in xylene.

Specific gravity d° 20/40: 0.990 – 1.002

Melting point: 80 – 86°C

Acid value <1.13> Not more than 10.0. Use a mixture of xylene and ethanol (95) (2:1) as solvent.

Saponification value <1.13> 78 – 95 Weigh accurately about 3 g of Carnauba Wax in a 300-mL flask, add 25 mL of xylene, and dissolve by warming. To this solution add 50 mL of ethanol (95) and exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and proceed as directed in the Saponification value. The time of heating should be 2 hours and the titration should be done by warming.

Iodine value <1.13> 5 – 14 (Dissolve the sample by shaking a glass-stoppered flask in warm water.)

Containers and storage Containers—Well-closed containers.

Carteolol Hydrochloride

カルテオロール塩酸塩

Carteolol Hydrochloride occurs as white crystals or crystalline powder.

It is soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of Carteolol Hydrochloride (1 in 100) is between 5.0 and 6.0.

The solution of Carteolol Hydrochloride (1 in 20) shows no optical rotation.

Melting point: about 277°C (with decomposition).

Identification (1) Dissolve 0.1 g of Carteolol Hydrochloride in 5 mL of water, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Carteolol Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Carteolol Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Carteolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Carteolol Hydrochloride in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Carteolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g
of Carteolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Carteolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28:50:20:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Carteolol Hydrochloride, previously dried, add 30 mL of acetic acid (100), and in ethanol (99.5). Weigh accurately about 0.5 g of Carteolol Hydrochloride, and make any necessary correction. Pipet 3 hours).

Containers and storage Containers—Well-closed containers.

Carumonam Sodium
カルモナムナトリウム

\[
\text{C}_{12}\text{H}_{12}\text{N}_{6}\text{Na}_{2}\text{O}_{10}\text{S}_{2} \cdot 466.40
\]

Carumonam Sodium contains not less than 850 μg (potency) and not more than 920 μg (potency) per mg, calculated on the anhydrous basis. The potency of Carumonam Sodium is expressed as mass (potency) of carumonam (\(\text{C}_{12}\text{H}_{14}\text{N}_{6}\text{O}_{10}\text{S}_{2}\cdot 466.40\)).

Description Carumonam Sodium occurs as a white to yellowish white, crystals or crystalline powder. It is freely soluble in water, soluble in formamide, very slightly soluble in methanol, and practically insoluble in acetic acid (100) and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Carumonam Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Carumonam Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carumonam Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Carumonam Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the H spectrum of a solution of Carumonam Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.27>, using sodium-3-trimethylsilylpropionate-d₄ for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal A at around δ 5.5 ppm, and a single signal B at around δ 7.0 ppm. The ratio of the integrated intensity of these signals, A:B, is about 1:1.

(4) Carumonam Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> [α]D²⁺ : +18.5 – +21.0° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Carumonam Sodium in 10 mL of water is between 5.0 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Carumonam Sodium in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Carumonam Sodium according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 15 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Carumonam Sodium according to Method 4, and perform the test (not more than 1 ppm).

(4) Related substance 1—Weigh accurately about 0.1 g of Carumonam Sodium, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Carumonam Sodium RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amount of the related substance having the relative retention time of 0.7 to the peak of carumonam is not more than 4.0%, and each amount of the related substances other than the related substance having the relative retention time of 0.7 to the peak of carumonam is not more than 1.0%.
Amount (%) of related substance

\[ M_s / M_f \times A_f / A_s \]

- **Mₜ**: Amount (g) of Carumonam Sodium RS
- **Mₛ**: Amount (g) of the sample
- **Aₛ**: Peak area of carumonam from the standard solution
- **Aₜ**: Each peak area other than carumonam from the sample solution

**Operating conditions**

- **Detector**: column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
- **Time span of measurement**: About 3 times as long as the retention time of carumonam.

**System suitability**

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of carumonam obtained from 10 µL of this solution is equivalent to 7 to 13% of that from 10 µL of the standard solution.

Operating conditions:

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 10 times as long as the retention time of carumonam.

**System performance**

- **Test for required detectability**: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of carumonam obtained from 10 µL of this solution is equivalent to 7 to 13% of that from 10 µL of the standard solution.
- **System repeatability**: When the test is repeated 3 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carumonam is not more than 2.0%.

**Related substance 2**

Weigh accurately about 0.1 g of Carumonam Sodium, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Carumonam Sodium RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.017) according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related substance is not more than 1.0%.

Amount (%) of related substance

\[ M_s / M_f \times A_f / A_s \]

- **Mₜ**: Amount (mg (potency)) of Carumonam (C₁₂H₁₄N₆O₁₀S₂)
- **Mₛ**: Amount (mg (potency)) of carumonam (C₁₂H₁₄N₆O₁₀S₂)

Flow rate: Dissolve 0.01 g of phthalic acid in the mobile phase to make 100 mL. Adjust the flow rate so that the retention time of phthalic acid is about 6.5 minutes when the procedure is run with 10 µL of this solution.

**Time span of measurement**: About 10 times as long as the retention time of carumonam.

**System suitability**

- **Test for required detectability**: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of carumonam obtained from 10 µL of this solution is equivalent to 7 to 13% of that from 10 µL of the standard solution.

System performance: Dissolve 40 mg of Carumonam Sodium in 20 mL of the mobile phase. To 5 mL of this solution add 5 mL of a solution of resorcinol in the mobile phase (9 in 1000) and the mobile phase to make 25 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, resorcinol and carumonam are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 3 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carumonam is not more than 2.0%.

Total amount of related substances—The total of the amounts of the related substances obtained in the Related substance 1 and the Related substance 2 is not more than 6.0%.

**Water**

Not more than 2.0% (0.2 g, volumetric titration, direct titration; Use a mixture of formamide for water determination and methanol for water determination (3:1) instead of methanol for water determination).

**Assay**

Weigh accurately an amount of Carumonam Sodium and Carumonam Sodium RS, equivalent to about 40 mg (potency), and dissolve each in the mobile phase to make exactly 20 mL. Measure exactly 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.017) according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of carumonam to that of the internal standard.

Amount [µg (potency)] of carumonam (C₁₂H₁₄N₆O₁₀S₂) = \( M_s \times Q_1 / Q_2 \times 1000 \)

- **Mₜ**: Amount (mg (potency)) of Carumonam Sodium RS

**Internal standard solution**—A solution of Carumonam Sodium RS equivalent to about 0.1 g (potency), and dissolve in the mobile phase to make exactly 20 mL. Measure exactly 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.017) according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of carumonam to that of the internal standard.

Amount (mg (potency)) of Carumonam Sodium RS

**Operating conditions**

- **Detector**: An ultraviolet absorption photometer (wavelength: 254 nm).

- **Column**: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

- **Column temperature**: A constant temperature of about 25°C.

- **Mobile phase**: A mixture of a solution of ammonium sulfate (1 in 10,000), methanol and acetic acid (100) (97:2:1).

Flow rate: Adjust the flow rate so that the retention time of
of carumonam is about 10 minutes.

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and carumonam are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of carumonam to that of the internal standard is not more than 1.0%.

**Containers and storage**  Containers—Hermetic containers.

Storage—Light-resistant.

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**Carvedilol**

カルベジロール

![Chemical Structure](https://example.com/structure.png)

C₂₅H₂₆N₂O₄: 406.47

(2RS)-1-[9H-Carbazol-4-yloxy]-3-[[2-(2-methoxyphenoxy)ethyl]amino]propan-2-ol [72956-09-3]

Carvedilol, when dried, contains not less than 99.0% and not more than 101.0% of C₂₅H₂₆N₂O₄.

**Description**  Carvedilol occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Carvedilol in methanol (1 in 100) shows no optical rotation.

**Identification (1)**  Determine the absorption spectrum of a solution of Carvedilol in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Identification (2)**  Determine the infrared absorption spectrum of Carvedilol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point**  <2.60> 114 - 119°C

**Purity (1)**  Heavy metals <1.07>—Wrap 2.0 g of Carvedilol with a filter paper for quantitative analysis, then proceed according to Method 4, and perform the test. Prepare the control solution as follows: Put a filter paper for quantitative analysis in a crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), then proceed as directed for the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 10 ppm).

(2) Related substances—Dissolve 65 mg of Carvedilol in 100 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than carvedilol obtained from the sample solution is not larger than 3/20 times the peak area of carvedilol from the standard solution, and the total area of the peaks other than carvedilol is not larger than 1/2 times the peak area of carvedilol from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 55°C.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 2.0 with phosphoric acid, and add water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of carvedilol is about 4 minutes.

Time span of measurement: About 9 times as long as the retention time of carvedilol, beginning after the solvent peak.

**System suitability—**

Test for required detectability: Pipet 2 mL of the standard solution, add the mobile phase to make exactly 20 mL. Confirm that the peak area of carumonam obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of carvedilol are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carvedilol is not more than 2.0%.

(3) Residual solvent—Being specified separately.

**Loss on drying**  <2.41>  Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition**  <2.44>  Not more than 0.1% (1 g).

**Assay**  Weigh accurately about 0.5 g of Carvedilol, previously dried, dissolve in 60 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 40.65 mg of C₂₅H₂₆N₂O₄

**Containers and storage**  Containers—Tight containers.
Carvedilol Tablets

カルベジロール錠

Carvedilol Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of carvedilol (C₂₄H₂₆N₂O₄: 406.47).

Method of preparation Prepare as directed under Tablets, with Carvedilol.

Identification Powder Carvedilol Tablets. To a portion of the powder, equivalent to 20 mg of Carvedilol according to the labeled amount, add 10 mL of methanol, shake well, and filter. To 0.5 mL of the filtrate add methanol to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \( \lambda_1 \). It exhibits maxima between 222 nm and 226 nm, between 241 nm and 245 nm, between 284 nm and 288 nm, between 317 nm and 321 nm and between 350 nm and 334 nm.

Purity Related substances—In this procedure the sample solution should be stored not exceeding 5°C and used within 24 hours after preparation. Powder Carvedilol Tablets. Dissolve a portion of the powder, equivalent to 12.5 mg of Carvedilol according to the labeled amount, add an adequate amount of the mobile phase and disperse the particles with the aid of ultrasonic waves, if necessary, add the mobile phase to make 100 mL, and shake for 30 minutes. Filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \)m, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( \lambda_2 \) according to the following conditions, and determine each peak area by the automatic integration method: the area of the two peaks, having the relative retention time between 1.7 and 1.9, and between 2.0 and 3.1 with respect to carvedilol, obtained from the sample solution of 1.25-mg or 2.5-mg tablet is not larger than 3/10 times and 4/5 times the area of carvedilol from the standard solution, respectively, the area of the peak other than carvedilol and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of carvedilol from the standard solution, and the total area of the peaks other than carvedilol from the sample solution is not larger than 2.2 times the peak area of carvedilol from the standard solution. The area of the two peaks, having the relative retention time between 1.7 and 1.9 and between 2.0 and 3.1 with respect to carvedilol, obtained from the sample solution of 10-mg or 20-mg tablet is not larger than 1/10 times and 2/5 times the peak area of carvedilol from the standard solution, respectively, the area of the peak other than carvedilol and other than the area mentioned above from the sample solution is not larger than 1/10 times the peak area of carvedilol from the standard solution, and the total area of the peaks other than carvedilol from the sample solution is not larger than 3/5 times the peak area of carvedilol from the standard solution. For these calculations use the area of the peak, having the relative retention time between 1.7 and 1.9, after multiplying by the relative response factor 1.25.

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 10 times as long as the retention time of carvedilol, beginning after the solvent peak.
System suitability—
Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL, and confirm that the peak area of carvedilol obtained with 50 \( \mu \)L of this solution is equivalent to 3.5 to 6.5% of that with 50 \( \mu \)L of the standard solution.
System performance: When the procedure is run with 50 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of carvedilol are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 50 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carvedilol is not more than 1.0%.

Uniformity of dosage units Perform the test according to the following method: it meets the requirement of the Content uniformity test.
To 1 tablet of Carvedilol Tablets add 70 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1), shake until the tablet is completely disintegrated, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \)m. Discard the first 10 mL of the filtrate, and the volume of the subsequent filtrate, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 20 mL so that each mL contains about 5 \( \mu \)g of carvedilol (C₂₄H₂₆N₂O₄), and use this solution as the standard solution. Separately, weigh accurately about 25 mg of carvedilol for assay, previously dried at 105°C for 2 hours, and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_{Sz} \), of the sample solution and standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry \( \lambda_2 \).

Amount (mg) of carvedilol (C₂₄H₂₆N₂O₄)
\[ M_S \times \frac{A_T}{A_{Sz}} \times \frac{V}{V} \times \frac{100}{50} \]
\[ M_S \]: Amount (mg) of carvedilol for assay

Dissolution

10-mg tablet and 20-mg tablet
When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 as the dissolution medium, the dissolution rate in 30 minutes of Carvedilol Tablets is not less than 80%.

Start the test with 1 tablet of Carvedilol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \)m. Discard the first 10 mL of the filtrate, and the filter volume of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 11 \( \mu \)g of carvedilol (C₂₄H₂₆N₂O₄) accord-
ing to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of carvedilol for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, $A_{T}$ and $A_{s}$, at 285 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry $<224>$, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of carvedilol (C$_{24}$H$_{26}$N$_{2}$O$_{4}$) = $M_{S} \times A_{T}/A_{S} \times V/\sqrt{V} \times 1/C \times 36$

$M_{S}$: Amount (mg) of carvedilol for assay
$C$: Labeled amount (mg) of carvedilol (C$_{24}$H$_{26}$N$_{2}$O$_{4}$) in 1 tablet

(2) 1.25-mg tablet and 2.5-mg tablet. When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 as the dissolution medium, the dissolution rate in 20 minutes is not less than 75%.

Start the test with 1 tablet of Carvedilol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add the dissolution medium to make exactly $V$ mL so that each mL contains about 1.4 μg of carvedilol (C$_{24}$H$_{26}$N$_{2}$O$_{4}$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of carvedilol for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 200 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, $A_{T}$ and $A_{s}$, at 240 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry $<224>$, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of carvedilol (C$_{24}$H$_{26}$N$_{2}$O$_{4}$) = $M_{S} \times A_{T}/A_{S} \times V/\sqrt{V} \times 1/C \times 9/2$

$M_{S}$: Amount (mg) of carvedilol for assay
$C$: Labeled amount (mg) of carvedilol (C$_{24}$H$_{26}$N$_{2}$O$_{4}$) in 1 tablet

**Assay** Weigh accurately the mass of not less than 20 Carvedilol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of carvedilol (C$_{24}$H$_{26}$N$_{2}$O$_{4}$), add exactly 5 mL of the internal standard solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make 250 mL, and shake for 30 minutes. To 2 mL of this solution, add the mobile phase to make 20 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of carvedilol for assay, previously dried at 105°C for 2 hours, and add exactly 5 mL of the internal standard solution, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make 250 mL. To 2 mL of this solution add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<$2.01$>$ under the following conditions, and calculate the ratios, $Q_{T}$ and $Q_{S}$, of the peak area of carvedilol to that of the internal standard.

\[
\text{Amount (mg) of carvedilol (C}_{24}\text{H}_{26}\text{N}_{2}\text{O}_{4}) = M_{S} \times Q_{T}/Q_{S}
\]

$M_{S}$: Amount (mg) of carvedilol for assay

**Internal standard solution**—A solution of isomyl parahydroxybenzoate in the mobile phase (1 in 70).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 2.7 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 5.0 with a solution prepared by dissolving 0.7 g of dipotassium hydrogen phosphate in water to make 200 mL. To 450 mL of this solution add 550 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of carvedilol is about 5 minutes.

**System suitability**—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, carvedilol and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of carvedilol to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

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**Castor Oil**

**Oleum Ricini**

ヒマシ油

Castor Oil is the fixed oil obtained by compression from the seeds of *Ricinus communis* Linné (*Euphorbiaceae*).

**Description** Castor Oil is a colorless or pale yellow, clear, viscous oil. It has a slight, characteristic odor, and has a bland at first, and afterwards slightly acid taste. It is miscible with ethanol (99.5) and with diethyl ether. It is freely soluble in ethanol (95), and practically insoluble in water.

When cooled to 0°C, it becomes more viscous, and turbidity is gradually formed.

**Identification** To 3 g of Castor Oil add 1 g of potassium hydroxide, and heat the mixture carefully to fuse: a charac-
Containers—Tight containers. Containers and storage of ethanol (95): no turbidity is produced. Add 15 mL of ethanol (95): it dissolves clearly. Add 15 mL of ethanol (95): no turbidity is produced.

Hydroxyl value \(<1.13\)
Saponification value \(<1.13\)
Iodine value \(<1.13\)

Purity
Adulteration—Shake to mix 1.0 g of Castor Oil with 4.0 mL of ethanol (95): it dissolves clearly. Add 15 mL of ethanol (95): no turbidity is produced.

Containers and storage—Containers—Tight containers.

**Aromatic Castor Oil**

加香ヒマシ油

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castor Oil</td>
<td>990 mL</td>
</tr>
<tr>
<td>Orange Oil</td>
<td>5 mL</td>
</tr>
<tr>
<td>Mentha Oil</td>
<td>5 mL</td>
</tr>
<tr>
<td><strong>To make</strong></td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Mix the above ingredients.

**Description**

Aromatic Castor Oil is a colorless or yellowish, clear, viscous liquid. It has an aromatic odor.

**Identification**

To 3 g of Aromatic Castor Oil add 1 g of potassium hydroxide, and heat the mixture carefully to fuse: a characteristic odor is perceptible. Dissolve the fused matter in 30 mL of water, add an excess of magnesium oxide, and filter. Acidify the filtrate with hydrochloric acid: white crystals are produced.

Containers and storage—Containers—Tight containers.

**Cefaclor**

セファクロル

![Cefaclor molecule](image)

C_{13}H_{14}ClN_{3}O_{4}S: 367.81

\((6R,7R)-7\text{-}([2R]\text{-}2\text{-}\text{Amino}\text{-}2\text{-}\text{phenylacetlamino}\text{-}3\text{-}\text{chloro}\text{-}8\text{-}\text{oxo}\text{-}5\text{-}\text{thia}\text{-}1\text{-}azabicyclo}\{4.2.0\}\text{oct}\text{-}2\text{-}\text{ene}\text{-}2\text{-}\text{carboxylic acid}\)

Cefaclor occurs as a white to yellowish white crystalline powder.

It is slightly soluble in water and in methanol, and practically insoluble in \(N, N\text{-}dimethylformamide\) and in ethanol (99.5).

**Identification**

1. Determine the absorption spectrum of a solution of Cefaclor (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Cefaclor as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

3. Dissolve 40 mg of Cefaclor in 0.5 mL of heavy water for nuclear magnetic resonance spectroscopy and 1 drop of deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy, and determine the \(^1H\) spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy \(<2.2D\rangle\), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits an AB type quartet signal A at around \(\delta 3.7\text{ ppm}\), and a single signal or a sharp multiple signal B at around \(\delta 7.6\text{ ppm}\). The ratio of the integrated intensity of each signal, A:B, is about 2:5.

4. Perform the test with Cefaclor as directed under Flame Coloration Test \(<1.04\rangle\) (2): a green color appears.

**Optical rotation** \(<2.49\rangle\ [\alpha]_{D}^{20}: +105 – +120^\circ\ (0.1 \text{ g calculated on the anhydrous basis}, \text{ water, 25 mL, 100 mm}).

**Purity**

1. Heavy metals \(<1.07\rangle\)—Proceed with 1.0 g of Cefaclor according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

2. Arsenic \(<1.11\rangle\)—Prepare the test solution by suspending 1.0 g of Cefaclor in 10 mL of \(N, N\text{-}dimethylformamide\), and perform the test (not more than 2 ppm).

3. Related substances—Dissolve 50 mg of Cefaclor in 10 mL of sodium dihydrogen phosphate TS, \(pH \ 2.5\), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add sodium dihydrogen phosphate TS, \(pH \ 2.5\) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \(\mu\text{L}\) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\rangle\) according to the following conditions, and determine each peak area by the automatic integration method: the peak areas other than cefaclor from the sample solution are not larger than 1/2 times the peak area of cefaclor from the standard solution, and the total of the peak areas other than cefaclor is not larger than 2 times of the peak area of cefaclor from the standard solution. If necessary, proceed with 20 \(\mu\text{L}\) of sodium dihydrogen phosphate TS, \(pH \ 2.5\) in the same manner as above to compensate the base line.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\text{m in particle diameter}).
Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 4.0 with phosphoric acid.

Mobile phase B: To 550 mL of the mobile phase A add 450 mL of acetonitrile for liquid chromatography.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>95 → 75</td>
<td>5 → 25</td>
</tr>
<tr>
<td>30 – 45</td>
<td>75 → 0</td>
<td>25 → 100</td>
</tr>
<tr>
<td>45 – 55</td>
<td>0</td>
<td>100</td>
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</tbody>
</table>

Flow rate: 1.0 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of cefaclor beginning after the solvent peak.

System suitability—
Test for required detectability: Measure exactly 1 mL of the standard solution, and add sodium dihydrogen phosphate TS, pH 2.5 to make exactly 20 mL. Confirm that the peak area of cefaclor obtained from 20 μL of this solution is equivalent to 4 to 6% of that from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefaclor are not less than 40,000 and 0.8 to 1.3, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas and the retention times of cefaclor are not more than 2.0%, respectively.

Water (2.48) Not more than 6.5% (0.2 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefaclor and Cefaclor RS, equivalent to about 50 mg (potency), and dissolve each in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.04) according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of cefaclor to that of the internal standard.

Amount [μg (potency)] of C₁₅H₁₄ClN₃O₄S
\[ M_s \times Q_1/Q_2 \times 1000 \]

Mₙ: Amount [μg (potency)] of Cefaclor RS

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.4 with diluted phosphoric acid (3 in 500). To 940 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefaclor is about 7 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefaclor and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefaclor to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cefaclor Capsules

Cefaclor Capsules contain not less than 90.0% and not more than 110.0% of the labeled amount of cefaclor (C₁₅H₁₄ClN₃O₄S: 367.81).

Method of preparation Prepare as directed under Capsules, with Cefaclor.

Identification Shake vigorously a quantity of the contents of one capsule of Cefaclor Capsules, equivalent to 20 mg (potency) of Cefaclor according to the labeled amount, with 10 mL of water, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg of Cefaclor RS in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetonitrile, water, ethyl acetate and formic acid (30:10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same R₁ value.

Purity Related substances—Weigh accurately not less than 5 Cefaclor Capsules, open the capsules and carefully take out the contents, mix well, and powder, if necessary. Wash the empty capsules with a little amount of diethyl ether, if necessary, allow the capsules to stand at room temperature to vaporize adhering diethyl ether, and weigh accurately the capsules to calculate the mass of the contents. Weigh accurately a quantity of the contents, equivalent to about 0.25 g
(potency) of Cefaclor, shake with 40 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 10 minutes, add the same buffer solution to make exactly 50 mL, and filter through a 0.45-μm pore-size membrane filter. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 20 mL. Pipet 2.5 mL of this solution, add the same buffer solution to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography. Calculate the amount of each related substance by automatic integration method. Calculate the amount of each liquid chromatography of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry.

System performance: When the procedure is run with 20 μL of the standard solution, and add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 20 mL. Confirm that the peak area of cefaclor obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that of cefaclor obtained with 20 μL of the standard solution.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefaclor is not more than 2.0%.

Water: Not more than 8.0% (0.2 g, volumetric titration, back titration).

Uniformity of dosage units: It meets the requirement of the Mass variation test.

Dissolution: When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefaclor Capsules is not less than 80%.

Start the test with 1 capsule of Cefaclor Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 20 μg (potency) of Cefaclor according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefaclor RS, and dissolve in water to make exactly 20 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₅, at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry. Dissolution rate (%) with respect to the labeled amount of cefaclor (C₆₁₆H₁₄Cl₁₅N₅O₆S) = Mₛ × A₁/₅/A₅ × V/V × 1/C × 90

M₅: Amount [mg (potency)] of Cefaclor RS
C: Labeled amount [mg (potency)] of Cefaclor in 1 capsule

Assay: Weigh accurately not less than 5 Cefaclor Capsules, open the capsules and carefully take out the contents, mix well, and powder, if necessary. Wash the empty capsules with a little amount of diethyl ether, if necessary, allow the capsules to stand at room temperature to vaporize adhering diethyl ether, and weigh accurately the capsules to calculate the mass of the contents. Weigh accurately a quantity of the contents, equivalent to about 0.1 g (potency) of Cefaclor according to the labeled amount, shake vigorously with 60 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 10 minutes, add the same buffer solution to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefaclor.

Amount [mg (potency)] of cefaclor (C₆₁₆H₁₄Cl₁₅N₅O₆S) = Mₛ × Qₛ/Qₛ × 2

M₅: Amount [mg (potency)] of Cefaclor RS

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

Containers and storage—Containers—Tight containers. Storage—Light-resistant.
Cefaclor Fine Granules

セファクロル細粒

Cefaclor Fine Granules contain not less than 90.0% and not more than 110.0% of the labeled amount of cefaclor (C15H14ClN3O4S: 367.81).

Method of preparation  Prepare as directed under Granules, with Cefaclor.

Identification  Shake vigorously a quantity of Cefaclor Fine Granules, equivalent to 20 mg (potency) of Cefaclor according to the labeled amount, with 10 mL of water, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg (potency) of Cefaclor RS in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.08>. Spot 2 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetonitrile, water, ethyl acetate and formic acid (30:10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same Rf value.

Purity  Related substances—Weigh accurately about 20 mg (potency) of Cefaclor Fine Granules after powdered if necessary, equivalent to about 0.1 g (potency) of Cefaclor according to the labeled amount, shake with 40 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 10 minutes, add the same buffer solution to make exactly 50 mL, and filter through a 0.45-µm pore-size membrane filter. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefaclor RS, and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 10 mL. Pipet 2 mL of this solution, add the same buffer solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetonitrile, water, ethyl acetate and formic acid (30:10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the standard solution and the spot from the sample solution show the same Rf value.

System suitability— Test for required detectability: Pipet 1 mL of the standard solution, and add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 20 mL. Confirm that the peak area of cefaclor obtained with 50 µL of this solution is equivalent to 3.5 to 6.5% of that of cefaclor obtained with 50 µL of the standard solution.

System performance: When the procedure is run with 50 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefaclor are not less than 40,000 and between 0.8 and 1.3, respectively.

System repeatability: When the test is repeated 3 times with 50 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefaclor is not more than 2.0%.

Water <2.48>  Not more than 1.5% (1 g, volumetric titration, back titration).

Uniformity of dosage units <6.02>  The granules in single-unit containers meet the requirements of the Mass variation test.

Dissolution <6.10>  When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefaclor Fine Granules is not less than 85%.

Start the test with an accurately weighed amount of Cefaclor Fine Granules, equivalent to about 0.25 g (potency) of Cefaclor according to the labeled amount, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 µm. Discard the first 10 mL of the filtrate, pipet 4 V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 20 µg (potency) of Cefaclor according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefaclor RS, and dissolve in water to make exactly 20 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A4 and A8, at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.22>.

Dissolution rate (%) with respect to the labeled amount of cefaclor (C15H14ClN3O4S)

\[ \frac{M_s}{M_f} \times A_T/A_S \times \frac{V}{V + 1/C \times 90} \]

M6: Amount [mg (potency)] of Cefaclor RS
Mf: Amount [mg (potency)] of sample
C: Labeled potency [mg (potency)] of cefaclor (C15H14ClN3O4S) per g of the sample

Particle size <6.03>  It meets the requirements of Fine granules.

Assay  Weigh accurately a quantity of Cefaclor Fine Granules after powdered if necessary, equivalent to about 0.1 g
Cefaclor Compound Granules

**Method of preparation** Prepare as directed under Granules, with Cefaclor, and divide into packs.

**Identification** Shake vigorously a quantity of Cefaclor Compound Granules, equivalent to 20 mg (potency) of Cefaclor according to the labeled total potency, with 10 mL of water, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with exactly 50 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.04> according to the following conditions, and determine each peak area of both solutions by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related substance is not more than 0.6%, and the total amount of the related substances is not more than 2.8%. If necessary, correct the fluctuation of the base line by performing the test in the same way with 50 μL of 0.1 mol/L phosphate buffer solution, pH 4.5.

**Operating conditions**

Proceed as directed in the operating conditions in the Purity (3) under Cefaclor.

**System suitability**

Test for required detectability: Pipet 1 mL of standard solution, and add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 20 mL. Perform the test with exactly 50 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.04> according to the following method: it meets the requirement of the Content uniformity test.

(1) Total potency—Take out the total contents of 1 Cefaclor Compound Granules, add a little amount of 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly V mL so that each mL contains about 5 mg (potency) of Cefaclor according to the labeled total potency. Pipet 10 mL of this solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 25 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solutions, pH 4.5, to make exactly 20 mL. Pipet 2 mL of this solution, add 0.1 mol/L phosphate buffer solutions, pH 4.5, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.04> according to the following method, and determine each peak area of both solutions by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related substance is not more than 0.6%, and the total amount of the related substances is not more than 2.8%. If necessary, correct the fluctuation of the base line by performing the test in the same way with 50 μL of 0.1 mol/L phosphate buffer solution, pH 4.5.

**Purity** Related substances—Take out the total contents of not less than 5 packs of Cefaclor Compound Granules, add a small amount of 0.1 mol/L phosphate buffer solution, pH 4.5, grind, add 0.1 mol/L phosphate buffer solution, pH 4.5, shake vigorously for 10 minutes, and add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly V mL so that each mL contains about 5 mg (potency) of Cefaclor according to the labeled total potency. Pipet 10 mL of this solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 25 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solutions, pH 4.5, to make exactly 20 mL. Pipet 2 mL of this solution, add 0.1 mol/L phosphate buffer solutions, pH 4.5, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.04> according to the following conditions, and determine each peak area of both solutions by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related substance is not more than 0.6%, and the total amount of the related substances is not more than 2.8%. If necessary, correct the fluctuation of the base line by performing the test in the same way with 50 μL of 0.1 mol/L phosphate buffer solution, pH 4.5.
mol/L phosphate buffer solution, pH 4.5, grind well, add the same buffer solutions to make exactly V mL so that each mL contains about 3.8 mg (potency) of Cefaclor according to the labeled total potency after shaking vigorously for 10 minutes, and centrifuge. Pipet 3 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefaclor.

Amount [mg (potency)] of cefaclor (C15H14ClN3O4S)  
\[ M_S = \frac{Q_T/Q_S \times V}{15} \]

\( M_S \): Amount [mg (potency)] of Cefaclor RS

**Internal standard solution**—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

(2) Potency of gastric granule—Take out the total contents of 1 Cefaclor Compound Granules stir gently with 60 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 5 minutes, add the same buffer solution to make exactly V mL so that each mL contains about 1.5 mg (potency) of Cefaclor according to the labeled potency of gastric granule, and centrifuge. Pipet 7 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefaclor.

Amount [mg (potency)] of cefaclor (C15H14ClN3O4S)  
\[ M_S = \frac{Q_T/Q_S \times V}{35} \]

\( M_S \): Amount [mg (potency)] of Cefaclor RS

**Internal standard solution**—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

**Dissolution** (6.10) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Cefaclor Compound Granules is not less than 70%.

Start the test with the total content of 1 pack of Cefaclor Fine Granules, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.01 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 20 μg (potency) of Cefaclor according to the labeled total potency, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 20 mg (potency), dissolve in the dissolution medium to make exactly 20 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry (2.24), using 0.01 mol/L hydrochloric acid TS as the blank.

Dissolution rate (%) of cefaclor (C15H14ClN3O4S) with respect to the labeled potency  
\[ M_S = \frac{A_T/A_S \times V/V \times 1/C \times 90}{C} \]

\( M_S \): Amount [mg (potency)] of Cefaclor RS

C: Labeled total potency [mg (potency)] of Cefaclor in 1 pack

Separately, when the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Cefaclor Compound Granules is not less than 70%.

Start the test with the total content of 1 pack of Cefaclor Fine Granules, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.01 mol/L hydrochloric acid TS to make exactly V mL, and warm at 37°C for 60 minutes. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry (2.24), using 0.01 mol/L hydrochloric acid TS as the blank.

Dissolution rate (%) of cefaclor (C15H14ClN3O4S) with respect to the labeled potency  
\[ M_S = \frac{A_T/A_S \times V/V \times 1/C \times 90}{C} \]

\( M_S \): Amount [mg (potency)] of Cefaclor RS

C: Labeled total potency [mg (potency)] of Cefaclor in 1 pack

**Assay** (1) Total potency—Take out the total contents of not less than 5 Cefaclor Compound Granules, add a small amount of 0.1 mol/L phosphate buffer solution, pH 4.5, grind well, add the same buffer solution to make a solution containing about 5 mg (potency) of Cefaclor per mL according to the labeled total potency after shaking vigorously for 10 minutes, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 50 mg (potency), and dissolve in the dissolution medium to make exactly 20 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry (2.24).

Dissolution rate (%) of cefaclor (C15H14ClN3O4S) with respect to the labeled potency  
\[ M_S = \frac{A_T/A_S \times V/V \times 1/C \times 90}{C} \]

\( M_S \): Amount [mg (potency)] of Cefaclor RS

C: Labeled total potency [mg (potency)] of Cefaclor in 1 pack

Separately, when the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Cefaclor Compound Granules is not less than 70%.

Start the test with the total content of 1 pack of Cefaclor Fine Granules, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.01 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 20 μg (potency) of Cefaclor according to the labeled total potency, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 20 mg (potency), dissolve in the dissolution medium to make exactly 20 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry (2.24), using 0.01 mol/L hydrochloric acid TS as the blank.

Dissolution rate (%) of cefaclor (C15H14ClN3O4S) with respect to the labeled potency  
\[ M_S = \frac{A_T/A_S \times V/V \times 1/C \times 90}{C} \]

\( M_S \): Amount [mg (potency)] of Cefaclor RS

C: Labeled total potency [mg (potency)] of Cefaclor in 1 pack
Cefadroxil

Cefadroxil occurs as a white to light yellow-white powder. It is sparingly soluble in water, slightly soluble in methanol, and very slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefadroxil (1 in 50,000) as directed under Ultra-violet-visible Spectrophotometry <2.49>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefadroxil RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefadroxil as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefadroxil RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ¹H spectrum of a solution of Cefadroxil in a mixture of heavy water for nuclear magnetic resonance spectroscopy and deuterated hydrochloric acid (3:1) (1 in 10), using sodium 3-(trimethylsilyl)propionate-d₄ for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>: it exhibits a single signal A at around δ 2.1 ppm, a double signal B at around δ 7.0 ppm, and a double signal C at around δ 7.5 ppm. The ratio of integrated intensity of each signal, A:B:C, is about 3:2:2.

Optical rotation <2.49> [α]D²⁰: +164° to +182° (0.6 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH <2.49> Dissolve 1.0 g of Cefadroxil in 200 mL of water: pH of the solution is between 4.0 and 6.0.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Cefadroxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.1 g of Cefadroxil in 4 mL of a mixture of ethanol (99.5), water and dilute hydrochloric acid (1 in 5) (75:22:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (99.5), water and dilute hydrochloric acid (1 in 5) (75:22:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.67>. Spot 2 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ethyl acetate, water, ethanol (99.5) and formic acid (14:5:5:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not less than 4.2% and not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefadroxil and Cefadroxil RS equivalent to about 50 mg (potency), dissolve each in water to make exactly 500 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of cefadroxil of the solutions.

\[
M₅ = \frac{A₁}{A₅} \times 1000
\]

Amount [µg (potency)] of C₁₆H₁₇N₃O₅S
\[
= M₅ \times \frac{Q₅}{Q₅} \times 1/5
\]

M₅: Amount [µg (potency)] of Cefadroxil RS

Internal standard solution—A solution of 4-aminoacetoephonone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

(2) Potency of gastric granule—Stir gently the total contents of not less than 5 Cefadroxil Compound Granules with about 100 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 5 minutes, the same buffer solution so that each mL containing about 2 mg (potency) of Cefadroxil according to the labeled potency of gastric granule, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefadroxil RS, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefadroxil.

Amount [µg (potency)] of cefadroxil (C₁₆H₁₇N₃O₅S)
\[
= M₅ \times \frac{Q₅}{Q₅} \times 1/5
\]

M₅: Amount [µg (potency)] of Cefadroxil RS

Internal standard solution—A solution of 4-aminoacetoephonone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefadroxil

C₁₆H₁₇N₃O₅S: 363.39
(6R,7R)-7-[(2R)-2-Amino-2-(4-hydroxyphenyl)acetylamino]-3-methyl-8-oxo-5-thia-1-azabicycle[4.2.0]oct-2-ene-2-carboxylic acid [50370-12-2]
Cefadroxil Capsules

セファドロキシルカプセル

Cefadroxil Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of cefadroxil (C_{16}H_{17}N_{3}O_{5}S: 363.39).

Method of preparation Prepare as directed under Capsules, with Cefadroxil.

Identification Dissolve the contents of Cefadroxil Capsules, equivalent to 10 mg (potency) of Cefadroxil according to the labeled amount, in 500 mL of water, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry 〈2.24〉: it exhibits maxima between 228 nm and 232 nm, and between 261 nm and 265 nm.

Water 〈2.48〉 Not more than 7.0% (0.15 g, volumetric titration, direct titration).

Uniformity of dosage units 〈6.02〉 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 capsule of Cefadroxil Capsules in 300 mL of water, disperse with the aid of ultrasonic waves, shake for 30 minutes, and add water to make exactly 500 mL. Pipet 5 mL of this solution, and add water to make exactly V mL so that each mL contains about 0.1 mg (potency) of Cefadroxil. Filter the solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefadroxil RS, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefadroxil.

Amount [mg (potency)] of cefadroxil (C_{16}H_{17}N_{3}O_{5}S) = M_{S} × A_{T} × V/90

M_{S} = Amount [mg (potency)] of Cefadroxil RS

Dissolution 〈6.10〉 When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 as the dissolution medium, the dissolution rate in 90 minutes of Cefadroxil Capsules is not less than 80%.

Start the test with 1 capsule of Cefadroxil Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V’ mL so that each mL contains about 22 μg (potency) of Cefadroxil according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Cefadroxil RS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_{T} and A_{S}, at 263 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 〈2.24〉, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of cefadroxil (C_{16}H_{17}N_{3}O_{5}S) = M_{S} × A_{T} × V’/V × V × 1/C × 90

M_{S} = Amount [mg (potency)] of Cefadroxil RS

C Labeled amount [mg (potency)] of cefadroxil in 1 capsule

Cefadroxil for Syrup

シロップ用セファドロキシル

Cefadroxil for Syrup is a preparation for syrup, which is suspended before use.

It contains not less than 95.0% and not more than 110.0% of the labeled amount of cefadroxil (C_{16}H_{17}N_{3}O_{5}S: 363.39).

Method of preparation Prepare as directed under Preparations for Syrups, with Cefadroxil.
Identification Dissolve an amount of Cefadroxil for Syrup, equivalent to 10 mg (potency) of Cefadroxil according to the labeled amount, in 500 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \( \lambda_{\text{max}} < 230 \text{ nm} \) and \( \lambda_{\text{max}} > 265 \text{ nm} \). Make a standard solution of Cefadroxil RS, equivalent to about 22 mg (potency) per g, in 20 mL of dimethylformamide, and compare the Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water \(< 2.48\) Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Uniformity of dosage units \(< 6.02\) The syrup in single-unit containers meets the requirement of the Mass variation test.

Dissolution \(\text{JP XVI} <6.10\) When the test is performed at 50 revolutions per minute according to the Paddle method (put the sample in the dissolution medium so that it disperses), using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefadroxil for Syrup is not less than 85%.

Start the test with accurately weighed amount of Cefadroxil for Syrup, equivalent to about 0.1 g (potency) of Cefadroxil according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \text{m} \). Discard the first 10 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Cefadroxil RS, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, \( A_1 \) and \( A_9 \), at 263 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \( \lambda_{\text{max}} < 230 \text{ nm} \) and \( \lambda_{\text{max}} > 265 \text{ nm} \).

Cefadroxil contains not less than 950 \( \mu \text{g} \) (potency) and not more than 1030 \( \mu \text{g} \) (potency) per mg, calculated on the anhydrous basis. The potency of Cefadroxil is expressed as mass (potency) of cefalexin (C\(_{16}\)H\(_{17}\)N\(_3\)O\(_4\)S) per mg.

Description Cefadroxil occurs as a white to light yellowish white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, and practically insoluble in ethanol (95) and in \( N,N \)-dimethylformamide.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Cefadroxil (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( \lambda_{\text{max}} < 230 \text{ nm} \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefadroxil as directed in the potassium bromide disk method under Infrared Spectrophotometry \( \lambda_{\text{max}} < 230 \text{ nm} \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the \( \text{H} \) spectrum of a solution of Cefadroxil in heavy water for nuclear magnetic resonance spectroscopy (1 in 200) as directed under Nuclear Magnetic Resonance Spectroscopy \( \lambda_{\text{max}} < 230 \text{ nm} \), using sodium 3-trimethylsilylpropionate-2,2,2-trifluoroacetate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around \( \delta 1.8 \text{ ppm} \), and a single or a sharp multiple signal B at around \( \delta 7.5 \text{ ppm} \). The ratio of integrated intensity of these signals, A:B, is about 3:5.

Optical rotation \(< 2.49\) \([\alpha]_D^\text{23} + 144° \pm 158° \) (0.125 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity (1) Heavy metals \(< 1.07\) Proceed with 2.0 g of Cefadroxil according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(< 1.11\) Prepare the test solution with 1.0 g of Cefadroxil by suspending in 10 mL of \( N,N \)-dimethylformamide, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve about 25 mg of Cefadroxil in a solution of potassium dihydrogenphosphate (9 in 500) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of potassium dihydrogenphosphate (9 in 500) to make ex-
Actly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and determine the areas of each peak by the automatic integration method. If necessary, correct the change of the base-line due to the potassium dihydrogenphosphate solution by proceeding in the same manner with 20 \( \mu L \) of a solution of potassium dihydrogenphosphate (9 in 500): each peak area other than cefalexin from the sample solution is not larger than the peak area of cefalexin from the standard solution, and the total of the peak areas which are larger than 1/50 times the peak area of cefalexin from the standard solution and those other than cefalexin from the sample solution is not larger than 5 times of the peak area of cefalexin from the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 1.0 g of sodium 1-pentanesulfonate in 1000 mL of water, add 15 mL of triethylamine, and adjust to pH 2.5 with phosphoric acid.

Mobile phase B: Dissolve 1.0 g of sodium 1-pentanesulfonate in 300 mL of water, add 15 mL of triethylamine, and adjust to pH 2.5 with phosphoric acid. To this solution add 350 mL of acetonitrile and 350 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1 – 34.5</td>
<td>100 – 0</td>
<td>0 – 100</td>
</tr>
<tr>
<td>34.5 – 35.5</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute.

Time span of measurement: About 2 times as long as the retention time of cefalexin beginning after the solvent peak.

**System suitability**

Test for required detection: Pipet 2 mL of the standard solution, add a solution of potassium dihydrogenphosphate (9 in 500) to make exactly 100 mL. Confirm that the peak area of cefalexin obtained from 20 \( \mu L \) of this solution is equivalent to 1.8 to 2.2% of that of cefalexin obtained from 20 \( \mu L \) of the standard solution.

System performance: When the procedure is run with 20 \( \mu L \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefalexin are not less than 150,000 steps and between 0.8 and 1.3, respectively.

System repeatability: When the test is repeated 3 times with 20 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the retention time and the peak areas of cefalexin are not more than 2.0%, respectively.

**Water** Not more than 8.0% (0.2 g, volumetric titration, back titration).

**Assay** Weigh accurately an amount of Cefalexin and Cefalexin RS, equivalent to about 25 mg (potency), dissolve each in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 25 mL. Pipet 10 mL of these solutions, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L phosphate buffer solution, pH 4.5 to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of cefalexin to that of the internal standard.

\[
\text{Amount} [\mu g \text{ (potency)}] \ of \ C_{16}H_{17}N_{3}O_{4}S = M_S \times Q_1/Q_2 \times 1000
\]

Where:

- \( M_S \): amount [mg (potency)] of Cefalexin RS

**Internal standard solution—**A solution of \( m \)-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 1500).

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in 1000 mL of water, adjust to pH 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefalexin is about 7 minutes.

**System suitability**

System performance: When the procedure is run with 10 \( \mu L \) of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefalexin to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

**Cefalexin Capsules**

セファレキシンカプセル

Cefalexin Capsules contain not less than 93.0% and not more than 107.0% of the labeled potency of cefalexin (\( C_{16}H_{17}N_{3}O_{4}S \): 347.39).

**Method of preparation** Prepare as directed under Capsules, with Cefalexin.

**Identification** Take out the contents of Cefalexin Capsules, to a quantity of the contents, equivalent to 70 mg (potency)
of Cefalexin according to the labeled amount, add 25 mL of water, shake vigorously for 5 minutes, and filter. To 1 mL of the filtrate add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \( <2.24 > \); it exhibits a maximum between 260 nm and 264 nm.

**Water** \(<2.48 >\) Not more than 10.0% (0.2 g, volumetric titration, back titration).

**Uniformity of dosage units** \(<6.02 >\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Open 1 capsule of Cefalexin Capsules, add 3\( V/5 \) mL of 0.1 mol/L phosphate buffer solution, pH 4.5, shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly \( V \) mL so that each mL contains about 1.25 mg (potency) of Cefalexin. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add about 10 mL of the internal standard solution, and add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the standard solution. Perform the test with 10 \( \mu \)L of each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07 >\) according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of cefalexin to that of the internal standard in each solution.

\[
\text{Amount [mg (potency)] of cefalexin (C}_{16}\text{H}_{17}\text{N}_{3}\text{O}_{4}\text{S)} = M_S \times \frac{Q_1}{Q_2} \times \frac{V}{20}
\]

*\( M_S \): Amount [mg (potency)] of Cefalexin RS

**Internal standard solution**—A solution of \( m \)-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 15,000).

**Operating conditions**—

Proceed as directed in the operating conditions in the Assay.

**System suitability**—

- System performance: When the procedure is run with 10 \( \mu \)L of the standard solution under the above operating conditions, cefalexin and the internal standard substance are eluted in this order with the resolution between these peaks being not less than 8.
- System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefalexin to that of the internal standard substance is not more than 1.0%.

**Dissolution** \(<6.10 >\) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 30 minutes of 125-mg (potency) capsule and in 60 minutes of 250-mg (potency) capsule are not less than 75% and 80%, respectively.

Start the test with 1 capsule of Cefalexin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 \( \mu \)m. Discard the first 10 mL of the filtrate, pipet \( V \) mL of the subsequent filtrate, add water to make exactly \( V \) mL so that each mL contains about 22 \( \mu \)g (potency) of Cefalexin according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 22 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24 >\), and determine the absorbances, \( A_T \) and \( A_S \), at 262 nm.

Dissolution rate (%) with respect to the labeled amount of cefalexin (\( \text{C}_{16}\text{H}_{17}\text{N}_{3}\text{O}_{4}\text{S) }\) 
\[
M_S \times \frac{A_T}{A_S} \times \frac{V}{V \times \frac{1}{C} \times 90}
\]

*\( M_S \): Amount [mg (potency)] of Cefalexin RS

C: Labeled amount [mg (potency)] of cefalexin (\( \text{C}_{16}\text{H}_{17}\text{N}_{3}\text{O}_{4}\text{S) in 1 capsule.}

**Assay** Take out the contents of not less than 20 capsules of Cefalexin Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefalexin, add 60 mL of 0.1 mol/L phosphate buffer solution, pH 4.5, shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 100 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the standard solution. Perform the test with 10 \( \mu \)L of each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07 >\) according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of cefalexin to that of the internal standard in each solution.

\[
\text{Amount [mg (potency)] of cefalexin (C}_{16}\text{H}_{17}\text{N}_{3}\text{O}_{4}\text{S) = M_S \times \frac{Q_1}{Q_2} \times 5}}
\]

*\( M_S \): Amount [mg (potency)] of Cefalexin RS

**Internal standard solution**—A solution of \( m \)-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 15,000).

**Operating conditions**—

- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 3.0 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 \( \mu \)m in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of cefalexin is about 6 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefalexin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefalexin for Syrup
シロップ用セファレキシン

Cefalexin for Syrup is a preparation for syrup, which is dissolved or suspended before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of cefalexin (C16H17N3O4S: 347.39).

Method of preparation Prepare as directed under Preparations for Syrups, with Cefalexin.

Identification Dissolve a quantity of Cefalexin for Syrup, equivalent to 3 mg (potency) of Cefalexin according to the labeled amount, in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24); it exhibits a maximum between 260 nm and 264 nm.

Water Not more than 5.0% (0.4 g, volumetric titration, back titration).

Uniformity of dosage units (6.02) Perform the test according to the following method: Cefalexin for Syrup in single-unit containers meets the requirement of the Content uniformity test.

Take out the total contents of 1 pack of Cefalexin for Syrup, add 3 V/5 mL of 0.1 mol/L phosphate buffer solution, pH 4.5, shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly V mL so that each mL contains about 1 mg (potency) of Cefalexin, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of cefalexin (C16H17N3O4S) = M5 × Q5/Q8 × V/20

M5: Amount [mg (potency)] of Cefalexin RS

Internal standard solution—A solution of m-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 15,000).

Dissolution (6.10b) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefalexin for Syrup is not less than 80%.

Start the test with an accurately weighed amount of Cefalexin, equivalent to about 0.25 g (potency) of Cefalexin for Syrup according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 22 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry (2.24), and determine the absorbances, A1 and A8, at 262 nm.

Dissolution rate (%) with respect to the labeled amount of cefalexin (C16H17N3O4S) = M5/M8 × A8/A5 × 1/C × 1125

M5: Amount [mg (potency)] of Cefalexin RS
M8: Amount (g) of Cefalexin for Syrup
C: Labeled amount [mg (potency)] of cefalexin (C16H17N3O4S) in 1 g

Assay Powder Cefalexin for Syrup, if necessary, and weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefalexin, add 60 mL of 0.1 mol/L phosphate buffer solution, pH 4.5, shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 100 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, Q1 and Q8, of the peak area of cefalexin to that of the internal standard in each solution.

Amount [mg (potency)] of cefalexin (C16H17N3O4S) = M5 × Q8/Q1 × 5

M5: Amount [mg (potency)] of Cefalexin RS

Internal standard solution—A solution of m-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 15,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 3.0 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 2.72 g of potassium dihydrogen...
phosphate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefalexin is about 6 minutes.

System suitability—

- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefalexin to that of the internal standard is not more than 1.0%.

Containers and storage — Containers—Tight containers.
Storage—Light-resistant.

Cefalotin Sodium

Cefalotin Sodium occurs as white to light yellowish white, crystals or crystalline powder.

It is freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetone.

Identification (1) Determine the absorption spectrum of a solution of Cefalotin Sodium (1 in 5,000) as directed under Ultraviolet-visible Spectrophotometry 2.2.4, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefalotin Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefalotin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.2.5, and compare the spectrum with the Reference Spectrum or the spectrum of Cefalotin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the 1H spectrum of a solution of Cefalotin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy 2.2.17, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around 3.1 ppm, a single or sharp multiple signal B at around 3.9 ppm, and a multiple signal C at around 7.0 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:2:2.

(4) Cefalotin Sodium responds to the Qualitative Tests 1.09 (1) for sodium salt.

Optical rotation 2.4.9 [α]D: +124° – +134° (5 g, water, 100 mL, 100 mm).

pH 2.5.4 The pH of a solution obtained by dissolving 1.0 g of Cefalotin Sodium in 10 mL of water is between 4.5 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefalotin Sodium in 10 mL of water: the solution is clear. The absorbance of this solution at 450 nm, determined as directed under Ultraviolet-visible Spectrophotometry 2.2.4, is not more than 0.20.

(2) Heavy metals 1.07—Proceed with 1.0 g of Cefalotin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic 1.11—Prepare the test solution with 1.0 g of Cefalotin Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Pipet 1 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution obtained in the Assay and the standard solution prepared here as directed under Liquid Chromatography 2.01 according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than cefalotin from the sample solution is not larger than the peak area of cefalotin from the standard solution, and the total area of the peaks other than cefalotin from the sample solution is not larger than 3 times the peak area of cefalotin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of cefalotin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefalotin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Heat the standard solution in a water bath of 90°C for 10 minutes, and cool. Measure exactly 2.5 mL of this solution, and add the mobile phase to make exactly 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of cefalotin and the peak, having the relative retention time of about 0.5 with respect to cefalotin, is not less than 9, and the symmetry factor of the peak of cefalotin is not more than 1.8.

System repeatability: When the test is repeated 3 times
Cefatrizine Propylene Glycolate

C_{18}H_{18}N_{6}O_{5}S_{2}: C_{3}H_{8}O_{2}: 538.60
(6R,7R)-7-[(2R)-2-Amino-2-(4-hydroxyphenyl)acetyl]-amino]-8-oxo-3-[1(1H,1,2,3-triazol-4-yl)sulfonylmethyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monopropionate,1,2-diolate (1/1)

Cefatrizine Propylene Glycolate contains not less than 816 µg (potency) and not more than 876 µg (potency) per mg, calculated on the anhydrous basis. The potency of Cefatrizine Propylene Glycolate is expressed as mass (potency) of cefatrizine (C_{18}H_{18}N_{6}O_{5}S_{2}: 462.50).

Description  
Cefatrizine Propylene Glycolate occurs as a white to yellowish white powder. It is sparingly soluble in water, and practically insoluble in methanol and in ethanol (95).

Identification (1)  
Determine the absorption spectrum of a solution of Cefatrizine Propylene Glycolate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefatrizine Propylene Glycolate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefatrizine Propylene Glycolate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefatrizine Propylene Glycolate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the 1H spectrum of a solution of Cefatrizine Propylene Glycolate in a mixture of heavy water for nuclear magnetic resonance spectroscopy and deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy (3:1) (1 in 10), using sodium 3-(trimethylsilyl)propionate-d_{4} for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>: it exhibits a double signal A at around δ 1.2 ppm, a double signal B at around δ 7.0 ppm, a double signal C at around δ 7.5 ppm and a single signal D at around δ 8.3 ppm. The ratio of integrated intensity of these signals, A:B:C:D, is about 3:2:2:1.

Optical rotation <2.49>  
[α]_{D}: +52 – +58° (2.5 g calculated on the anhydrous bases, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity (1)  
Heavy metals <1.07>—Proceed with 1.0 g of
Cefatrizine Propylene Glycolate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic $<\text{1.1}>$—Prepare the test solution with 1.0 g of Cefatrizine Propylene Glycolate according to Method 3, and perform the test (not more than 2 ppm). Use a solution of magnesium nitrate hexahydrate in ethanol (1 in 25).

(3) Related substances—Dissolve 25 mg of Cefatrizine Propylene Glycolate in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.01>$. Spot 5 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of 1-butanol, water and acetic acid (100:3:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water $<2.48>$—Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefatrizine Propylene Glycolate and Cefatrizine Propylene Glycolate RS equivalent to about 0.1 g (potency), dissolve each in water to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 $\mu$L of each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and determine the peak areas, $A_1$ and $A_5$, of cefatrizine of these solutions.

Amount $[\mu g \text{ (potency)}]$ of cefatrizine $(C_{18}H_{18}N_6O_5S_2)$

$$M_2: \text{Amount } [\text{mg } \text{ (potency)}] \text{ of Cefatrizine Propylene Glycolate RS}$$

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 270 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilaized silica gel for liquid chromatography (5 $\mu$m in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of a solution of potassium dihydrogenphosphate (17 in 12,500) and methanol (17:3).
Flow rate: Adjust the flow rate so that the retention time of cefatrizine is about 11 minutes.

System suitability—
System performance: Dissolve about 5 mg (potency) of Cefadroxil and about 10 mg (potency) of Cefatrizine Propylene Glycolate in 50 mL of water. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, cefadroxil and cefatrizine are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of peak areas of cefatrizine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefatrizine Propylene Glycolate for Syrup シロップ用セファトリジンプロピレングリコール

Cefatrizine Propylene Glycolate for Syrup is a preparation for syrup, which is dissolved before use. It contains not less than 90.0% and not more than 105.0% of the labeled potency of Cefatrizine $(C_{18}H_{18}N_6O_5S_2$: 462.50).

Method of preparation Prepare as directed under Preparations for Syrup, with Cefatrizine Propylene Glycolate.

Identification Powder Cefatrizine Propylene Glycolate for Syrup, weigh a portion of the powder, equivalent to 10 mg (potency) of Cefatrizine Propylene Glycolate according to the labeled amount, and dissolve in 10 mL of water. To 2 mL of this solution add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $<2.24>$: it exhibits maxima between 225 nm and 229 nm, and between 266 nm and 271 nm.

pH $<2.5>$—Take an amount of Cefatrizine Propylene Glycolate for Syrup, equivalent to 0.4 g (potency) of Cefatrizine Propylene Glycolate according to the labeled amount, and suspend in 10 mL of water: the pH of this suspension is between 4.0 and 6.0.

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L of each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than cefatrizine obtained from the sample solution is not larger than the peak area of cefatrizine from the standard solution, and the total area of the peaks other than the peak of cefatrizine is not larger than 2 times the peak area of cefatrizine from the standard solution.

Operating conditions—
Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Cefatrizine Propylene Glycolate.

Time span of measurement: About 2.5 times as long as the retention time of cefatrizine, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 2 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of cefatrizine obtained from 10 $\mu$L of this solution is equivalent to 15 to 25% of that of cefatrizine from 10 $\mu$L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay under Cefatrizine Propylene Glycolate.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operat-
ing conditions, the relative standard deviation of the peak area of cefatrizine is not more than 2.0%.

**Uniformity of dosage units** 6.02\(\Delta\) Cefatrizine Propylene Glycolate for Syrup in single-unit containers meets the requirement of the Mass variation test.

**Assay** Powder Cefatrizine Propylene Glycolate for Syrup, weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefatrizine Propylene Glycolate, dissolve in water to make exactly 500 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefatrizine Propylene Glycolate RS, equivalent to about 20 mg (potency), dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefatrizine Propylene Glycolate.

Amount \([\text{mg (potency)}]\) of cefatrizine \(\left(C_{13}H_{18}N_{8}O_{4}S_{3}\right)\)

\[M_5 = \frac{M \times A_T}{A_S} \times 5\]

\(M_5:\) Amount \([\text{mg (potency)}]\) of Cefatrizine Propylene Glycolate RS

**Containers and storage** Containers—Tight containers.

## Cefazolin Sodium

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![Cefazolin Sodium structure](image)

\(C_{14}H_{13}N_{8}NaO_{4}S_{3}: 476.49\)

Monosodium \((6R,7R)-3-(5\text{-methyl}-1,3,4\text{-thiadiazol-2-ylsulfanylmethyl})-8\text{-oxo-7-(2-(1H-tetrazol-1-yl)acetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate}\)

\([27164-46-1]\)

Cefazolin Sodium contains not less than 900 \(\mu\)g (potency) and not more than 975 \(\mu\)g (potency) per mg, calculated on the anhydrous basis. The potency of Cefazolin Sodium is expressed as mass (potency) of cefazolin \(\left(C_{14}H_{13}N_{8}O_{4}S_{3}\right): 454.51\)

**Description** Cefazolin Sodium occurs as a white to light yellow-white, crystals or crystalline powder.

It is freely soluble in water and in formamide, slightly soluble in methanol, and practically insoluble in ethanol (95).

**Identification** (1) Determine the absorption spectrum of a solution of Cefazolin Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \(\leq 2.24\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefazolin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry \(\leq 2.25\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the \(^1\)H spectrum of a solution of Cefazolin Sodium in heavy water for nuclear magnetic resonance spectroscopy \(1 \text{ in } 10\), using sodium 3-trimethylsilylpropionate-\(d_4\) for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy \(\leq 2.22\): it exhibits single signals, A and B, at around \(\delta\) 2.7 ppm and at around \(\delta\) 9.3 ppm, respectively. The ratio of integrated intensity of these signals, A:B, is about 3:1.

(4) Cefazolin Sodium responds to the Qualitative Tests \(\leq 1.09\) (1) for sodium salt.

**Optical rotation** \(\leq 2.49\) \([\alpha]_{D}^{20}\): \(-19 \sim -23^\circ\) (2.5 \(g\) calculated as the anhydrous basis, water, 25 \(mL\), 100 \(mm\)).

**pH** \(\leq 2.54\) Dissolve 1.0 \(g\) of Cefazolin Sodium in 10 \(mL\) of water: pH of the solution is between 4.8 and 6.3.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 \(g\) of Cefazolin Sodium in 10 \(mL\) of water: the solution is clear and colorless to pale yellow, and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry \(\leq 2.24\) is not more than 0.35. The test should be performed within 10 minutes after preparing of the solution.

(2) Heavy metals \(\leq 2.07\)—Proceed with 2.0 \(g\) of Cefazolin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 \(mL\) of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic \(\leq 1.11\)—Prepare the test solution with 2.0 \(g\) of Cefazolin Sodium according to Method 3, and perform the test. When prepare the test solution, add 1.5 \(mL\) of hydrogen peroxide (30) after addition of 10 \(mL\) of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), and then ignite (not more than 1 ppm).

(4) Related substances—Dissolve 0.10 \(g\) of Cefazolin Sodium in 20 \(mL\) of 0.1 \(mol/L\) phosphate buffer solution, pH 7.0 and use this solution as the sample solution. Prepare the sample solution before use. Perform the test with 5 \(\muL\) of the sample solution as directed under Liquid Chromatography \(\leq 2.07\) according to the following conditions, and measure the areas of a peak appeared at the relative retention time of about 0.2 to the retention time of cefazolin and peaks other than cefazolin by the automatic integration method, and calculate the amounts of these peak areas by the area percentage method: the amount of each peak area is not more than 1.5%, and the total area of the peaks other than cefazolin is not more than 2.5%. The area of the peak appeared at the relative retention time of about 0.2 to the retention time of cefazolin obtained here is used after multiplying by its relative response factor, 1.43.

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cefazolin beginning after the solvent peak.

**System suitability**—

Test for required detection: Dissolve about 80 \(mg\) of Cefazolin RS in 0.1 \(mol/L\) phosphate buffer solution, pH 7.0 to make 100 \(mL\), and use this solution as the solution for system suitability test. Pipet 1 \(mL\) of the solution for system suitability test, and add 0.1 \(mol/L\) phosphate buffer solu-
Cefazolin Sodium for Injection

Cefazolin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of cefazolin (C14H14N8O4S3: 454.51).

Method of preparation

Prepare as directed under Injections, with Cefazolin Sodium.

Description

Cefazolin Sodium for Injection occurs as white to light yellowish white crystals or crystalline powder or masses.

Identification (1) Determine the absorption spectrum of a solution of Cefazolin Sodium for Injection (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.242>: it exhibits a maximum between 270 nm and 274 nm.

(2) Cefazolin Sodium for Injection responds to the Qualitative Tests <1.09> (1) for chloride.

Osmotic pressure ratio

Being specified separately.

pH <2.54> The pH of a solution prepared by dissolving an amount of Cefazolin Sodium for Injection, equivalent to 1.0 g (potency) of Cefazolin Sodium according to the labeled amount, in 10 mL of water is 4.5 to 6.5.

Purity (1) Clarity and color of solution—Conduct this procedure within 10 minutes after the preparation of the solutions. A solution prepared by dissolving an amount of Cefazolin Sodium for Injection, equivalent to 1.0 g (potency) of Cefazolin Sodium according to the labeled amount, in 10 mL of water is clear, and the absorbance of this solution at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry <2.242>, is not more than 0.35.

(2) Related substances—Dissolve an amount of Cefazolin Sodium for Injection, equivalent to 0.10 g (potency) of Cefazolin Sodium according to the labeled amount, in 20 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Prepare the sample solution before use. Perform the test with 5 µL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of each peak by the area percentage method: each area of the peaks other than cefazolin is not more than 1.5%. Furthermore the total area of the peaks other than cefazolin is not more than 2.5%. For these calculations, use the area of the peak, having the relative retention time of about 0.2 with respect to cefazolin, after multiplying by the relative response factor, 1.43.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Cefazolin Sodium.

Time span of measurement: About 3 times as long as the retention time of cefazolin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 8 mL of the sample solution, add 0.1 mol/L phosphate buffer solution, pH 7.0

Assay

Weigh accurately an amount of Cefazolin Sodium and Cefazolin RS, equivalent to about 0.1 g (potency), dissolve each in the internal standard solution to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 µL of each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of cefazolin to that of the internal standard.

Amount [µg (potency)] of cefazolin (C14H14N8O4S3) = M6 × Q1/Q2 × 1000

M6: Amount [µg (potency)] of Cefazolin RS

Internal standard solution—A solution of p-acetoanisidide in 0.1 mol/L phosphate buffer solution, pH 7.0 (11 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.27 g of disodium hydrogen phosphate dodecahydrate and 0.47 g of citric acid monohydrate in water to make 935 mL, and add 65 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefazolin is about 8 minutes.

System suitability—

System performance: Proceed as directed in the system suitability test. The relative standard deviation of the ratios of the peak areas of cefazolin is not more than 1.0%.

System repeatability: When the test is repeated 6 times with 5 µL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefazolin is not more than 1.0%.

Water <2.48> Not more than 2.5% (1.0 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Containers and storage—Tight containers.

Cefazolin Sodium for Injection

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Cefazolin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of cefazolin (C14H14N8O4S3: 454.51).

Method of preparation

Prepare as directed under Injections, with Cefazolin Sodium.

Description

Cefazolin Sodium for Injection occurs as white to light yellowish white crystals or crystalline powder or masses.

Identification (1) Determine the absorption spectrum of a solution of Cefazolin Sodium for Injection (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.242>: it exhibits a maximum between 270 nm and 274 nm.

(2) Cefazolin Sodium for Injection responds to the Qualitative Tests <1.09> (1) for chloride.

Osmotic pressure ratio

Being specified separately.

pH <2.54> The pH of a solution prepared by dissolving an amount of Cefazolin Sodium for Injection, equivalent to 1.0 g (potency) of Cefazolin Sodium according to the labeled amount, in 10 mL of water is 4.5 to 6.5.

Purity (1) Clarity and color of solution—Conduct this procedure within 10 minutes after the preparation of the solutions. A solution prepared by dissolving an amount of Cefazolin Sodium for Injection, equivalent to 1.0 g (potency) of Cefazolin Sodium according to the labeled amount, in 10 mL of water is clear, and the absorbance of this solution at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry <2.242>, is not more than 0.35.

(2) Related substances—Dissolve an amount of Cefazolin Sodium for Injection, equivalent to 0.10 g (potency) of Cefazolin Sodium according to the labeled amount, in 20 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Prepare the sample solution before use. Perform the test with 5 µL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of each peak by the area percentage method: each area of the peaks other than cefazolin is not more than 1.5%. Furthermore the total area of the peaks other than cefazolin is not more than 2.5%. For these calculations, use the area of the peak, having the relative retention time of about 0.2 with respect to cefazolin, after multiplying by the relative response factor, 1.43.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Cefazolin Sodium.

Time span of measurement: About 3 times as long as the retention time of cefazolin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 8 mL of the sample solution, add 0.1 mol/L phosphate buffer solution, pH 7.0

Assay

Weigh accurately an amount of Cefazolin Sodium and Cefazolin RS, equivalent to about 0.1 g (potency), dissolve each in the internal standard solution to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 µL of each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of cefazolin to that of the internal standard.

Amount [µg (potency)] of cefazolin (C14H14N8O4S3) = M6 × Q1/Q2 × 1000

M6: Amount [µg (potency)] of Cefazolin RS

Internal standard solution—A solution of p-acetoanisidide in 0.1 mol/L phosphate buffer solution, pH 7.0 (11 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.27 g of disodium hydrogen phosphate dodecahydrate and 0.47 g of citric acid monohydrate in water to make 935 mL, and add 65 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefazolin is about 8 minutes.

System suitability—

System performance: Proceed as directed in the system suitability test. The relative standard deviation of the ratios of the peak areas of cefazolin is not more than 1.0%.

System repeatability: When the test is repeated 6 times with 5 µL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefazolin is not more than 1.0%.

Water <2.48> Not more than 2.5% (1.0 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Containers and storage—Tight containers.
to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 20 mL. Confirm that the peak area of cefazolin obtained from 5 μL of this solution is equivalent to 3 to 7% of that from 5 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay under Cefazolin Sodium.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cefazolin is not more than 1.0%.

Water <2.48> Not more than 3.0% (0.5 g, volumetric titration, direct titration). Use a mixture of formamide for Karl Fischer method and methanol for Karl Fischer method (2:1) instead of methanol for Karl Fischer method.

Bacterial endotoxins <4.01> Less than 0.05 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Cefazolin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of Cefazolin Sodium, dissolve in the internal standard solution to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefazolin RS, equivalent to about 50 mg (potency), dissolve in the internal standard solution to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefazolin Sodium.

Amount [mg (potency)] of cefazolin (C_{14}H_{13}N_{8}NaO_{4}S_{3})

\[ M_s \times \frac{Q_t}{Q_i} \]

\( M_s \): Amount [mg (potency)] of Cefazolin RS

Internal standard solution—A solution of p-acetanisidide in 0.1 mol/L phosphate buffer solution, pH 7.0 (11 in 20,000).

Containers and storage Containers—hermetic containers. Plastic containers for aqueous injections may be used.

Cefazolin Sodium Hydrate セファゾリンナトリウム水和物

C_{14}H_{13}N_{8}NaO_{4}S_{3}.5H_{2}O: 566.57

Monosodium (6R,7R)-3-(5-methyl-1,3,4-thiadiazol-2-ylsulfonyl[methyl])-8-oxo-7-[2-(1H-tetrazol-1-yl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate pentahydrate [115850-11-8]

Cefazolin Sodium Hydrate contains not less than 920 μg (potency) and not more than 975 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefazolin Sodium Hydrate is expressed as mass (potency) of cefazolin (C_{14}H_{13}N_{8}O_{4}S_{3}: 454.51).

Description Cefazolin Sodium Hydrate occurs as white to pale yellowish white crystals.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Cefazolin Sodium Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefazolin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the \(^1\)H spectrum of a solution of Cefazolin Sodium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 10), using sodium 3-trimethylsilylpropionate-\(d_2\) for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.22>: it exhibits single signals, A and B, at around \(\delta 2.7\) ppm and at around \(\delta 9.3\) ppm. The ratio of integrated intensity of each signal, A:B, is about 3:1.

(4) Cefazolin Sodium Hydrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Absorbance <2.24> \(E_{1\text{cm}}^{1\text{cm}}\) (272 nm): 272 - 292 (80 mg calculated on the anhydrous basis, water, 5000 mL).

Optical rotation <2.49> \([\alpha]_D^{20}\): -20 - -25° (2.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefazolin Sodium Hydrate in 10 mL of water: the pH of the solution is between 4.8 and 6.3.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefazolin Sodium Hydrate in 10 mL of water: the solu-
tion is clear, and when determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry, it is not more than 0.15.

(2) Heavy metals \(< 0.07\) — Proceed with 2.0 g of Cefazolin Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances — Dissolve 0.10 g of Cefazolin Sodium Hydrate in 20 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Perform the test with 5.0 mL of the sample solution as directed under Liquid Chromatography \(< 0.01\) according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the area of the peak having the relative retention time of about 0.2 with respect to cefazolin is not more than 1.0%, the area of the peak other than cefazolin and the peak mentioned above is not more than 0.5%, and the total area of the peaks other than cefazolin is not more than 2.0%. For this calculation use the area of the peak, having the relative retention time of about 0.2 with respect to cefazolin, after multiplying by its relative response factor 1.43.

Operating conditions —

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cefazolin, beginning after the solvent peak.

System suitability —

Test for required detectability: To 1 mL of the sample solution add 0.1 mol/L phosphate buffer solution, pH 7.0 to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Confirm that the peak area of cefazolin obtained with 5.0 mL of this solution is equivalent to 7 to 13% of that with 5.0 mL of the solution for system suitability test.

System performance: Dissolve 20 mg of Cefazolin Sodium Hydrate in 20 mL of a solution of \(p\)-acetoanisidide in 0.1 mol/L phosphate buffer solution, pH 7.0 (11 in 20,000). When the procedure is run with 5.0 mL of this solution under the above operating conditions, cefazolin and \(p\)-acetoanisidide are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5.0 mL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cefazolin is not more than 2.0%.

(4) Residual solvents — Be specified separately.

Water \(< 2.48\) — Not less than 13.7% and not more than 16.0%, 0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination.

Bacterial endotoxins \(< 0.01\) — Less than 0.10 EU/mg (potency).

Assay Weigh accurately an amount of Cefazolin Sodium Hydrate and Cefazolin RS, equivalent to about 20 mg (potency), dissolve in exactly 20 mL of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 5.0 mL each of the sample solution and standard solution as directed under Liquid Chromatography \(< 0.01\) according to the following conditions, and calculate the ratios, \(Q_1\) and \(Q_2\), of the peak area of cefazolin to that of the internal standard.

Amount [\(\mu g\) (potency)] of cefazolin (C\(_{14}\)H\(_{14}\)N\(_8\)O\(_4\)S\(_3\))

\[
M_5 = \frac{M_5}{2} \times \frac{Q_1}{Q_2} \times 1000
\]

\(M_5\): Amount [mg (potency)] of Cefazolin RS

Internal standard solution — A solution of \(p\)-acetoanisidide in 0.1 mol/L phosphate buffer solution, pH 7.0 (11 in 20,000).

Operating conditions —

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 \(\mu m\) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.27 g of disodium hydrogen phosphate dodecahydrate and 0.47 g of citric acid monohydrate in water to make 935 mL. To this solution, add 65 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefazolin is about 8 minutes.

System suitability —

System performance: When the procedure is run with 5.0 mL of the standard solution under the above operating conditions, cefazolin and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5.0 mL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefazolin is not more than 1.0%.

Containers and storage Containers — Hermetic containers.

Storage — Light-resistant.

Cefbuperazone Sodium

セフブペラゾンナトリウム

C\(_{22}\)H\(_{28}\)N\(_9\)NaO\(_9\)S\(_2\): 649.63

Monosodium (6\(R\),7\(S\))-7-\(R\)-\(2,3\)-dioxopiperazine-1-carbonyl]amino]-3-hydroxybutanoylamino]-7-methoxy-3-\(R\)\(-\text{methyl}-1H-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

Cefbuperazone Sodium contains not less than 870 \(\mu g\) (potency) per mg, calculated on the anhydrous
Cefbuperazone Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol and in pyridine, sparingly soluble in ethanol (95), and very slightly soluble in acetonitrile.

**Identification (1)** Determine the absorption spectrum of a solution of Cefbuperazone Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g of Cefbuperazone Sodium in 0.5 mL of deuterated pyridine for nuclear magnetic resonance spectroscopy and 1 drop of heavy water for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal A at around δ 1.1 ppm, and two doublet signals, B and C, at around δ 1.6 ppm and at around δ 5.1 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(3) Cefbuperazone Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

**Optical rotation** <2.49> [α]D: +48° - +56° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

**pH** <2.54> Dissolve 1.0 g of Cefbuperazone Sodium in 4 mL of water: the pH of the solution is between 4.0 and 6.0.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Cefbuperazone Sodium in 4 mL of water: the solution is clear and light yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cefbuperazone Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefbuperazone Sodium according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Cefbuperazone Sodium in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the percentages of each peak area of related substances from the sample solution against 50 times of the peak area of cefbuperazone from the standard solution; the amount of related substance I having the relative retention time of about 0.2 to cefbuperazone is not more than 2.0%, the amount of related substance II having the relative retention time of about 0.6 to cefbuperazone is not more than 4.5% and the amount of related substance III having the relative retention time of about 1.6 to cefbuperazone is not more than 1.0%, and the total amount of these related substances is not more than 6.0%. For these calculations, use the values of the peak areas of the related substances I and III obtained by the automatic integration method after multiplying by each relative response factors, 0.72 and 0.69, respectively.

**System suitability—**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of cefbuperazone.

**System performance**—When the procedure is run with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefbuperazone is not more than 2.0%.

**Water** <2.48> Not more than 1.0% (3 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Cefbuperazone Sodium and Cefbuperazone RS, equivalent to about 0.1 g (potency), and dissolve each in the mobile phase to make exactly 100 mL. Measure exactly 10 mL of each of these solutions, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q1 and Q2, of the peak area of cefbuperazone to that of the internal standard.

\[
\text{Amount [μg (potency)] of cefbuperazone (C}_{22}\text{H}_{29}\text{N}_{9}\text{O}_{9}\text{S}_{2}) = M_3 \times Q_1/Q_2 \times 1000
\]

M3: Amount [mg (potency)] of Cefbuperazone RS

**Internal standard solution—** A solution of acetonitrile in the mobile phase (1 in 4000).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.0 g of tetra-n-propylammonium bromide in 1000 mL of a mixture of water, acetonitrile and acetic acid-sodium acetate buffer solution, pH 5.0 (83:13:4). Flow rate: Adjust the flow rate so that the retention time of cefbuperazone is about 16 minutes.

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating con-
Cefcapene Pivoxil Hydrochloride Hydrate

セフカペン ピボキシル塩酸塩水和物

C$_2$H$_3$N$_2$O$_5$S$_2$·HCl·H$_2$O: 622.11
2,2-Dimethylpropanoyloxymethyl (6R,7R)-7-{(2Z)-2-(2-
aminothiazol-4-yl)pent-2-enylamino}-3-
carbamoyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]
2-
ene-2-carboxylate monohydrochloride monohydrate
[147816-24-8]

Cefcapene Pivoxil Hydrochloride Hydrate contains
not less than 722 μg (potency) and not more than 764
μg (potency) per mg, calculated on the anhydrous
basis. The potency of Cefcapene Pivoxil Hydrochloride Hydrate is expressed as mass (potency) of cefca-
pene (C$_{17}$H$_{19}$N$_2$O$_5$S$_2$: 453.49).

Description Cefcapene Pivoxil Hydrochloride Hydrate occurs as a white to pale yellowish white, crystalline powder or mass. It has slightly a characteristic odor.

It is freely soluble in N,N-dimethylformamide and in methanol, soluble in ethanol (99.5), slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Cefcapene Pivoxil Hydrochloride Hydrate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefcapene Pivoxil Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectra of Cefcapene Pivoxil Hydrochloride Hydrate and Cefcapene Pivoxil Hydrochloride RS as directed in the paste method under In-
frared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the 'H spectrum of a solution of Cefcapene Pivoxil Hydrochloride Hydrate in deuterated methanol for nuclear magnetic resonance spectroscopy (1 in 50) as dis-
rected under Nuclear Magnetic Resonance Spectroscopy <2.22>, using tetramethylsilane for nuclear magnetic reso-
nance spectroscopy as an internal reference compound: it exhibits a triplet signal A at around δ 6.3 ppm, and a single signal B at around δ 6.7 ppm, and the ratio of integrated intensity of each signal, A:B, is about 1:1.

(4) Dissolve 10 mg of Cefcapene Pivoxil Hydrochloride Hydrate in 2 mL of a mixture of water and methanol (1:1), and add 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation <2.49> [α]$_D^{20}$: +51 – +54° (0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07> Proceed with 2.0 g of Cefcapene Pivoxil Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substance I—Dissolve an amount of Cefca-
pen Pivoxil Hydrochloride Hydrate, equivalent to about 10
mg (potency), in 2 mL of methanol, add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Perform the test with 30 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method. If neces-

Ssary, compensate the baseline by performing in the same
manner as the test with 30 μL of a mixture of water and methanol (1:1). Measure the amount of the peak other than cefcapene pivoxil by the area percentage method: the amounts of the peaks, having the relative retention times of about 1.5 and about 1.7 with respect to cefcapene pivoxil, are not more than 0.2%, respectively. The amount of the peak other than the peaks mentioned above is not more than 0.1%, and the total of them is not more than 1.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-
length: 265 nm).

Column: A stainless steel column 4.6 mm in inside diam-
ter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase A: Dissolve 5.99 g of potassium dihydrogen phosphate in water to make 1100 mL. To this solution add 0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07> Proceed with 2.0 g of Cefcapene Pivoxil Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substance I—Dissolve an amount of Cefca-
pen Pivoxil Hydrochloride Hydrate, equivalent to about 10
mg (potency), in 2 mL of methanol, add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Perform the test with 30 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method. If neces-

Ssary, compensate the baseline by performing in the same
manner as the test with 30 μL of a mixture of water and methanol (1:1). Measure the amount of the peak other than cefcapene pivoxil by the area percentage method: the amounts of the peaks, having the relative retention times of about 1.5 and about 1.7 with respect to cefcapene pivoxil, are not more than 0.2%, respectively. The amount of the peak other than the peaks mentioned above is not more than 0.1%, and the total of them is not more than 1.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-
length: 265 nm).

Column: A stainless steel column 4.6 mm in inside diam-
ter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase A: Dissolve 5.99 g of potassium dihydrogen phosphate in water to make 1100 mL. To this solution add 0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm).

Mobile phase B: A mixture of methanol and water (22:3). Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 20</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>20 – 40</td>
<td>98 – 50</td>
<td>2 – 50</td>
</tr>
<tr>
<td>40 – 50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Flow rate: 0.8 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of cefcapene pivoxil.
**System suitability**—

Test for required detectability: To exactly 1 mL of the sample solution add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of cefcapene pivoxil obtained from 30 μL of this solution is equivalent to 7 to 13% of that of cefcapene pivoxil obtained from 30 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Cefcapene Pivoxil Hydrochloride Hydrate and 10 mg of propyl parahydroxybenzoate in 25 mL of methanol, and add water to make 50 mL. To 5 mL of this solution add the mixture of water and methanol (1:1) to make 50 mL. When the procedure is run with 30 μL of this solution under the above operating conditions, cefcapene pivoxil and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 7. System repeatability: When the test is repeated 3 times with 30 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cefcapene pivoxil is not more than 4.0%.

(3) Related substance II—Dissolve an amount of Cefcapene Pivoxil Hydrochloride Hydrate, equivalent to about 2 mg (potency), in N,N-dimethylformamide for liquid chromatography to make 20 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography to make 20 mL, and use this solution as the system suitability test. Pipet 1 mL of the solution add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of cefcapene pivoxil obtained from 30 μL of this solution is equivalent to 7 to 13% of that of cefcapene pivoxil obtained from 30 μL of the solution for system suitability test.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase: A solution of lithium bromide in N,N-dimethylformamide for liquid chromatography (13 in 5000).

Flow rate: Adjust the flow rate so that the retention time of cefcapene pivoxil is about 22 minutes.

Time span of measurement: About 1.8 times as long as the retention time of cefcapene pivoxil.

**System suitability**—

Test for required detection: To exactly 1 mL of the sample solution add N,N-dimethylformamide for liquid chromatography to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 3 mL of the solution for system suitability test, and add N,N-dimethylformamide for liquid chromatography to make exactly 10 mL. Confirm that the peak area of cefcapene pivoxil obtained from 20 μL of this solution is equivalent to 7 to 13% of that of cefcapene pivoxil obtained from 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates of the peak of cefcapene pivoxil is not less than 12,000 steps.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefcapene pivoxil is not more than 4.0%.

**Water [2.48]** Not less than 2.8% and not more than 3.7% (0.5 g, volumetric titration, back titration).

**Assay**—

Weigh accurately an amount of Cefcapene Pivoxil Hydrochloride Hydrate and Cefcapene Pivoxil Hydrochloride RS, equivalent to about 20 mg (potency), and dissolve each in a mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of each of these solutions, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (1:1) to them to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography [2.07] according to the following conditions, and calculate the ratios, Qc and Qo, of the peak area of cefcapene pivoxil to that of the internal standard of these solutions.

\[
\text{Amount [μg (potency)] of cefcapene (C}_{17}\text{H}_{19}\text{N}_{5}\text{O}_{6}\text{S}_2} = M_S \times Q_c / Q_o \times 1000
\]

\[
M_S: \text{Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride RS}
\]

**Internal standard solution**—A solution of p-benzylphenol in a mixture of water and methanol (1:1) (7 in 4000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 7.5 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.56 g of sodium dihydrogenphosphate dihydrate and 1.22 g of sodium 1-decanesulfonate in water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile and 100 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefcapene pivoxil is about 5 minutes.

**System suitability**—

System performance: Dissolve 0.2 g of Cefcapene Pivoxil Hydrochloride Hydrate in 10 mL of methanol, and warm in a water bath at 60°C for 20 minutes. After cooling, pipet 1 mL of this solution, and add exactly 10 mL of the internal standard solution and the mixture of water and methanol (1:1) to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cefcapene pivoxil, trans-cefcapene pivoxil and the internal standard are eluted in this order, the ratios of the retention time of trans-cefcapene pivoxil and the internal standard to that of cefcapene pivoxil are about 1.7 and 2.0, respectively, and the resolution between the peaks of trans-cefcapene pivoxil and the internal standard is not less than 1.5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of
Cefcapene Pivoxil Hydrochloride Fine Granules

Cefcapene Pivoxil Hydrochloride Fine Granules contain not less than 90.0% and not more than 110.0% of cefcapene (C<sub>17</sub>H<sub>19</sub>N<sub>5</sub>O<sub>6</sub>S<sub>2</sub>: 453.49).

**Method of preparation** Prepare as directed under Granules, with Cefcapene Pivoxil Hydrochloride Hydrate.

**Identification** Powder Cefcapene Pivoxil Hydrochloride Fine Granules. To a portion of the powder, equivalent to 10 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 40 mL of methanol, shake vigorously, and add methanol to make 50 mL. To 4 mL of this solution add methanol to make 50 mL, and filter through a membrane filter with a pore size of 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits a maximum between 264 nm and 268 nm.

**Purity** (1) Related substances I—Powder Cefcapene Pivoxil Hydrochloride Fine Granules. To a portion of the powder, equivalent to 5 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 1 mL of methanol, shake, and shake vigorously for 5 minutes, and filter through a membrane filter with a pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 30 μL of the sample solution as directed under Liquid Chromatography 2.01 according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks eluted before that of cefcapene pivoxil is not more than 4.0% of the total area of all peaks other than the solvent peak.

**Operating conditions**—
Proceed as directed in the operating conditions in the Purity (3) under Cefcapene Pivoxil Hydrochloride Hydrate.

**System suitability**—
Proceed as directed in the system suitability in the Purity (3) under Cefcapene Pivoxil Hydrochloride Hydrate.

**Water** Not more than 1.4% (0.5 g, volumetric titration, back titration). Perform the test without pulverizing the sample, and handling the sample under a relative humidity of less than 30%.

**Uniformity of dosage units** The granules in single-unit containers meet the requirement of the Mass variation test.

**Dissolution** Being specified separately.

**Particle size** It meets the requirements of Fine granules.

**Assay** Weigh accurately an amount of Cefcapene Pivoxil Hydrochloride Fine Granules, equivalent to about 0.2 g (potency) of and Cefcapene Pivoxil Hydrochloride Hydrate, add 100 mL of the mixture of water and methanol (1:1), shake vigorously for 10 minutes, add the mixture of water and methanol (1:1) to make exactly 200 mL, and centrifuge at 3000 rpm for 5 minutes. Filter the supernatant liquid through a membrane filter with a pore size of 0.45 μm, discard the first 1 mL of the filtrate, pipet the subsequent 2 mL of the filtrate, add exactly 5 mL of the internal standard solution and the mixture of water and methanol (1:1) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefcapene Pivoxil Hydrochloride RS, and dissolve in the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefcapene Pivoxil Hydrochloride Hydrate.

\[
\text{Amount [mg (potency)] of cefcapene (C}_{17}\text{H}_{19}\text{N}_{5}\text{O}_{6}\text{S}_{2}) = \frac{M_S}{M_C} \times \frac{Q_T}{Q_S} \times 10
\]

\(M_S\): Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride RS

**Internal standard solution**—A solution of p-benzylphenol in the mixture of water and methanol (1:1) (7 in 4000).

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.
Cefcapene Pivoxil Hydrochloride Tablets

セフカペン ピボキシル塩酸塩錠

Cefcapene Pivoxil Hydrochloride Tablets contain not less than 90.0% and not more than 105.0% of the labeled amount of cefcapene (C17H19N5O6S2: 453.49).

**Method of preparation** Prepare as directed under Tablets, with Cefcapene Pivoxil Hydrochloride Hydrate.

**Identification** To an amount of powdered Cefcapene Pivoxil Hydrochloride Tablets, equivalent to about 10 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate according to the labeled amount, add 40 mL of methanol, shake vigorously, and add methanol to make 50 mL. To 4 mL of this solution add methanol to make 50 mL, filter through a membrane filter with pore size of 0.45 μm, and use the filtrate as the sample solution. Determine the absorbance spectrum of the sample solution as directed under Ultraviolet-Visible Spectrophotometry \(<2.24\): it exhibits a maximum between 263 nm and 267 nm.

**Purity (1)** Related substances—To an amount of powdered Cefcapene Pivoxil Hydrochloride Tablets, equivalent to about 5 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate according to the labeled amount, add 1 mL of methanol, and shake. Add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 5 minutes, and filter through a membrane filter with pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 30 μL of the sample solution as directed under Liquid Chromatography \(<2.07\) according to the following conditions, and determine each peak area by the automatic integration method. If necessary, proceed with 30 μL of the mixture of water and methanol (1:1) in the same manner as the sample solution to compensate the base line. Calculate the amounts of the peaks other than cefcapene pivoxil by the area percentage method: the peak, having the relative retention time of about 1.3 with respect to cefcapene pivoxil, is not more than 0.4%, the peak of cefcapene pivoxil trans-isomer, having the relative retention time of about 1.5, is not more than 0.5%, any other peaks are not more than 0.3%, respectively, and the total of these peaks is not more than 2.0%.

**Operating conditions**—Proceed as directed in the operating conditions in the Purity (2) under Cefcapene Pivoxil Hydrochloride Hydrate.

**System suitability**—Proceed as directed in the system suitability in the Purity (2) under Cefcapene Pivoxil Hydrochloride Hydrate.

**Water** \(<2.48\) Not more than 3.9% (0.5 g, volumetric titration, back titration). Powdering of the sample tablets and handling of the powder are performed under the relative humidity of not exceeding 30%.

**Uniformity of dosage units** \(<6.02\> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cefcapene Pivoxil Hydrochloride Tablets add 5 mL of water, and shake vigorously for 5 minutes to disintegrate. Add 20 mL of methanol, shake vigorously for 5 minutes, add a mixture of methanol and water (4:1) to make exactly 50 mL, and centrifuge at 3000 rpm for 5 minutes. Filter the supernatant liquid through a membrane filter with pore size of 0.45 μm, and discard the first 1 mL of the filtrate. Pipet 0.5 mL of the subsequent filtrate, equivalent to about 6 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate according to the labeled amount, add exactly 15 mL of the internal standard solution, add a mixture of water and methanol (1:1) to make 75 mL, and use this solution as the sample solution. Hereinafter, proceed as directed in the Assay.

\[
\text{Amount [mg (potency)] of cefcapene (C}_{17}\text{H}_{19}\text{N}_{5}\text{O}_{6}\text{S}_2} = \frac{M_S \times Q_T}{Q_S} \times \frac{15}{V}
\]

\(M_S\): Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride RS

**Internal standard solution**—A solution of p-benzylphenol in a mixture of water and methanol (1:1) (7 in 4000).

**Dissolution** Being specified separately.

**Assay** To an amount of Cefcapene Pivoxil Hydrochloride Tablets, equivalent to about 0.6 g (potency) of Cefcapene Pivoxil Hydrochloride Hydrate according to the labeled amount, add 20 mL of water, and shake for 5 minutes to disintegrate. Add 80 mL of methanol, shake vigorously for 5 minutes, add a mixture of methanol and water (4:1) to make exactly 200 mL, and centrifuge at 3000 rpm for 5 minutes. Filter the supernatant liquid through a membrane filter with pore size of 0.45 μm, and discard the first 1 mL of the filtrate. Pipet 2 mL of the subsequent filtrate, add exactly 15 mL of the internal standard solution, add the mixture of water and methanol (1:1) to make 75 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefcapene Pivoxil Hydrochloride RS, equivalent to about 20 mg (potency), and dissolve in the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefcapene Pivoxil
Cefdinir / Official Monographs

Hydrochloride Hydrate.

Amount [mg (potency)] of cefcapene (C_{17}H_{18}N_{5}O_{6}S_{2})

\[ M_s = M_s \times Q_t / Q_o \times 30 \]

M_s: Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride RS

Internal standard solution—A solution of p-benzylphenol in the mixture of water and methanol (1:1) (7 in 4000).

Containers and storage—Tight containers.

Cefdinir

セフジニル

![Cefdinir structure](image)

C_{14}H_{13}N_{5}O_{5}S_{2}: 395.41

(6R,7R)-7-[[2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetylamino]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [91832-40-5]

Cefdinir contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg. The potency of Cefdinir is expressed as mass (potency) of cefdinir (C_{14}H_{13}N_{5}O_{5}S_{2}).

Description—Cefdinir occurs as a white to light yellow crystalline powder. It is practically insoluble in water, in ethanol (95%) and in diethyl ether.

It dissolves in 0.1 mol/L phosphate buffer solution, pH 7.0.

Identification (1) Determine the absorption spectra of solutions of Cefdinir and Cefdinir RS in 0.1 mol/L phosphate buffer solution, pH 7.0 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.20>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectra of Cefdinir and Cefdinir RS as directed in the past method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefdinir in a mixture of deuterated dimethyl sulfoxide and heavy water for nuclear magnetic resonance spectroscopy (4:1) (1 in 10), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>:

Absorbance <2.24> \[ E_{1%}^{10} (287 nm): 570 - 610 \]

Optical rotation <2.49> \[ [\alpha]_{D}^{20}: -58 - -66^\circ \]
System performance: Dissolve 0.03 g of Cefdinir RS and 2 mg of cefdinir lactam ring-cleavage lactones in 3 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and add tetramethylammonium hydroxide TS, pH 5.5, to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, peak 1 and peak 2 of cefdinir lactam ring-cleavage lactones separated into 4 peaks, cefdinir, peak 3 and peak 4 of remaining cefdinir lactam ring-cleavage lactones are eluted in this order. Relative retention time of peak 3 of cefdinir lactam ring-cleavage lactone to the retention time of cefdinir is not less than 1.09. The number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 7000 steps and not more than 3.0, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefdinir is not more than 2.0%.

Water Not more than 2.0% (% g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Cefdinir and Cefdinir RS equivalent to about 20 mg (potency), dissolve each in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 5 μL of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A₁ and A₃, of cefdinir of the solutions.

\[
\text{Amount [μg (potency)] of } C_{14}H_{13}N_{5}O_{5}S_{2} = M_o \times A_1 / A_3 \times 1000
\]

\[
M_o: \text{Amount [mg (potency)] of Cefdinir RS}
\]

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 1000 mL of tetramethylammonium hydroxide TS, pH 5.5, add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS. To 900 mL of this solution add 60 mL of acetonitrile for liquid chromatography and 40 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefdinir is about 8 minutes.

System suitability—

System performance: Dissolve 2 mg of Cefdinir RS and 5 mg of cefdinir lactam ring-cleavage lactones in 10 mL of 0.1 mol/L phosphate buffer solution, pH 7.0. When the procedure is run with 5 μL of this solution under the above operating conditions, peak 1 and peak 2 of cefdinir lactam ring-cleavage lactones separated into 4 peaks, cefdinir, peak 3 and peak 4 of remaining cefdinir lactam ring-cleavage lactones are eluted in this order. The resolution between the peak 2 of cefdinir lactam ring-cleavage lactone and that of cefdinir is not less than 1.2. The number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefdinir is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Cefdinir Capsules

Cefdinir Capsules contain not less than 90.0% and not more than 110.0% of the labeled amount of cefdinir (C₁₄H₁₃N₅O₅S₂: 395.41).

Method of preparation Prepare as directed under Capsules, with Cefdinir.

Identification To an amount of the contents of Cefdinir Capsules, equivalent to 10 mg (potency) of Cefdinir according to the labeled amount, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, exposure to ultrasonic waves for 1 minute, and filter. To 2 mL of the filtrate add 0.1 mol/L phosphate buffer solution, pH 7.0 to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 221 nm and 225 nm and between 285 nm and 289 nm.

Uniformity of dosage unit It meets the requirement of the Mass variation test.

Dissolution When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate of a 50-mg capsule in 30 minutes is not less than 80%, and that of a 100-mg capsule in 45 minutes is not less than 75%.

Start the test with 1 capsule of Cefdinir Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 56 μg (potency) of Cefdinir according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg (potency) of Cefdinir RS, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography, and determine the peak areas, A₁ and A₃, of cefdinir.

Dissolution rate (%) with respect to the labeled amount of cefdinir (C₁₄H₁₃N₅O₅S₂)

\[
= M_o \times A_1 / A_3 \times V/V \times 1/C \times 180
\]

\[
M_o: \text{Amount [mg (potency)] of Cefdinir RS}
\]

C: Labeled amount [mg (potency)] of cefdinir (C₁₄H₁₃N₅O₅S₂) in 1 capsule
Operating conditions—
Proceed as directed in the operating conditions in the Assay under Cefdinir.

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefdinir is not more than 1.0%.

Assay
Weigh accurately not less than 5 Cefdinir Capsules, take out the contents, and powder. Wash the empty capsules with a little amount of diethyl ether, if necessary, to allow to stand at a room temperature to vaporize the adhering diethyl ether, and weigh accurately the mass of the capsules to calculate the mass of the contents. Weigh accurately an amount of the contents, equivalent to about 0.1 g (potency) of Cefdinir according to the labeled amount, add 70 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL. Centrifuge this solution at 3000 revolutions per minute for 10 minutes, and use this solution as the sample solution.

Separately, weigh accurately an amount of Cefdinir RS, equivalent to about 0.1 g (potency) of Cefdinir according to the labeled amount, add 70 mL of 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefdinir.

Assay under Cefdinir
Proceed as directed in the operating conditions in the Assay under Cefdinir.

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefdinir is not more than 1.0%.

Cefdinir Fine Granules
セフジニル細粒

Cefdinir Fine Granules contain not less than 93.0%, and not more than 107.0% of the labeled amount of cefdinir (C14H13N5O5S2: 395.41).

Method of preparation
Prepare as directed under Granules, with Cefdinir.

Identification
To an amount of Cefdinir Fine Granules, equivalent to 10 mg (potency) of Cefdinir according to the labeled amount, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, exposure to ultrasonic waves for 1 minute, and filter. To 2 mL of the filtrate add 0.1 mol/L phosphate buffer solution, pH 7.0 to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 221 nm and 225 nm and between 285 nm and 289 nm.

Uniformity of dosage units <6.02>
The granules in single-unit containers meet the requirement of the Mass variation test.

Dissolution <6.10>
When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Cefdinir Fine Granules is not less than 75%.

Start the test with an accurate amount of Cefdinir Fine Granules, equivalent to about 0.1 g (potency) of Cefdinir according to the labeled amount, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 µm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg (potency) of Cefdinir RS, and dissolve in the dissolution medium to make exactly 50 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and determine the peak areas, A1 and A5, of cefdinir.

Dissolution rate (%) with respect to the labeled amount of cefdinir (C14H13N5O5S2)

\[
M_s = \frac{A_5}{A_1} \times \frac{A_1}{A_5} \times \frac{1}{C} \times 360
\]

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Cefdinir.

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefdinir is not more than 1.0%.

Particle size <6.03>
It meets the requirements of Fine granules.

Assay
Powder, if necessary, and weigh accurately an amount of Cefdinir Fine Granules, equivalent to about 0.1 g (potency) of Cefdinir according to the labeled amount, add 70 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL. Centrifuge at 3000 revolutions per minute for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefdinir RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefdinir.

Assay under Cefdinir

\[
M_s = \frac{A_5}{A_1} \times \frac{A_1}{A_5} \times 5
\]
Cefditoren Pivoxil

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Cefditoren Pivoxil**

C₂₅H₂₈N₆O₇S₃: 620.72
2,2-Dimethylpropanoyloxymethyl (6R,7R)-7-([Z]-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-([Z]-2-(4-methylthiazol-5-yl)ethenyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

C₂₂H₂₃N₇O₇S₃: 506.58

**Description** Cefditoren Pivoxil occurs as a light yellowish white to light yellow crystalline powder.

It is sparingly soluble in methanol, slightly soluble in acetonitrile and in ethanol (95), very slightly soluble in diethyl ether and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

**Identification** (1) Dissolve 5 mg of Cefditoren Pivoxil in 3 mL of hydroxyammonium chloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS and shake: a red-brown color develops.

(2) Dissolve 1 mg of Cefditoren Pivoxil in 1 mL of dilute hydrochloric acid and 4 mL of water, add 3 drops of sodium nitrite TS under ice-cooling, shake, and allow to stand for 2 minutes. Then add 1 mL of ammonium amidosulfate TS, shake well, and allow to stand for 1 minute, and add 1 mL of N,N-diethyl-N'-1-naphthylethylenediamine oxalate TS: a purple color develops.

(3) Determine the absorption spectrum of a solution of Cefditoren Pivoxil in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefditoren Pivoxil RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the 1H spectrum of a solution of Cefditoren Pivoxil in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 50), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.27>:

- It exhibits single signals A, B and C, at around δ 1.1 ppm, at around δ 2.4 ppm and at around δ 6.0 ppm
- Double signals D and E, at around δ 4.0 ppm
- Single signals F and G, at around δ 8.6 ppm
- The ratio of integrated intensity of each signal A:B:C:D:E:F is 9:3:3:1:1:1.

**Assay** Conduct this procedure using light-resistant vessels. Weigh accurately an amount of Cefditoren Pivoxil and Cefditoren Pivoxil RS, equivalent to about 40 mg (potency), dissolve in 40 mL of acetonitrile, add exactly 10 mL each of the internal standard solution, and add acetonitrile to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> and calculate the ratios, Q₂ and Q₃, of the peak area of cefditoren pivoxil to that of the internal standard.

Amount [μg (potency)] of cefditoren (C₁₉H₁₈N₆O₅S₃) = \[\frac{M₅ \times Q₂}{Q₃} \times 1000\]

M₅: Amount [mg (potency)] of Cefditoren Pivoxil RS

**Related substances**—Being specified separately.

**Residue on ignition** Being specified separately.

**Assay** Conduct this procedure using light-resistant vessels. Weigh accurately an amount of Cefditoren Pivoxil and Cefditoren Pivoxil RS, equivalent to about 40 mg (potency), dissolve in 40 mL of acetonitrile, add exactly 10 mL each of the internal standard solution, and add acetonitrile to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> and calculate the ratios, Q₂ and Q₃, of the peak area of cefditoren pivoxil to that of the internal standard.

Amount [μg (potency)] of cefditoren (C₁₉H₁₈N₆O₅S₃) = \[\frac{M₅ \times Q₂}{Q₃} \times 1000\]

M₅: Amount [mg (potency)] of Cefditoren Pivoxil RS

**Internal standard solution**—A solution of propyl p-hydroxybenzoate in acetonitrile (1 in 200).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.58 g of ammonium formate in 900 mL of water, adjust to pH 6.0 with dilute formic acid (1 in 250), and add water to make 1000 mL. To 450 mL of this solution add 275 mL of acetonitrile and 275 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefditoren pivoxil is about 15 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefditoren pivoxil are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefditoren pivoxil to that of the internal standard is not more than 1.0%.
Cefditoren Pivoxil Fine Granules

Cefditoren Pivoxil Fine Granules contain not less than 90.0% and not more than 110.0% of the labeled amount of cefditoren (C19H18N6O5S3: 506.58).

Method of preparation Prepare as directed under Granules, with Cefditoren Pivoxil.

Identification To an amount of powdered Cefditoren Pivoxil Fine Granules, equivalent to 0.1 g (potency) of Cefditoren Pivoxil according to the labeled amount, add 10 mL of acetonitrile, shake vigorously, and filter. To 1 mL of the filtrate add acetonitrile to make 50 mL. To 1 mL of this solution add acetonitrile to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits a maximum between 229 nm and 233 nm.

Purity Related substances—Being specified separately.

Loss on drying 2.41 Not more than 4.5% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Uniformity of dosage units 6.02 The granules in single-unit containers meet the requirement of the Mass variation test.

Dissolution Being specified separately.

Particle size 6.03 It meets the requirements of Fine granules.

Assay Conduct this procedure using light-resistant vessels. Weigh accurately an amount of powdered Cefditoren Pivoxil Fine Granules, equivalent to about 40 mg (potency) of Cefditoren Pivoxil according to the labeled amount, add 70 mL of diluted acetonitrile (3 in 4), and shake vigorously. To this solution add exactly 10 mL of the internal standard solution, then add acetonitrile to make 100 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefditoren Pivoxil RS, equivalent to about 20 mg (potency), dissolve in 20 mL of acetonitrile, shake, and filter. To 5 mL of the filtrate add methanol to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits a maximum between 229 nm and 233 nm.

Purity Related substances—Being specified separately.

Loss on drying 2.41 Not more than 4.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Uniformity of dosage units 6.02 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Cefditoren Pivoxil Tablets

Cefditoren Pivoxil Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of cefditoren (C19H18N6O5S3: 506.58).

Method of preparation Prepare as directed under Tablets, with Cefditoren Pivoxil.

Identification To an amount of powdered Cefditoren Pivoxil Tablets, equivalent to 35 mg (potency) of Cefditoren Pivoxil according to the labeled amount, add 100 mL of methanol, shake, and filter. To 5 mL of the filtrate add methanol to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits a maximum between 229 nm and 233 nm.

Purity Related substances—Being specified separately.

Loss on drying 2.41 Not more than 4.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Uniformity of dosage units 6.02 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Start the test with 1 tablet of Cefditoren Pivoxil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent 5 V/mL of the filtrate add to make exactly 5 V/mL, and use this solution as the standard solution. Separately, weigh accurately an amount of Cefditoren
Pivoxil RS, equivalent to about 22 mg (potency), dissolve in 20 mL of diluted acetonitrile (3 in 4), then add the dissolution medium to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, at 272 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry $<2.24>$ using water as the control.

Dissolution rate (%) with respect to the labeled amount

$$A_T = \frac{M_s \times A_t/A_s \times V/V \times 1/C \times 45}{M_s}$$

$M_S$: Amount [mg (potency)] of Cefditoren Pivoxil RS
$C$: Labeled amount [mg (potency)] of cefditoren pivoxil 
($C_{19}H_{24}N_6O_{5}S_2$) in 1 tablet

**Assay** Conduct this procedure using light-resistant vessels. To an amount of Cefditoren Pivoxil Tablets, equivalent to 0.5 g (potency) of Cefditoren Pivoxil according to the labeled amount, add 63 mL of the 1st fluid for disintegration test, shake vigorously, add about 125 mL of acetonitrile, shake again, and add acetonitrile to make exactly 250 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add acetonitrile (3 in 4) to make 50 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefditoren Pivoxil RS, equivalent to about 20 mg (potency), dissolve in 20 mL of acetonitrile, add exactly 5 mL of the internal standard solution, then add acetonitrile to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefditoren Pivoxil.

Amount [mg (potency)] of cefditoren ($C_{19}H_{24}N_6O_{5}S_2$)

$$A_T = \frac{M_s \times A_t/A_s \times V/V \times 1/C \times 25}{M_s}$$

$M_S$: Amount [mg (potency)] of Cefditoren Pivoxil RS

**Internal standard solution**—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 200).

**Containers and storage** Containers—Tight containers.

### Cefepime Dihydrochloride Hydrate

セフェピム塩酸塩水和物

Cefepime Dihydrochloride Hydrate contains not less than 835 μg (potency) and not more than 886 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefepime Dihydrochloride Hydrate is expressed as mass (potency) of cefepime ($C_{19}H_{24}N_6O_{5}S_2$: 480.56).

**Description** Cefepime Dihydrochloride Hydrate occurs as a white to yellowish white, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (95), and practically in soluble in diethyl ether.

**Identification** (1) Dissolve 0.02 g of Cefepime Dihydrochloride Hydrate in 2 mL of water, add 1 mL of a solution of hydroxylationmonium chloride (1 in 10) and 2 mL of sodium hydroxide TS, allow to stand for 5 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS: a red-brown color develops.

(2) Determine the absorption spectra of solutions (1 in 20,000) of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride RS as directed under Ultraviolet-visible Spectrophotometry $<2.24>$, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectra of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25>$, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Determine the pH spectrum of a solution of Cefepime Dihydrochloride Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy $<2.21>$, using sodium 3-trimethylsilylpropionate-d$_4$ for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 3.1 ppm and at around δ 7.2 ppm, respectively, and the ratio of integrated intensity of each signal, A:B, is about 3:1.

(5) Dissolve 15 mg of Cefepime Dihydrochloride Hydrate in 5 mL of water, and add 2 drops of silver nitrate TS: a white turbidity is produced.

**Absorbance** $<2.24>$ $E_{1cm}^{1\%}$ (259 nm): 310 - 340 (50 mg calculated on the anhydrous basis, water, 1000 mL).

**Optical rotation** $<2.49> [\alpha]_{D}^{20} + 39 - +47^\circ$ (60 mg calculated on the anhydrous basis, water, 20 mL, 100 mm)

**pH** $<2.54>$ Dissolve 0.1 g of Cefepime Dihydrochloride Hydrate in 10 mL of water: the pH of this solution is between 1.6 and 2.1.

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Cefepime Dihydrochloride Hydrate in 5 mL of a solution of L-arginine (3 in 50): the solution is clear and has no more color than Matching Fluid H.

(2) Heavy metals $<1.07>$—Proceed with 1.0 g of Cefepime Dihydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) N-Methylpyrrrolidine—Weigh accurately an amount of Cefepime Dihydrochloride Hydrate equivalent to about 80 mg (potency), dissolve in diluted nitric acid (2 in 3125) to make exactly 10 mL, and use this solution as the sample solution. Separately, put 30 mL of water in a 100-mL volumetric flask, weigh accurately the mass of flask, then add about 0.125 g of N-methylpyrrrolidine, weigh accurately the mass of the flask again, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add diluted nitric acid (2 in 3125) to make exactly 100 mL, and use this solution as the
standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0> according to the following conditions, and determine the peak areas, $A_t$ and $A_S$, of N-methylpyrrolidine by the automatic integration method. Calculate the amount of N-methylpyrrolidine per 1 mg (potency) of Cefepime Dihydrochloride Hydrate by the following equation: not more than 0.5%. The sample solution must be tested within 20 minutes after preparation.

$$\text{Amount (mg) of N-methylpyrrolidine} = \frac{M_S \times f}{M_t} \times \frac{A_t}{A_S} \times \frac{1}{250}$$

$M_S$: Amount (mg) of N-methylpyrrolidine  
$M_t$: Amount [mg (potency)] of sample  
$f$: Purity (%) of N-methylpyrrolidine

**Operating conditions—**

Detector: An electric conductivity detector

Column: A plastic tube 4.6 mm in inside diameter and 5 cm in length, packed with hydrophilic silica gel for liquid chromatography carrying sulfonic acid groups having the exchange capacity of about 0.3 meq per g (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 990 mL of diluted nitric acid (2 in 3125) add 10 mL of acetonitrile.

Flow rate: 1.0 mL per minute.

**System suitability—**

System performance: To 20 mL of a solution of sodium chloride (3 in 1000) add 0.125 g of N-methylpyrrolidine, and add water to make 100 mL. To 4 mL of this solution add diluted nitric acid (2 in 3125) to make 100 mL. When the procedure is run with 100 μL of this solution under the above operating conditions, sodium and N-methylpyrrolidine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 3 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of N-methylpyrrolidine is not more than 4.0%.

(4) Related substances—Dissolve about 0.1 g of Cefepime Dihydrochloride Hydrate in the mobile phase A to make 50 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.0> according to the following conditions, and determine the area of each peak by the automatic integration method. Calculate the total of the peak areas other than cefepime: not more than 0.5%.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 0.57 g of ammonium dihydrogenphosphate in 1000 mL of water.

Mobile phase B: Acetonitrile.

Flow of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 25</td>
<td>100 → 75</td>
<td>0 → 25</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of cefepime is about 9.5 minutes.

Time span of measurement: About 2.5 times as long as the retention time of cefepime.

**System suitability—**

Test for required detection: To 1 mL of the sample solution add the mobile phase A to make 10 mL, and use this solution as the solution for system suitability test. To 1 mL of the solution for system suitability test add the mobile phase A to make 10 mL, and use this solution as the solution for test for required detection. Pipet 1 mL of the solution for test for required detection, add the mobile phase A to make exactly 10 mL. Conform that the peak area of cefepime obtained from 5 μL of this solution is equivalent to 7 to 13% of that of cefepime obtained from 5 μL of the solution for test for required detection.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates of the peak of cefepime is not less than 6000 steps.

System repeatability: When the test is repeated 3 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefepime is not more than 2.0%.

**Water <2.4>** Not less than 3.0% and not more than 4.5% (Weigh accurately about 50 mg, and add exactly 2 mL of methanol for water determination to dissolve. Use exactly 0.5 mL of this solution as the test solution; coulometric titration).

**Residue on ignition <2.4>** Not more than 0.1% (1 g).

**Bacterial endotoxins <4.0>** Less than 0.04 EU/mg (potency).

**Assay** Weigh accurately an amount of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride RS, equivalent to about 60 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.0> according to the following conditions, and determine the peak areas, $A_t$ and $A_S$, of cefepime of each solution.

$$\text{Amount [μg (potency)] of cefepime (C_{19}H_{24}N_{6}O_{5}S_{2})} = \frac{M_S \times A_t}{A_S} \times 1000$$

$M_S$: Amount [μg (potency)] of Cefepime Dihydrochloride RS

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.
Cefepime Dihydrochloride for Injection

Cefepime Dihydrochloride for Injection is a preparation for injection, which is dissolved before use. It contains not less than 95.0% and not more than 110.0% of the labeled amount of Cefepime (C₁₉H₂₄N₆O₅S₂: 480.56).

Method of preparation
Prepare as directed under Injections, with Cefepime Dihydrochloride Hydrate.

Description
Cefepime Dihydrochloride for Injection occurs as a white to pale yellow powder.

Identification
(1) Dissolve 40 mg of Cefepime Dihydrochloride in 2 mL of water, add 1 mL of a solution of hydroxylammonium chloride (1 in 10) and 2 mL of sodium hydroxide TS, allow to stand for 5 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS: a red-brown color develops.

(2) Determine the absorption spectrum of a solution of Cefepime Dihydrochloride for Injection (1 in 12,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 233 nm and 237 nm and between 255 nm and 259 nm.

pH <2.54> The pH of a solution obtained by dissolving an amount of Cefepime Dihydrochloride for Injection, equivalent to 0.5 g (potency) of Cefepime Dihydrochloride Hydrate according to the labeled amount, in 5 mL of water is between 4.0 and 6.0.

Purity
(1) Clarity and color of solution—Dissolve an amount of Cefepime Dihydrochloride for Injection, equivalent to 0.5 g (potency) of Cefepime Dihydrochloride Hydrate according to the labeled amount, in 5 mL of water: the solution is clear and colorless or light yellow. The color is not darker than Matching Fluid 1.

(2) N-Methylpyrrolidine—Weigh accurately an amount of Cefepime Dihydrochloride for Injection, equivalent to about 0.2 g (potency) of Cefepime Dihydrochloride Hydrate according to the labeled amount, dissolve in diluted nitric acid (2 in 625) to make exactly 20 mL, and use this solution as the sample solution. Separately, transfer 30 mL of water into a 100-mL volumetric flask, weigh accurately the mass of the flask, add about 0.125 g of N-methylpyrrolidine, then weigh accurately the mass, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add diluted nitric acid (2 in 3125) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas of N-methylpyrrolidine, A₁ and A₂, by the automatic integration method within 20 minutes after the sample solution is prepared. Calculate the amount of N-methylpyrrolidine per mg (potency) of Cefepime Dihydrochloride for Injection by the following formula: not more than 1.0%.

\[
\text{Amount (mg) of N-methylpyrrolidine} = \frac{M_s \times A_2}{A_1} \times \frac{A_1}{A_2} \times 125
\]

Mₛ: Amount (mg) of N-methylpyrrolidine
M₁: Amount [mg (potency)] of cefepime in the sample
f: Purity (%) of N-methylpyrrolidine

Operating conditions—Proceed as directed in the operating conditions in the Purity (3) under Cefepime Dihydrochloride Hydrate.

System suitability—Proceed as directed in the system suitability in the Purity (3) under Cefepime Dihydrochloride Hydrate.

Water <2.48> Not more than 4.0% (Weigh accurately about 50 mg of Cefepime Dihydrochloride for Injection, dissolve in exactly 2 mL of methanol for Karl Fischer method, and perform the test with exactly 0.5 mL of this solution. Coulometric titration).

Bacterial endotoxins <4.01> Less than 0.06 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to the Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay
Weigh accurately the mass of the contents of not less than 10 Cefepime Dihydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 60 mg (potency) of Cefepime Dihydrochloride Hydrate according to the labeled amount, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefepime Dihydrochloride RS, equivalent to about 60 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefepime Dihydrochloride Hydrate.

Amount [μg (potency)] of cefepime (C₁₉H₂₄N₆O₅S₂)
\[
= M_S \times \frac{A_1}{A_2} \times 1000
\]
Mₕ: Amount [mg (potency)] of Cefepime Dihydrochloride RS

Containers and storage  Containers—Hermetic containers. Storage—Light-resistant.

Cefixime Hydrate

Cefixime Hydrate occurs as a white to light yellow crystalline powder. It is freely soluble in methanol and in dimethylsulfoxide, sparingly soluble in ethanol (99.5), and practically insoluble in water.

Identification  (1) Determine the absorption spectrum of a solution of Cefixime Hydrate in 0.1 mol/L phosphate buffer solution, pH 7.0 (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.22>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefixime RS prepared in the same manner as the sample solution; both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefixime Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefixime RS; both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.05 g of Cefixime Hydrate in 0.5 mL of a mixture of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy and heavy water for nuclear magnetic resonance spectroscopy (4:1). Determine the ¹H spectrum of this solution, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.27>; it exhibits a single signal A at around δ 4.7 ppm, and a multiple signal B between δ 6.5 ppm and δ 7.4 ppm. The ratio of integrated intensity of these signals, A:B, is about 1:1.

Optical rotation <2.49> [α]D: −75° to −88° (0.45 g calculated on the anhydrous basis, a solution of sodium hydrogen carbonate (1 in 50), 50 mL, 100 mm).

Purity  Dissolve 0.1 g of Cefixime Hydrate in 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, measure the areas of the peaks by the automatic integration method, and calculate the amounts of these peak areas by the area percentage method: the amount of each peak area other than cefixime is not more than 1.0%, and the total area of the peaks other than cefixime is not more than 2.5%.

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 3 times as long as the retention time of cefixime beginning after the solvent peak.

System suitability—
Test for required detection: Pipet 1 mL of the sample solution, and add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL. Confirm that the peak height of cefixime obtained from 10 μL of this solution is equivalent to 20 to 60 mm.

System performance: Dissolve about 2 mg of Cefixime RS in 200 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the solution for system suitability test. When the procedure is run with 10 μL of the solution according to the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefixime is not more than 2.0%.

Water <2.48> Not less than 9.0 and not more than 12.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay  Weigh accurately an amount of Cefixime Hydrate and Cefixime RS, equivalent to about 0.1 g (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL each. Pipet 10 mL each of these solutions, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 50 mL each, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₃, of cefixime of these solutions.

Amount [μg (potency)] of C₁₆H₁₅N₅O₇S₂ = Mₕ × A₁/A₃ × 1000

Mₕ: Amount [mg (potency)] of Cefixime RS

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 125 mm in length, packed with octadecylsilylized silica gel for liquid chromatography (4 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To 25 mL of a solution of tetrabutylammonio-
Cefixime Capsules

Cefixime Capsules contain not less than 90.0% and not more than 105.0% of the labeled potency of cefixime (C16H15N5O7S2: 453.45).

Method of preparation
Prepare as directed under Capsules, with Cefixime Hydrate.

Identification
Take out the contents of Cefixime Capsules, to a quantity of the contents of Cefixime Capsules, equivalent to 70 mg (potency) of Cefixime Hydrate according to the labeled amount, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, shake for 30 minutes, and filter. To 1 mL of the filtrate add 0.1 mol/L phosphate buffer solution, pH 7.0, to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\): it exhibits a maximum between 286 nm and 290 nm.

Purity
Related substances—Take out the contents of Cefixime Capsules, to a quantity of the contents of Cefixime Capsules, equivalent to 0.1 g (potency) of Cefixime Hydrate according to the labeled amount, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, shake for 30 minutes, and filter, and use the filtrate as the sample solution. Perform the test with 10 mL of the sample solution as directed under Liquid Chromatography \(<2.26\): the retention time of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System suitability
System performance: When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of peak areas of cefixime is not more than 2.0%.

Containers and storage
Containers—Tight containers.
Storage—Light-resistant.

Dissolution
When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 7.5 as the dissolution medium, the dissolution rates in 60 minutes of 50-mg (potency) capsule and in 90 minutes of 100-mg (potency) capsule are not less than 80%, respectively.

Start the test with 1 capsule of Cefixime Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add the dissolution medium to make exactly \(V\) mL so that each mL contains about 56 \(\mu\)g (potency) of Cefixime Hydrate according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefixime RS, equivalent to about 28 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefixime Hydrate.

Amount [mg (potency)] of cefixime \((C_{16}H_{15}N_{5}O_{7}S_{2})\) = \(M_{S} \times A_{T} / A_{S} \times V / 20\)

\(M_{S}\): Amount [mg (potency)] of Cefixime RS

Note: Not more than 12.0% (0.1 g of the contents, volumetric titration, direct titration).

Water \(<2.40\): Not more than 12.0% (0.1 g of the contents, volumetric titration, direct titration).

Uniformity of dosage units \(<6.02\)
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Related substances—Take out the contents of 1 capsule of Cefixime Capsules, and to the contents and the capsule shells add 7\(V\)/10 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly \(V\) mL so that each mL contains about 1 mg (potency) of Cefixime Hydrate. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefixime RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefixime Hydrate.

Amount [mg (potency)] of cefixime \((C_{16}H_{15}N_{5}O_{7}S_{2})\) = \(M_{S} \times A_{T} / A_{S} \times V / 20\)

\(M_{S}\): Amount [mg (potency)] of Cefixime RS

Note: Not more than 12.0% (0.1 g of the contents, volumetric titration, direct titration).

Operating conditions—
Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Cefixime Hydrate.

Time span for measurement: Proceed as directed in the operating conditions in the Purity under Cefixime Hydrate.

System suitability—
Test for required detectability: Pipet 1 mL of the sample solution, and add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 10 mL. Confirm that the peak area of cefixime obtained from 10 mL of this solution is equivalent to 17 to 13% of that from 10 \(\mu\)L of the solution for system suitability test.

System performance: When the procedure is run with 10 \(\mu\)L of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cefixime is not more than 2.0%.
Cefmenoxime Hydrochloride

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefixime is not more than 2.0%.

Assay Take out the contents of not less than 20 Cefixime Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefixime Hydrate, add 70 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and shake for 30 minutes, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL.

Amount [mg (potency)] of cefixime (C_{16}H_{15}N_{5}O_{7}S_{2})
= M_{S} \times A_{T} / A_{S} \times V / V \times 1 / C \times 180

= M_{S} \times A_{T} / A_{S} \times V / V \times 1 / C \times 180

M_{S}: Amount [mg (potency)] of Cefixime RS
C: Labeled amount [mg (potency)] of Cefixime Hydrate in 1 capsule

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Cefixime Hydrate.

Dissolution rate (%) with respect to the labeled amount of cefixime (C_{16}H_{15}N_{5}O_{7}S_{2})
= M_{S} \times A_{T} / A_{S} \times V / V \times 1 / C \times 180

= M_{S} \times A_{T} / A_{S} \times V / V \times 1 / C \times 180

M_{S}: Amount [mg (potency)] of Cefixime RS
C: Labeled amount [mg (potency)] of Cefixime Hydrate in 1 capsule

Containers and storage Containers—Tight containers.

Cefmenoxime Hydrochloride

セフメノキシム塩酸塩

(C_{16}H_{17}N_{9}O_{5}S_{3}.)_{2}.HCl: 1059.58
(6R,7R)-7-[[Z]-2-(2-Aminothiazol-4-yl)-2-
(methoxylimino)acetamino]-3-(1-methyl-1H-tetrazol-5-
ylsulfanyl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-
ene-2-carboxylic acid hemihydrochloride

(C_{16}H_{17}N_{9}O_{5}S_{3}.)_{2}.HCl: 1059.58
(6R,7R)-7-[[Z]-2-(2-Aminothiazol-4-yl)-2-
(methoxylimino)acetamino]-3-(1-methyl-1H-tetrazol-5-
ylsulfanyl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-
ene-2-carboxylic acid hemihydrochloride

[C = 511.56].

Cefmenoxime Hydrochloride contains not less than 890 µg (potency) and not more than 975 µg (potency) per mg, calculated on the dehydrated basis. The potency of Cefmenoxime Hydrochloride is expressed as mass (potency) of cefmenoxime (C_{16}H_{17}N_{9}O_{5}S_{3}.)_{2}: 511.56).

Description Cefmenoxime Hydrochloride occurs as white to light orange-yellow crystals or crystalline powder.

It is freely soluble in formamide and in dimethylsulfoxide, slightly soluble in methanol, very slightly soluble in water, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefmenoxime Hydrochloride in 0.1 mol/L phosphate buffer solution, pH 6.8 (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry 2<.2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefmenoxime Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefmenoxime Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry 2<.2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefmenoxime Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefmenoxime Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy 2<.2.21>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits two single signals, A and B, at around δ 3.9 ppm, and a single signal C at around δ 6.8 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Dissolve 10 mg of Cefmenoxime Hydrochloride in 1 mL of diluted sodium carbonate TS (1 in 20), add 5 mL of acetic acid (100) and 2 drops of silver nitrate TS: a white precipitate is formed.

Optical rotation 2<.2.49> [α]_{D}: −27 – −35° (1 g, 0.1 mol/L phosphate buffer solution, pH 6.8, 100 mL, 100 mm).
Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cefmenoxime Hydrochloride in 10 mL of dilute sodium carbonate TS (1 in 4) is clear and colorless to light yellow.

(2) Heavy metals <1.0%—Proceed with 1.0 g of Cefmenoxime Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.1%—Prepare the test solution with 1.0 g of Cefmenoxime Hydrochloride according to Method 4 and adding 10 mL of dilute hydrochloric acid to the residue after cooling, and perform the test (not more than 2 ppm).

(4) Related substances—Weigh accurately about 0.1 g of Cefmenoxime Hydrochloride RS, dissolve in 20 mL of 0.1 mol/L phosphate buffer solution, pH 6.8, and add the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of 1-methyl-1H-tetrazol-5-thiol, and dissolve in the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution (1). Weigh accurately about 0.1 g of Cefmenoxime Hydrochloride RS, dissolve in 20 mL of 0.1 mol/L phosphate buffer solution, pH 6.8, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution (2). Perform the test immediately after preparation of these solutions with exactly 10 µL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area obtained from the chromatograms of these solutions by the automatic integration method, and calculate the amounts of 1-methyl-1H-tetrazol-5-thiol and the total related substance by the following formula: the amount of 1-methyl-1H-tetrazol-5-thiol and the total related substance by the following formula:

\[
\text{Amount of 1-methyl-1H-tetrazol-5-thiol} = \frac{M_{5s}}{M_f} \times \frac{A_{1s}}{A_{5s}} \times 20
\]

\[
\text{Amount of total related substances} = \frac{M_{5s}}{M_f} \times \frac{A_{1s}}{A_{5s}} \times 20 + \frac{M_{5s}}{M_f} \times \frac{S_l}{A_{5s}} \times 5
\]

\[M_{5s}: \text{Amount (g) of 1-methyl-1H-tetrazol-5-thiol}\]

\[M_{5s}: \text{Amount (g) of Cefmenoxime Hydrochloride RS}\]

\[M_f: \text{Amount (g) of the sample}\]

\[A_{1s}: \text{Peak area of 1-methyl-1H-tetrazol-5-thiol from the standard solution (1)}\]

\[A_{5s}: \text{Peak area of cefmenoxime from the standard solution (2)}\]

\[A_{1s}: \text{Peak area of 1-methyl-1H-tetrazol-5-thiol from the sample solution}\]

\[S_l: \text{Total area of the peaks other than 1-methyl-1H-tetrazol-5-thiol and other than cefmenoxime from the sample solution}\]

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of cefmenoxime.

System suitability—
Test for required detectability: Measure exactly 5 mL of the standard solution (1), add the mobile phase to make exactly 100 mL. Confirm that the peak area of 1-methyl-1H-tetrazol-5-thiol obtained from 10 µL of this solution is equivalent to 4.5 to 5.5% of that from 10 µL of the standard solution (1). Then, measure exactly 2 mL of the standard solution (2), add the mobile phase to make exactly 100 mL. Confirm that the peak area of cefmenoxime obtained from 10 µL of this solution is equivalent to 1.5 to 2.5% of that from 10 µL of the standard solution (2).

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of 1-methyl-1H-tetrazol-5-thiol is not more than 1.0%.

Water <2.48> Not more than 1.5% (1 g, volumetric titration, direct titration. Use a mixture of formamide for Karl Fisher method and methanol for Karl Fisher method (2:1)).

Assay Weigh accurately an amount of Cefmenoxime Hydrochloride and Cefmenoxime Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in 10 mL of 0.1 mol/L phosphate buffer solution, pH 6.8, and add the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, add exactly 20 mL of the internal standard solution and mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_{S_l}\), of the peak area of cefmenoxime to that of the internal standard.

Amount [µg (potency)] of cefmenoxime (\(C_{16}H_{17}N_4O_5S_3\))

\[\frac{M_s}{A_{1s}} \times Q_T/Q_{S_l} \times 1000\]

\[M_s: \text{Amount [mg (potency)] of Cefmenoxime Hydrochloride RS}\]

Internal standard solution—A solution of phthalimide in methanol (3 in 2000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100:50:10:1).

Flow rate: Adjust the flow rate so that the retention time of cefmenoxime is about 8 minutes.

System suitability—
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, cefmenoxime and the internal standard are eluted in
Cefmetazole Sodium

セフェメタゾールナトリウム

C$_{15}$H$_{17}$N$_7$O$_5$S$_3$: 471.53
Monosodium (6R,7R)-7-[(cyanomethylsulfanyl)acetylamino]-7-methoxy-3-(1-methyl-1H-tetrazol-5-ylsulfanyl)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

[56796-20-4]

Cefmetazole Sodium contains not less than 860 μg (potency) and not more than 965 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefmetazole Sodium is expressed as mass (potency) of cefmetazole (C$_{15}$H$_{17}$N$_7$O$_5$S$_3$: 471.53).

Description

Cefmetazole Sodium occurs as a white to light yellowish white, powder or mass.

It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in tetrahydrofuran.

It is hygroscopic.

Identification

(1) Determine the absorption spectrum of a solution of Cefmetazole Sodium (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefmetazole Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the $^1$H spectrum of a solution of Cefmetazole Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.27>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ 3.6 ppm, at around δ 4.1 ppm and at around δ 5.2 ppm, respectively. The ratio of integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Cefmetazole Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> [α]$_D^2$: +73 - +85° (0.25 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefmetazole Sodium in 10 mL of water: the pH of the solution is between 4.2 and 6.2.

Purity

(1) Clarity and color of solution—Dissolve 1.0 g of Cefmetazole Sodium in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefmetazole Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefmetazole Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Cefmetazole Sodium in 2 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution (1). Separately, dissolve 0.10 g of 1-methyl-1H-tetrazole-5-thiol in water to make exactly 100 mL, and use this solution as the standard solution (2). Immediately perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 1 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of 1-buthanol, water and acetic acid (100) (4:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spot obtained from the sample solution corresponding to the spot from the standard solution (2) is not more intense than the spot from the standard solution (2), and the spots other than this spot and other than the principal spot are not more intense than the spot from the standard solution (1).

Water <2.48> Not more than 1.0% (1 g, volumetric titration, direct titration).

Assay

Weigh accurately an amount of Cefmetazole Sodium and Cefmetazole RS, equivalent to about 50 mg (potency), and dissolve each in mobile phase to make exactly 25 mL. Pipet 1 mL each of these solutions, add water to make exactly 10 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q$_T$ and Q$_S$, of the peak area of cefmetazole to that of the internal standard of each solution.

Amount [μg (potency)] of cefmetazole (C$_{15}$H$_{17}$N$_7$O$_5$S$_3$) = $M_S \times Q_T/Q_S \times 1000$

$M_S$: Amount [mg (potency)] of Cefmetazole RS

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecysilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 5.75 g of ammonium dihydrogen-phosphate in 700 mL of water, add 280 mL of methanol, 20 mL of tetrahydrofuran and 3.2 mL of 40% tetrabutylammonium hydroxide TS, and adjust to pH 4.5 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of cefmetazole is about 8 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefmetazole and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefmetazole to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Cefmetazole Sodium for Injection
注射用セフメタゾールナトリウム

Cefmetazole Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of cefmetazole (C\textsubscript{15}H\textsubscript{17}N\textsubscript{7}O\textsubscript{5}S\textsubscript{3}: 471.53).

Method of preparation Prepare as directed under Injections, with Cefmetazole Sodium.

Description Cefmetazole Sodium for Injection is a white to light yellow powder or masses.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Cefmetazole Sodium for Injection (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefmetazole Sodium for Injection as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Take an amount of Cefmetazole Sodium for Injection equivalent to 1.0 g (potency) of Cefmetazole Sodium according to the labeled amount, and dissolve in 10 mL of water: the pH of the solution is 4.2 to 6.2.

Purity (1) Clarity and color of solution—Dissolve an amount of Cefmetazole Sodium for Injection, equivalent to 1.0 g (potency) of Cefmetazole Sodium according to the labeled amount, in 10 mL of water: the solution is clear and the color is not darker than the following control solution.

Control solution: Pipet 5 mL of Iron (III) Chloride CS and 0.5 mL of Cobalt (II) Chloride CS, and add water to make exactly 50 mL. Pipet 15 mL of this solution, and add water to make exactly 20 mL.

(2) Related substances—Proceed as directed in the Purity (4) under Cefmetazole Sodium.

Bacterial endotoxins <4.01> Less than 0.06 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign particulate matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take 10 containers of Cefmetazole Sodium for Injection, dissolve the contents of each in the mobile phase, rinse each of the containers with the mobile phase, combine the rinse with the respective previous solution, and add the mobile phase to make exactly 500 mL. Take exactly a volume of this solution equivalent to about 0.2 g (potency) of Cefmetazole Sodium, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefmetazole RS, equivalent to about 50 mg (potency), and dissolve in the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefmetazole Sodium.

Amount [mg (potency)] of cefmetazole (C\textsubscript{15}H\textsubscript{17}N\textsubscript{7}O\textsubscript{5}S\textsubscript{3})
\[ M_5 = M_5 \times \frac{Q_1}{Q_5} \times 4 \]

\( M_5 \): Amount [mg (potency)] of Cefmetazole RS

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 10,000).

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Cefminox Sodium Hydrate
セフミノクスナトリウム水和物

C\textsubscript{16}H\textsubscript{20}N\textsubscript{7}NaO\textsubscript{7}S\textsubscript{3}·7H\textsubscript{2}O: 667.66

Cefminox Sodium Hydrate contains not less than 900 μg (potency) and not more than 970 μg (potency).
Cefodizime Sodium / Official Monographs

per mg, calculated on the anhydrous basis. The potency of Cefminox Sodium Hydrate is expressed as mass (potency) of cefminox (C_{16}H_{21}N_{7}O_{7}S_{3}: 519.58).

**Description** Cefminox Sodium Hydrate occurs as a white to light yellow crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

**Identification** (1) Determine the absorption spectrum of a solution of Cefminox Sodium Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefminox Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefminox Sodium Hydrate as directed in the potassium bromide method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefminox Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the 1H spectrum of a solution of Cefminox Sodium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 30) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a multiple signal, A, at around 3.2 ppm, a single signal, B, at around 3.5 ppm, a single signal, C, at around 4.0 ppm, and a single signal, D, at around 5.1 ppm. The ratio of integrated intensity of each signal, A:B:C:D, is about 2:3:3:1.

(4) Cefminox Sodium Hydrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

**Optical rotation** <2.49> [α]_D: +62° to +72° (50 mg, water, 10 mL, 100 mm).

**pH** <2.54> Dissolve 0.70 g of Cefminox Sodium Hydrate in 10 mL of water: the pH of the solution is between 4.5 and 6.0.

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 g of Cefminox Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11D>—Prepare the test solution with 2.0 g of Cefminox Sodium Hydrate according to Method 3, and perform the test (not more than 1 ppm).

**Water** <2.48> Not less than 18.0% and not more than 20.0% (0.1 g, volumetric titration, direct titration).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—Escherichia coli NIHJ
(ii) Culture medium—Use the medium iii in 3) under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.5 to 6.6 after sterilization.
(iii) Standard solution—Weigh accurately an amount of Cefminox Sodium RS, equivalent to about 40 mg (potency), dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 40 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solution—Weigh accurately an amount of Cefminox Sodium Hydrate equivalent to about 40 mg (potency), dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 40 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

(v) Procedure—Incubate between 32°C and 35°C.

**Containers and storage** Containers—Hermetic containers.

Cefodizime Sodium

セフォジジムナトリウム

C_{20}H_{18}N_{6}Na_{2}O_{7}S_{4}: 628.63

Cefodizime Sodium contains not less than 890 μg (potency) per mg, calculated on the anhydrous basis and corrected by the ethanol amount. The potency of Cefodizime Sodium is expressed as mass (potency) of cefodizime (C_{20}H_{20}N_{6}O_{7}S_{4}: 584.67).

**Description** Cefodizime Sodium occurs as a white to light yellowish white crystalline powder.

It is very soluble in water, and practically insoluble in acetonitrile and in ethanol (99.5).

**Identification** (1) Determine the absorption spectrum of a solution of Cefodizime Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefodizime Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefodizime Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefodizime Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.
(3) Determine the \(1^H\) spectrum of a solution of Cefodizime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy (2.21), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around \(\delta\) 2.3 ppm, at around \(\delta\) 4.0 ppm, and at around \(\delta\) 7.0 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:3:1.

(4) Cefodizime Sodium responds to the Qualitative Tests (1.099) (1) for sodium salt.

**Optical rotation**<sup>2.49</sup> \([\alpha]_D^{20}\) = \(-56 \pm -62^\circ\) (0.2 g calculated on the anhydrous basis and corrected by the ethanol amount, water, 20 mL, 100 mm).

**pH**<sup>2.54</sup> Dissolve 1.0 g of Cefodizime Sodium in 10 mL of water: the pH of the solution is between 5.5 and 7.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Cefodizime Sodium in 10 mL of water: the solution is clear and pale yellow to light yellow.

(2) Heavy metals<sup>1.077</sup>—Weigh 1.0 g of Cefodizime Sodium in a crucible, cover loosely, and carbonize by gentle heating. After cooling, add 2 mL of sulfuric acid, heat gradually until the white fumes are no longer evolved, and ignite between 500°C and 600°C. Proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic<sup>1.111</sup>—Prepare the test solution with 1.0 g of Cefodizime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 30 mg of Cefodizime Sodium in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography (2.07) according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than cefodizime from the sample solution is not larger than the peak area of cefodizime from the standard solution, and the total area of the peaks other than cefodizime from the sample solution is not larger than 3 times the peak area of cefodizime from the standard solution.

**Operating conditions**—
- Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
- Time span of measurement: About 4 times as long as the retention time of cefodizime beginning after the solvent peak.

**System suitability**—
- Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefodizime obtained from 5 \(\mu\)L of this solution is equivalent to 7 to 13% of that from 5 \(\mu\)L of the standard solution.
- System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

(5) Ethanol—Weigh accurately about 1 g of Cefodizime Sodium, and dissolve in water to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 2 g of ethanol for gas chromatography, and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 \(\mu\)L each of the sample solution and standard solution as directed under Gas Chromatography (2.02) according to the following conditions, and calculate the ratio, \(Q_1\) and \(Q_0\), of the peak area of ethanol to that of the internal standard: the amount of ethanol is not more than 2.0%.

\[
\text{Amount (\%) of ethanol} = \frac{M_S}{M_T} \times \frac{Q_1}{Q_S}
\]

\(M_S\): Amount (g) of ethanol for gas chromatography
\(M_T\): Amount (g) of the sample

**Internal standard solution**—A solution of 1-propanol (1 in 400).

**Operating conditions**—
- Detector: A hydrogen flame-ionization detector.
- Column: A glass column 3.2 mm in inside diameter and 3 m in length, packed with tetrafluoroethylene polymer for gas chromatography (180 – 250 \(\mu\)m in particle diameter) coated in 15% with polyethylene glycol 20 M.
- Column temperature: A constant temperature of about 100°C.
- Carrier gas: Nitrogen.
- Flow rate: Adjust the flow rate so that the retention time of ethanol is about 3 minutes.

**System suitability**—
- System performance: When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.
- System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 2.0%.

**Water**<sup>2.45</sup> Not more than 4.0% (0.5 g, volumetric titration, direct titration).

**Assay**—Weigh accurately an amount of Cefodizime Sodium and Cefodizime Sodium RS, equivalent to about 50 mg (potency), add exactly 10 mL of the internal standard solution to dissolve, add water to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography (2.07) according to the following conditions, and calculate the ratios, \(Q_1\) and \(Q_0\), of the peak area of cefodizime to that of the internal standard.

\[
\text{Amount [mg (potency)] of cefodizime (C\textsubscript{20}H\textsubscript{20}N\textsubscript{4}O\textsubscript{3}S\textsubscript{2})} = \frac{M_S}{M_T} \times \frac{Q_1}{Q_S} \times 1000
\]

\(M_S\): Amount [mg (potency)] of Cefodizime Sodium RS

**Internal standard solution**—A solution of anhydrous caffeine (3 in 400).

**Operating conditions**—
- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.80 g of potassium dihydrogen phosphate and 0.20 g of anhydrous disodium hydrogen phosphate in a suitable amount of water, and add 80 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cefodizime is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefodizime and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefodizime to that of the internal standard is not more than 2.0%.

Containers and storage—Containers—Tight containers.

Cefoperazone Sodium  

セフォペラゾンナトリウム

C₂₅H₂₆N₉NaO₈S₂: 667.65

Monosodium (6R,7R)-7-[(2R)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-(4-hydroxyphenyl)acetamido]-3-(1-methyl-1H-tetrazol-5-ylsulfanyl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [62893-20-3]

Cefoperazone Sodium contains not less than 871 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefoperazone Sodium is expressed as mass (potency) of cefoperazone (C₂₅H₂₇N₉O₈S₂: 645.67).

Description—Cefoperazone Sodium occurs as a white to yellowish white crystalline powder.

It is very soluble in water, soluble in methanol, and slightly soluble in ethanol (99.5).

Identification—(1) Determine the absorption spectrum of a solution of Cefoperazone Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ‘H spectrum of a solution of Cefoperazone Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.23>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal A at around δ 1.2 ppm, and double signals, B and C, at around δ 6.8 ppm and at around δ 7.3 ppm. The ratio of integrated intensity of these signals, A:B:C, is about 3:2:2.

(3) Cefoperazone Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation—[α]D²⁵ + 25 to +25° (1 g, water, 100 mL, 100 mm).

pH—Dissolve 1.0 g of Cefoperazone Sodium in 4 mL of water: the pH of the solution is between 4.5 and 6.5.

Purity—(1) Clarity and color of solution—Dissolve 1.0 g of Cefoperazone Sodium in 10 mL of water: the solution is clear and pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Cefoperazone Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Cefoperazone Sodium according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.1 g of Cefoperazone Sodium in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas of each peak by the automatic integration method. Calculate the percentages of each peak area from the sample solution to 50 times of the peak area of cefoperazone from the standard solution: the related substance I with the retention time of about 8 minutes is not more than 5.0%, the related substance II with that of about 17 minutes is not more than 1.5%, and the total of all related substances is not more than 7.0%. Use the peak areas of the related substances I and II after multiplying by their relative response factor, 0.90 and 0.75, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cefoperazone beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefoperazone obtained from 25 μL of this solution is equivalent to 3.5 to 6.5% of that from 25 μL of the standard solution.

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefoperazone are not less than 5000 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operat-
Cefotaxime Sodium

セフォタキシムナトリウム

C_{16}H_{16}N_{5}NaO_{7}S_{2}: 477.45

Monosodium (6R,7R)-3-acetoxymethyl-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [64485-93-4]

Cefotaxime Sodium contains not less than 916 µg (potency) per mg, calculated on the dried basis. The potency of Cefotaxime Sodium is expressed as mass (potency) of cefotaxime (C_{16}H_{17}N_{5}O_{7}S_{2}: 455.47).

Description

Cefotaxime Sodium occurs as white to light yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (95%).

Identification (1) Dissolve 2 mg of Cefotaxime Sodium in 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotaxime Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the {\textit{H}} spectrum of a solution of Cefotaxime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 125) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits three single signals, A, B and C, at around δ 2.1 ppm, at around δ 4.0 ppm and at around δ 7.0 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Cefotaxime Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> [\alpha]_D^20: +58° to +64° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Cefotaxime Sodium in 10 mL of water is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cefotaxime Sodium in 10 mL of water is clear and light yellow.

(2) Sulfate <1.14>—Dissolve 2.0 g of Cefotaxime Sodium in 40 mL of water, add 2 mL of dilute hydrochloric acid and water to make 50 mL, shake well, and filter. Discard first 10 mL of the filtrate, and to the subsequent 25 mL of the fil-

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefoperazone is not more than 2.0%.

Water <2.48> Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay

Weigh accurately an amount of Cefoperazone Sodium equivalent to about 0.1 g (potency), dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefoperazone RS equivalent to about 0.1 g (potency), dissolve in 5 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_1 and Q_2, of the peak area of cefoperazone to that of the internal standard.

Amout [µg (potency)] of cefoperazone (C_{25}H_{27}N_{9}O_{8}S_{2}) = M_5 \times Q_1/Q_2 \times 1000

M_5: Amount [µg (potency)] of Cefoperazone RS

Internal standard solution—A solution of acetanilide in a mixture of water and acetonitrile (43:7) (3 in 8000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 57 mL of acetic acid (100) add 139 mL of triethylamine and water to make 1000 mL. To 20 mL of this solution add 835 mL of water, 140 mL of acetonitrile and 5 mL of dilute acetic acid.

Flow rate: Adjust the flow rate so that the retention time of cefoperazone is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the internal standard and cefoperazone are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefoperazone to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Hermetic containers.

Storage—In a cold place.
rate add water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: To 1.0 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals \( \leq 0.07 \) — Proceed with 1.0 g of Cefotaxime Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic \( \leq 0.11 \) — Proceed with the test solution with 1.0 g of Cefotaxime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances — Proceed with the test with 10 \( \mu L \) of the sample solution obtained in the Assay as directed under Liquid Chromatography \( \leq 2.0 \) according to the following conditions, and determine each peak area obtained from the chromatogram by the automatic integration method, and calculated the amounts of them by the area percentage method: the each peak area other than cefotaxime is not more than 1.0% and the total of these peak areas is not more than 3.0%.

**Operating conditions**

Detector, column, column temperature, mobile phase A, mobile phase B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3.5 times as long as the retention time of cefotaxime beginning after the solvent peak.

**System suitability**

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase A to make exactly 100 mL. Pipet 2 mL of this solution, and add the mobile phase A to make exactly 20 mL. Confirm that the peak area of cefotaxime obtained from 10 \( \mu L \) of this solution is equivalent to 0.15 to 0.25% of that from 10 \( \mu L \) of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

**Loss on drying \( \leq 2.4\text{f} \) Not more than 3.0% (1 g, 105°C, 3 hours).

**Assay** Weigh accurately an amount of Cefotaxime Sodium and Cefotaxime RS, equivalent to about 40 mg (potency), dissolve each in the mobile phase A to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \( \leq 2.0 \) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of cefotaxime of these solutions.

\[
\text{Amount [} \mu g \text{ (potency)] of cefotaxime (C}_{16}H_{17}N_{5}O_{7}S_{2}) = M_S \times A_T/A_S \times 1000
\]

\( M_S \): Amount [mg (potency)] of Cefotaxime RS

**Operating conditions**


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 30°C.

**Mobile phase A**

To 0.05 mol/L disodium hydrogen phosphate TS add phosphoric acid to adjust the pH to 6.25. To 860 mL of this solution add 140 mL of methanol.

**Flowing of mobile phase**

Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 7</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7 – 9</td>
<td>100 → 80</td>
<td>0 → 20</td>
</tr>
<tr>
<td>9 – 16</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>16 – 45</td>
<td>80 → 0</td>
<td>20 → 100</td>
</tr>
<tr>
<td>45 – 50</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of cefotaxime is about 14 minutes (about 1.3 mL/min).

**System suitability**

System performance: To 1 mL of the standard solution add 7.0 mL of water and 2.0 mL of methanol, mix, then add 25 mg of sodium carbonate decahydrate, and shake. After allowing to stand for 10 minutes, add 3 drops of acetic acid (100) and 1 mL of the standard solution, and mix. When the procedure is run with 10 \( \mu L \) of this solution under the above operating conditions, desacetyl cefotaxime with the relative retention time being about 0.3 to cefotaxime and cefotaxime are eluted in this order with the resolution between these peaks being not less than 20, and the symmetry factor of the peak of cefotaxime is not more than 2.

System repeatability: When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefotaxime is not more than 2.0%.

**Containers and storage**

Containers—Tight containers.

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**Cefotetan**

セフォテタン

\( \text{C}_{17}H_{17}N_{7}O_{8}S_{4}: 575.62 \)

\( 6R,7\text{R})-7-[4-(\text{Carbamoylcarboxymethylidene}-1,3-dithietane-2-carbonyl]amino]-7-methoxy-3-(1-\text{methyl}-1H-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid \ [69712-56-7]

Cefotetan contains not less than 960 \( \mu g \) (potency) and not more than 1010 \( \mu g \) (potency) per mg, calculated on the anhydrous basis. The potency of Cefotetan is expressed as mass (potency) of cefotetan.
Cefotetan occurs as white to light yellowish white powder.

It is sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Cefotetan RS in phosphate buffer solution for antibiotics, pH 6.5 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( \text{UV-Vis} \), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotetan prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotetan as directed in the potassium bromide disk method under Infrared Spectrophotometry \( \text{IR} \), and compare and the spectrum with the Reference Spectrum or the spectrum of Cefotetan RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 50 mg of Cefotetan in 0.5 mL of a solution of sodium hydrogen carbonate in heavy water for nuclear magnetic resonance spectroscopy (1 in 25). Determine the \(^1\)H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy \( \text{NMR} \), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B, C and D, at around \( \delta 3.6 \) ppm, at around \( \delta 4.0 \) ppm, at around \( \delta 5.1 \) ppm and at around \( \delta 5.2 \) ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C:D, is about 3:3:1:1.

Optical rotation \( \text{OR} \) 
\[ \alpha_D^{20} = +112 \text{ to } +124^\circ \] (0.5 g calculated on the anhydrous basis, a solution of sodium hydrogen carbonate (1 in 200), 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefotetan in 10 mL of a solution of sodium hydrogen carbonate (1 in 30): the solution is clear, and colorless or light yellow.

(2) Heavy metals \( \text{HMT} \)—Proceed with 1.0 g of Cefotetan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately about 0.1 g of Cefotetan, dissolve in a suitable amount of methanol, add exactly 2 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of 1-methyl-1H-tetrazole-5-thiol for liquid chromatography, previously dried in a desiccator (in vacuum, silica gel) for 2 hours, and add 2 mg of Cefotetan RS, calculated on the anhydrous basis, dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the standard solution. Perform the test with 5 \( \mu L \) of the sample solution and standard solution as directed under Liquid Chromatography \( \text{LC} \) according to the following conditions, and calculate the ratios, \( Q_H \), \( Q_M \), \( Q_{1H} \), \( Q_{2H} \), \( Q_T \), and \( Q_{2C} \), of the peak areas of 1-methyl-1H-tetrazole-5-thiol, cefotetan, lactone having the relative retention time of about 0.5 with respect to cefotetan, \( \Delta_2 \)-cefotetan, isothiazole substance having the relative retention time of about 1.3 with respect to cefotetan, each of other related substances and the total of other related substances, to the peak area of the internal standard, respectively, obtained from the sample solution, and the ratios, \( Q_{H,T} \) and \( Q_{M,T} \), of the peak areas of 1-methyl-1H-tetrazole-5-thiol and cefotetan, to the peak area of the internal standard, respectively, obtained from the standard solution. Calculate the amount of 1-methyl-1H-tetrazole-5-thiol, cefotetan lactone, \( \Delta_2 \)-cefotetan, isothiazole substance, each of other related substances and the total of other related substances from the following equations: the amount of 1-methyl-1H-tetrazole-5-thiol is not more than 0.3\%, cefotetan lactone is not more than 0.3\%, \( \Delta_2 \)-cefotetan is not more than 0.5\%, isothiazole substance is not more than 0.5\%, each of other related substances is not more than 0.2\% and the total of other related substances is not more than 0.4\%.

1-Methyl-1H-tetrazole-5-thiol (\%) 
\[ = \frac{M_{\text{H,T}}}{M_{\text{T}}} \times \frac{Q_{\text{T,H}}}{Q_{\text{T}}} \times \frac{1}{100} \]
Cefotetan lactone (\%) 
\[ = \frac{M_{\text{H,T}}}{M_{\text{T}}} \times \frac{Q_{\text{T,H}}}{Q_{\text{T}}} \times \frac{1}{100} \]
\( \Delta_2 \)-Cefotetan (\%) 
\[ = \frac{M_{\text{H,T}}}{M_{\text{T}}} \times \frac{Q_{\text{T,h}}}{Q_{\text{T}}} \times \frac{1}{100} \]
Isothiazole substance (\%) 
\[ = \frac{M_{\text{H,T}}}{M_{\text{T}}} \times \frac{Q_{\text{T,h}}}{Q_{\text{T}}} \times \frac{1}{100} \]
Each of other related substances (\%) 
\[ = \frac{M_{\text{H,T}}}{M_{\text{T}}} \times \frac{Q_{\text{T,h}}}{Q_{\text{T}}} \times \frac{1}{100} \]
Total of other related substances (\%) 
\[ = \frac{M_{\text{H,T}}}{M_{\text{T}}} \times \frac{Q_{\text{T,h}}}{Q_{\text{T}}} \times \frac{1}{100} \]

Internal standard solution—A solution of anhydrous caffeine in methanol (3 in 10,000).

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3.5 times as long as the retention time of cefotetan.

System suitability—
Test for required detectability: Measure exactly 15 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of cefotetan obtained from 5 \( \mu L \) of this solution is equivalent to 12 to 18\% of that from 5 \( \mu L \) of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefotetan to that of the internal standard is not more than 2.0\%.

Water \( \text{W} \) Not more than 2.5\% (1 g, volumetric titration, direct titration).

Residue on ignition \( \text{ROI} \) Not more than 0.1\% (1 g).

Isomer ratio Dissolve 10 mg of Cefotetan in 20 mL of
methanol, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the adjacent two peaks appeared at around the retention time of 40 minutes, one having shorter retention time is l-substance and another having longer retention time is d-substance, by the area percentage method: the amount of l-substance is not less than 35% and not more than 45%.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecysilanized silica gel for liquid chromato- graphy (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, water, and a solution of tetrabutylammonium hydrogensulfate in acetonitrile (1 in 150) (9:9:2).
Flow rate: Adjust the flow rate so that the retention time of l-substance is about 40 minutes.

System suitability—
System performance: When the procedure is run with 5 μL of the sample solution under the above operating conditions, l-substance and d-substance are eluted in this order with the resolution between these peaks being not less than 1.5.
System repeatability: To exactly 1 mL of the sample solution add methanol to make exactly 10 mL. When the test is repeated 6 times with 5 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of l-substance is not more than 5.0%.

Assay
Weigh accurately an amount of Cefotetan and Cefotetan RS, equivalent to about 50 mg (potency), and dissolve each in phosphate buffer solution for antibiotics, pH 6.5 to make exactly 50 mL. Pipet 15 mL each of these solutions, and another having longer retention time is d-substance, by

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecysilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 11.53 g of phosphoric acid in 1000 mL of water. To 850 mL of this solution add 50 mL of acetonitrile, 50 mL of acetic acid (100) and 50 mL of metha-

Flow rate: Adjust the flow rate so that the retention time of cefotetan is about 17 minutes.

System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefotetan to that of the internal standard is not more than 1.0%.

Containers and storage
Containers—Tight containers.
Storage—Light-resistant, and at a temperature not exceeding 5°C.

Cefotiam Hexetil Hydrochloride

C72H73N2O8S2·2HCl·768.76

(1S)-1-Cyclohexyloxycarbonyloxyethyl (6R,7R)-7-
[2-(2-aminothiazol-4-yl)acetamidin]-3-[(2-
dimethylaminoethyl)-1H-tetrazol-5-ylsulfanyl)methyl]-
8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
dihydrochloride

Cefotiam Hexetil Hydrochloride contains not less than 615 μg (potency) and not more than 690 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefotiam Hexetil Hydrochloride is expressed as mass (potency) of cefotiam (C18H23N9O4S3·H2O: 525.63).

Description
Cefotiam Hexetil Hydrochloride occurs as a white to light yellow powder.
It is very soluble in water, in methanol and in ethanol (95), freely soluble in dimethylsulfoxide, and slightly soluble in acetonitrile.
It dissolves in 0.1 mol/L hydrochloric acid TS.
It is hygroscopic.

Identification
(1) Determine the absorption spectrum of a solution of Cefotiam Hexetil Hydrochloride in 0.1 mol/L hydrochloric acid TS (3 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.29>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotiam Hexetil Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the H spectrum of a solution of Cefotiam
Hexitil Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21D>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits two single signals, A and B, at around δ 2.8 ppm and at around δ 6.6 ppm, and a multiple signal, C, at around δ 6.9 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 6:1:1.

(3) To a solution of Cefotiam Hexitil Hydrochloride (1 in 200) add 2 mL of dilute nitric acid and 1 mL of silver nitrate TS, and mix: a white precipitate is formed.

**Optical rotation** <2.49>  [α]D¹⁰⁰ + 52° ± 6° (0.1 g calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 10 mL, 100 mm).

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 g of Cefotiam Hexitil Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 2.0 g of Cefotiam Hexitil Hydrochloride according to Method 3, and perform the test, using a solution of magnesium nitrate hexahydrate in ethanol (95%) (1 in 5) (not more than 1 ppm).

(3) Related substance 1—Weigh accurately about 50 mg of Cefotiam Hexitil Hydrochloride, and dissolve in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Cefotiam Hexitil Hydrochloride RS, and dissolve in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amount of the related substance having the relative retention time of about 1.2 to one of the peaks of cefotiam hexitil, which has the larger retention time, is not more than 2.0%, and each amount of the other related substances is not more than 0.5%. For this calculation, use the value of the peak area obtained by the automatic integration method of the related substance having the relative retention time of about 1.2 to one of the peaks of cefotiam hexitil, which has the larger retention time, after multiplying by its relative response factor, 0.78.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase A: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2), acetonitrile and acetic acid (100) (72:28:1).
Mobile phase B: A mixture of acetonitrile, diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2) and acetic acid (100) (60:40:1).
Flowing of the mobile phase: Adjust so that the mixing rate of the mobile phase A and the mobile phase B is changed linearly from 1:0 to 0:1 for 30 minutes.
Flow rate: 0.7 mL per minute.
Time span of measurement: As long as about 3 times of the retention time of one of the cefotiam hexitil peaks, which appears first, beginning after the solvent peak.

**System suitability**—
Test for required detectability: Measure exactly 1 mL of the standard solution, and add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Confirm that each area of the two peaks of cefotiam hexitil obtained from 10 μL of this solution is equivalent to 1.6 to 2.4% of that from 10 μL of the standard solution.
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the resolution between the two peaks of cefotiam hexitil is not less than 2.0.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the total of the two peak areas of cefotiam hexitil is not more than 2.0%.

(4) Related substance 2—Weigh accurately about 20 mg of Cefotiam Hexitil Hydrochloride, dissolve in exactly 2 mL of methanol, add a mixture of a solution of diammonium hydrogen phosphate (79 in 20,000) and acetic acid (100) (200:3) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Cefotiam Hydrochloride RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amounts of the related substances having the relative retention time of about 0.1 and 0.9 to cefotiam are not more than 1.0%, respectively, and each amount of the related substances other than the related substances having the relative retention time of about 0.1 and 0.9 to cefotiam is not more than 0.5%. For this calculation, use the value of the peak area of the related substance having the relative retention time of about 0.9 to cefotiam after multiplying by its sensitivity coefficient, 0.76.
Amount (%) of each related substance

\[ M_a = \frac{M_f}{M_i} \times \frac{A_f}{A_i} \times 4 \]

- $M_a$: Amount (g) of Cefotiam Hydrochloride RS
- $M_f$: Amount (g) of the sample
- $A_f$: Peak area of cefotiam from the standard solution
- $A_i$: Each peak area from the sample solution

**Operating conditions**

- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of a solution of diammonium hydrogen phosphate (79 in 20,000), methanol and acetic acid (100) (200:10:3).
- Flow rate: Adjust the flow rate so that the retention time of cefotiam is about 15 minutes.
- Time span of measurement: As long as about 2 times of the retention time of cefotiam beginning after the solvent peak.

**System suitability**

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of cefotiam obtained from 10 μL of this solution is equivalent to 1.6 to 2.4% of that from 10 μL of the standard solution.

System performance: To 1 mL of a solution of acetaminophen in the mobile phase (1 in 50,000) add 3 mL of the internal standard, and mix well. When the procedure is run with 10 μL of this solution under the above operating conditions, acetaminophen and cefotiam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefotiam is not more than 2.0%.

(5) Total amount of related substances—The total of the amount of related substances obtained in the Related substance 1 and the Related substance 2 is not more than 6.5%.

**Water** <2.44> Not more than 3.5% (0.1 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Isomer ratio** Proceed the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_i$ and $Q_o$, of the peak area of cefotiam hexetil to that of the internal standard. For this calculation, the total of the areas of the two peaks appeared closely each other at the retention time of around 10 minutes is used as the peak area of cefotiam hexetil.

Amount [μg (potency)] of cefotiam (C18H23N9O4S3)

\[ M_a = \frac{M_f}{M_i} \times \frac{A_f}{A_i} \times 1000 \]

- $M_a$: Amount [μg (potency)] of Cefotiam Hexetil Hydrochloride RS

**Internal standard solution**—A solution of benzoic acid in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) (7 in 10,000).

**Operating conditions**

- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of a solution of sodium dihydrogen phosphate TS (1 in 2), acetonitrile and acetic acid (100) (72:28:1).
- Flow rate: Adjust the flow rate so that the retention time of the faster peak of cefotiam hexetil is about 9 minutes.

**System suitability**—System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and cefotiam hexetil are eluted in this order with the resolution between the two peaks of cefotiam hexetil being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefotiam hexetil to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
Cefotiam Hydrochloride

Cefotiam Hydrochloride contains not less than 810 \( \mu g \) (potency) and not more than 890 \( \mu g \) (potency) per mg, calculated on the anhydrous basis. The potency of Cefotiam Hydrochloride is expressed as mass (potency) of cefotiam (C_{18}H_{23}N_{9}O_{4}S_{3}: 525.63).

**Description** Cefotiam Hydrochloride occurs as white to light yellow, crystals or crystalline powder. It is freely soluble in water, in methanol and in formamide, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

**Identification**

1. **Determine the absorption spectrum** of a solution of Cefotiam Hydrochloride (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry. Compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotiam Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. **Determine the infrared absorption spectrum** of Cefotiam Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry. Cefotiam Hydrochloride exhibits single signals at around 2.21 \(+\) 1.11 \(\times\) 1000 ppm.

3. **Determine the \( ^1H \) spectrum** of a solution of Cefotiam Hydrochloride in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy. Use sodium 3-trimethylsilylpropansulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound; it exhibits single signals at around 3.1 ppm and at around 6.7 ppm, respectively. The ratio of integrated intensity of each signal is about 6:1. Dissolve 0.1 g of Cefotiam Hydrochloride in 5 mL of dilute nitric acid, and immediately add 1 mL of silver nitrate TS: a white precipitate is formed.

**Optical rotation** \(<2.49\> [\alpha]^{20}_D: +60 – +72° (1 g calculated on the anhydrous bases, water, 100 mL, 100 mm).

**pH** \(<2.54\>\) Dissolve 1.0 g of Cefotiam Hydrochloride in 10 mL of water: the pH of the solution is between 1.2 and 1.7.

**Purity**

1. **Clarity of solution**—Dissolve 1.0 g of Cefotiam Hydrochloride in 10 mL of water: the solution is clear, and colorless to yellow.

2. **Heavy metals** \(<1.07\>—To 1.0 g of Cefotiam Hydrochloride add 1 mL of sulfuric acid, and heat gently to carbonate. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, then heat gradually to incinerate. If a carbonized residue still retains, moisten the residue with a little amount of sulfuric acid, and ignite again to incinerate. After cooling, add 2 mL of hydrochloric acid to the residue, heat on a water bath to dissolve, then heat to dryness. Add 10 mL of water, and heat to dissolve. After cooling, add ammonia TS dropwise to adjust to pH 3 – 4, if necessary, filter, wash the residue on the filter with 10 mL of water, and use this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution in the same manner as for preparation of the test solution (not more than 20 ppm).

3. **Arsenic** \(<1.12\>—Incinere 1.0 g of Cefotiam Hydrochloride according to Method 4. After cooling, add 10 mL of dilute hydrochloric acid to the residue, heat to dissolve on the water bath, and use this solution as the test solution. Perform the test (not more than 2 ppm).

**Water** \(<2.48\> Not more than 7.0% (0.25 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

**Assay** Weigh accurately an amount of Cefotiam Hydrochloride and Cefotiam Hydrochloride RS, equivalent to about 0.1 g (potency), and dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 \(\mu L\) each of the sample solution and standard solution as directed under Liquid Chromatography. To determine the peak areas, \( A_T \) and \( A_S \), of cefotiam of these solutions.

\[
M_S: \text{Amount [mg (potency)] of Cefotiam Hydrochloride RS} \\
M_T: \text{Amount [mg (potency)] of Cefotiam Hydrochloride} \\
M_S = M_T \times A_T / A_S \times 1000
\]

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu m\) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 800 mL of 0.05 mol/L disodium hydrogenphosphate TS add 0.05 mol/L potassium dihydrogenphosphate TS to adjust the pH to 7.7. To 440 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefotiam is about 14 minutes.

**System suitability**

System performance: Dissolve 0.04 g of orcin in 10 mL of
Cefotiam Hydrochloride for Injection

Cefotiam Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of cefotiam (C₁₈H₁₉N₅O₄S₃: 525.63).

Method of Preparation

Prepare as directed under Injection, with Cefotiam Hydrochloride.

Identification

Determine the absorption spectrum of a solution of Cefotiam Hydrochloride for Injection (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 257 nm and 261 nm.

Dissolve 50 mg of Cefotiam Hydrochloride for Injection in 0.5 mL of heavy water for nuclear magnetic resonance spectroscopy, and determine the spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using sodium 3-trimethylsilyl-propanesulfonate as an internal reference compound: it exhibits a single signal A between δ 2.7 ppm and δ 3.0 ppm, and a single signal B at around δ 6.5 ppm. The ratio of the integrated intensity of each signal, A:B, is about 6:1.

pH <2.54>

The pH of a solution prepared by dissolving an amount of Cefotiam Hydrochloride for Injection, equivalent to 0.5 g (potency) according to the labeled amount, in 5 mL of water is between 5.7 and 7.2.

Purity

Clarity and color of solution—Dissolve an amount of Cefotiam Hydrochloride for Injection, equivalent to 1.0 g (potency) of Cefotiam Hydrochloride according to the labeled potency, in 10 mL of water: the solution is clear, and the absorbance of this solution, determined at 450 nm 10 minutes after dissolving as directed under Ultraviolet-visible Spectrophotometry <2.24>, is not more than 0.20.

Loss on drying <2.41>

Not more than 6.0% (0.5 g, in vacuum, 60°C, 3 hours).

Bacterial endotoxins <4.01>

Less than 0.125 EU/mg (potency).

Uniformity of dosage units <6.02>

It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06>

Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07>

Perform the test according to Method 1: it meets the requirement.

Sterility <4.06>

Perform the test according to the Membrane filtration method: it meets the requirement.

Assay

Weigh accurately the contents of not less than 10 Cefotiam Hydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 50 mg (potency) of Cefotiam Hydrochloride according to the labeled amount, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg (potency) of Cefotiam Hydrochloride RS, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefotiam Hydrochloride.

\[
\text{Amount [µg (potency)] of cefotiam (C}_{18}\text{H}_{19}\text{N}_5\text{O}_4\text{S}_3) = M_s \times A_1/A_S \times 1000}
\]

\(M_s: \text{Amount [µg (potency)] of Cefotiam Hydrochloride RS}

Operating conditions—

Proceed as directed in the Assay under Cefotiam Hydrochloride.

System Suitability—

Proceed as directed in the Assay under Cefotiam Hydrochloride.

Containers and storage

Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

Cefozopran Hydrochloride

Cefozopran Hydrochloride occurs as a white to light yellow powder.

Description

Cefozopran Hydrochloride occurs as a white to pale yellow, crystals or crystalline powder.

C₁₉H₁₇N₉O₅S₂·HCl: 551.99

(6R,7R)-7-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(methoxyimino)acetylaminio]-3-(1H-imidazo[1,2-b]pyridazin-4-ium-1-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate monohydrochloride

[C13359-04-9, Cefozopran]

Cefozopran Hydrochloride contains not less than 860 µg (potency) and not more than 960 µg (potency) per mg, calculated on the anhydrous basis. The potency of Cefozopran Hydrochloride is expressed as mass (potency) of cefozopran (C₁₉H₁₇N₉O₅S₂: 515.53).

Cefozopran Hydrochloride occurs as a white to pale yellow, crystals or crystalline powder.

It is freely soluble in dimethylsulfoxide and in formamide, slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in acetonitrile and diethyl ether.
Identification (1) Dissolve 0.02 g of Cefozopran Hydrochloride in 10 mL of water, add 1 mL of a solution of hydroxyalumonium chloride (1 in 10) and 2 mL of sodium hydroxide TS, allow to stand for 5 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS, and mix: a red-purple color develops.

(2) Determine the absorption spectra of solutions of Cefozopran Hydrochloride and Cefozopran Hydrochloride RS in a mixture of sodium chloride TS and methanol (3:2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.22>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the \(^1H\) spectrum of a solution of Cefozopran Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around \(\delta 3.9 \text{ ppm}\), a double signal B at around \(\delta 5.2 \text{ ppm}\), and a quartet signal C at around \(\delta 8.0 \text{ ppm}\), and the ratio of integrated intensity of each signal, A:B:C, is about 3:1:1.

(4) Dissolve 0.01 g of Cefozopran Hydrochloride in 1 mL of water and 2 mL of acetic acid (100), add 2 drops of silver nitrate TS, and mix: a white turbidity is formed.

Absorbance <2.24> \(E_{1\%}^{10}(238 \text{ nm}): 455 - 485 (50 \text{ mg calculated on the anhydrous basis, a mixture of sodium chloride TS and methanol (3:2)}; 5000 \text{ mL})

Optical rotation <2.49> \([\alpha]_D^{23} = -73 - -78^\circ (0.1 \text{ g calculated on the anhydrous basis, a mixture of sodium chloride TS and methanol (3:2)}, 10 \text{ mL, 100 mm})

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cefozopran Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Being specified separately.

(4) Related substances—Being specified separately.

Water <2.48> Not more than 2.5% (0.5 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Residue on ignition Being specified separately.

Bacterial endotoxins <4.01> Less than 0.05 EU/mg (potency).

Assay Weigh accurately an amount of Cefozopran Hydrochloride and Cefozopran Hydrochloride RS, equivalent to about 50 mg (potency), and dissolve each in the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and the mobile phase to make 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_1\) and \(Q_2\), of the peak area of cefozopran to that of the internal standard of these solutions.

Amount [\(\mu\)g (potency)] of cefozopran (C\(_{19}\)H\(_{17}\)N\(_{9}\)O\(_{5}\)S\(_{2}\)) = \(M_5 \times Q_1/Q_2 \times 1000\)

\(M_5\): Amount [\(\mu\)g (potency)] of Cefozopran Hydrochloride RS

Internal standard solution—A solution of 2,4-dihydroxybenzoic acid in the mobile phase (1 in 1250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Mix 0.366 g of diethylamine with water to make 1000 mL, and add 60 mL of acetonitrile and 5 mL of acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of cefozopran is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, cefozopran and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefozopran to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Cefozopran Hydrochloride for Injection

Cefozopran Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled potency of cefozopran (C\(_{19}\)H\(_{17}\)N\(_{9}\)O\(_{5}\)S\(_{2}\): 515.53).

Method of Preparation Prepare as directed under the Injections, with Cefozopran Hydrochloride.

Description Cefozopran Hydrochloride for Injection occurs as a white to light yellow, powder or masses.

Identification (1) Determine the absorption spectrum of a solution of Cefozopran Hydrochloride for Injection (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.22>: it exhibits a maximum between 236 nm and 241 nm.

(2) To 50 mg of Cefozopran Hydrochloride for Injection add 0.8 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and filter after shaking, and determine the spectrum of the filtrate as directed under Nuclear Magnetic Resonance Spectroscopy <2.2>, \(^1H\),
Cefpiramide Sodium / Official Monographs

Cefpiramide Sodium

C₇H₁₅Na₂O₄S₃: 634.62

Cefpiramide Sodium contains not less than 900 μg (potency) and not more than 990 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefpiramide Sodium is expressed as mass (potency) of cefpiramide (C₂₅H₂₄N₈O₇S₂: 612.64).

Description

Cefpiramide Sodium occurs as white to yellowish white powder.

It is very soluble in dimethylsulfoxide, freely soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

Identification

(1) Determine the absorption spectrum of a solution of Cefpiramide Sodium in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $<2.24>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the $^1$H spectrum of a solution of Cefpiramide Sodium in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy $<2.21>$, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ 2.3 ppm, at around δ 3.9 ppm and at around δ 8.2 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C, is about 3:1:1.

(3) Cefpiramide Sodium responds to the Qualitative Tests $<1.09>$ (1) for sodium salt.

Optical rotation $<2.49>$: [α]D$^{20}$: $-33$ $-$ $-40^\circ$ (0.2 g calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 10 mL, 100 mm).

pH $<2.54>$ The pH of a solution obtained by dissolving 2.0 g of Cefpiramide Sodium in 20 mL of water is between 5.5 and 8.0.

Purity

(1) Clarity and color of solution—Dissolve 1.0 g of Cefpiramide Sodium in 10 mL of 0.05 mol/L phosphate buffer solution, pH 7.0: the solution is clear, and colorless or light yellow.

(2) Heavy metals $<1.077>$—Proceed with 1.0 g of Cefpiramide Sodium according to Method 2, and perform the
test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately about 25 mg of Cefpiramide Sodium, dissolve in 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of 1-methyl-1H-tetrazole-5-thiol for liquid chromatography, previously dried in a desiccator (in vacuum, silica gel) for 2 hours, and an amount of Cefpiramide RS, equivalent to about 75 mg (potency), dissolve them in 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 100 mL. Pipet 2 mL of this solution, add 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of 1-methyl-1H-tetrazole-5-thiol, each of the other related substances and the total of the other related substances by the following equations: the amount of 1-methyl-1H-tetrazole-5-thiol, each of the other related substances and the total of the other related substances are not more than 1.0%, not more than 1.5% and not more than 4.0%, respectively.

Amount (%) of 1-methyl-1H-tetrazole-5-thiol (C$_7$H$_{12}$N$_4$S)

\[ \frac{M_{5}}{M_{1}} \times \frac{A_{T}}{A_{S}} \]

Amount (%) of each of other related substances

\[ \frac{M_{S}}{M_{1}} \times \frac{A_{T}}{A_{S}} \]

$M_{S}$: Amount (mg) of 1-methyl-1H-tetrazole-5-thiol

$M_{1}$: Amount [mg (potency)] of Cefpiramide RS

$A_{T}$: Peak area of 1-methyl-1H-tetrazole-5-thiol from the standard solution

$A_{S}$: Peak area of cefpiramide from the standard solution

$A_{T}$: Area of each peak other than 1-methyl-1H-tetrazole-5-thiol and cefpiramide from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.01 mol/L phosphate buffer solution, pH 6.8, acetonitrile, methanol and tetrahydrofuran (22:1:1:1).

Flow rate: Adjust the flow rate so that the retention time of cefpiramide is about 7 minutes.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 50 mL. Confirm that the peak area of 1-methyl-1H-tetrazole-5-thiol obtained from 5 μL of this solution is equivalent to 8 to 12% of that from 5 μL of the standard solution.

System performance: Dissolve 25 mg of Cefpiramide RS and 7 mg of cinnamic acid in the mobile phase to make 50 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, cinnamic acid and cefpiramide are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 1-methyl-1H-tetrazole-5-thiol is not more than 2.0%.

Water <2.48> Not more than 7.0% (0.35 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefpiramide Sodium and Cefpiramide RS, equivalent to about 50 mg (potency), add exactly 5 mL of the internal standard solution to dissolve, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{T}$ and $Q_{S}$, of the peak area of cefpiramide to that of the internal standard.

Amount [μg (potency)] of cefpiramide (C$_7$H$_{12}$N$_4$O$_3$S$_3$)

\[ M_{S} \times \frac{Q_{T}}{Q_{S}} \times 1000 \]

$M_{S}$: Amount [μg (potency)] of Cefpiramide RS

Internal standard solution—A solution of 4-dimethylaminoantipyrine (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.01 mol/L phosphate buffer solution, pH 6.8, acetonitrile, methanol and tetrahydrofuran (22:1:1:1).

Flow rate: Adjust the flow rate so that the retention time of cefpiramide is about 7 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cefpiramide and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefpiramide to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding 5°C.
Cefpirome Sulfate

C₂₂H₂₂N₆O₅S₂·H₂SO₄: 612.66


Cefpirome Sulfate contains not less than 760 µg (potency) per mg, calculated on the anhydrous basis. The potency of Cefpirome Sulfate is expressed as mass (potency) of cefpirome (C₂₂H₂₂N₆O₅S₂: 514.58).

Description  Cefpirome Sulfate occurs as a white to pale yellowish white crystalline powder, and has a slight, characteristic odor.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification  (1) Dissolve 10 mg of Cefpirome Sulfate in 2 mL of water, add 3 mL of hydroxylammonium hydrochloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium ion (III) sulfate TS, and shake: a red-brown color develops.

(2) Dissolve 1 mg of Cefpirome Sulfate in 4 mL of water, add 1 mL of dilute hydrochloric acid while cooling in ice, add 1 mL of a freshly prepared solution of sodium nitrite (1 in 10), and allow to stand for 2 minutes. Add 1 mL of ammonium amidosulfuric acid TS while cooling in ice bath, allow to stand for 1 minute, and add 1 mL of a solution of N-1-naphthylethylene dihydrochloride (1 in 1000): a purple color develops.

(3) Take 5 mg of Cefpirome Sulfate, dissolve in 1 mL of ethanol (95) and 1 mL of water, add 100 mg of 1-chloro-2,4-dinitrobenezene, and heat on a water bath for 5 minutes. After cooling, add 2 or 3 drops of a solution of sodium hydroxide (1 in 10) and 3 mL of ethanol (95): a red-brown color develops.

(4) Determine the absorption spectra of solutions of Cefpirome Sulfate and Cefpirome Sulfate RS, equivalent to about 50 mg (potency), in 0.01 mol/L hydrochloric acid TS (1 in 50,000), as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the ¹H spectrum of a solution of Cefpirome Sulfate in heavy water for nuclear magnetic resonance spectroscopy (1 in 25) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 4.1 ppm, a double signal B at around δ 5.9 ppm, a single signal C at around δ 7.1 ppm, and a multiple signal D at around δ 7.8 ppm. The ratio of integrated intensity of each signal, A:B:C:D, is about 3:1:1:1.

A solution of Cefpirome Sulfate (1 in 250) responds to the Qualitative Tests <1.09> (1) for sulfate salt.

Absorbance  <2.24>  ε₁₃₀₄₅₆₇₈ (270 nm): 405 - 435 (50 mg calculated on the anhydrous basis, 0.01 mol/L hydrochloric acid TS, 2500 mL).

Optical rotation  <2.49>  [α]D₂⁰: −27 - −33° (50 mg calculated on the anhydrous basis, a solution prepared by addition of water to 25 mL of acetonitrile to make 50 mL, 20 mL, 100 mm).

pH  <2.54>  Dissolve 0.1 g of Cefpirome Sulfate in 10 mL of water: the pH of the solution is between 1.6 and 2.6.

Purity  (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals  <1.07>—Proceed with 1.0 g of Cefpirome Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Being specified separately.

(4) Related substances—Being specified separately.

(5) Residual solvents—Being specified separately.

Water  <2.48>  Not more than 2.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition—Being specified separately.

Bacterial endotoxins  <4.01>  Less than 0.10 EU/mg (potency).

Assay  Weigh accurately an amount of Cefpirome Sulfate and Cefpirome Sulfate RS, equivalent to about 50 mg (potency), dissolve each in water to make exactly 100 mL. Pipet 5 mL of these solutions, add each in water to make exactly 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 µL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and Aₛ, of cefpirome of each solution.

Amount [µg (potency)] of cefpirome (C₂₂H₂₂N₆O₅S₂) = Mₛ × A₁/Aₛ × 1000

Mₛ: Amount [µg (potency)] of Cefpirome Sulfate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.45 g of ammonium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.3 with phosphoric acid. To 800 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefpirome is about 7.5 minutes.

System suitability—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating con-
ditions, the number of theoretical plates of the peak of cepfi-rome is not less than 3600.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cepfiprome is not more than 1.0%.

Containers and storage  Containers—Hermetic containers.

Storage—At a temperature between 2 and 8°C.

Cefpodoxime Proxetil

Cefpodoxime Proxetil contains not less than 706 μg (potency) and not more than 774 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefpodoxime Proxetil is expressed as mass (potency) of Cefpodoxime (C_{21}H_{27}N_{5}O_{9}S_{2}: 557.60)

Description  Cefpodoxime Proxetil occurs as a white to light brownish white powder.

It is very soluble in acetonitrile, in methanol and in chloroform, freely soluble in ethanol (99.5), and very slightly soluble in water.

Identification  (1) Determine the absorption spectrum of a solution of Cefpodoxime Proxetil in acetonitrile (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.2>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefpodoxime Proxetil RS prepared in the same manner as the sample solution; both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefpodoxime Proxetil as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.2>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefpodoxime Proxetil RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the 1H spectrum of a solution of Cefpodoxime Proxetil in deuterochloroform for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.2>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits double signals, A and B, at around δ 1.3 ppm and at around δ 1.6 ppm, and single signals, C and D, at around δ 3.3 ppm and at around δ 4.0 ppm. The ratio of the integrated intensity of these signals, A:B:C:D, is about 2:1:1:1.

Optical rotation  <2.4> [α]_D^24: +24.0 – +31.4° (0.1 g calculated on the anhydrous basis, acetonitrile, 20 mL, 100 mm).

Purity  (1) Heavy metals <1.07>—Proceed with 1.0 g of Cefpodoxime Proxetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Cefpodoxime Proxetil in 50 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.0> according to the following conditions. If necessary, perform the test in the same manner with 20 μL of the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to compensate for the base line. Determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the peak, having the relative retention time of about 0.8 with respect to the isomer B of cefpodoxime proxetil, is not more than 2.0%, the peak other than cefpodoxime proxetil is not more than 1.0%, and the sum of the peaks other than cefpodoxime proxetil is not more than 6.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 22°C.

Mobile phase A: A mixture of water, methanol and a solution of formic acid (1 in 50) (11:8:1).

Mobile phase B: A mixture of methanol and a solution of formic acid (1 in 50) (19:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 65</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>65 – 145</td>
<td>95 → 15</td>
<td>5 → 85</td>
</tr>
<tr>
<td>145 – 155</td>
<td>15</td>
<td>85</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of the isomer B of cefpodoxime proxetil is about 60 minutes.

Time span of measurement: About 2.5 times as long as the retention time of the isomer B of cefpodoxime proxetil beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 5 mL of the sample solution, add the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make exactly 200 mL, and use this solution as the solution for required detectability test. Pipet 2 mL of the solution for required detectability test, and add the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make exactly 100 mL. Confirm that the peak areas of the isomer A and the isomer B of ce-
Cefroxadine Hydrate / Official Monographs

Cefroxadine Hydrate contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dehydrated basis. The potency of Cefroxadine Hydrate is expressed as mass (potency) of cefroxadine (C₁₆H₁₉N₃O₅S.₂H₂O: 401.43)

C₁₆H₁₉N₃O₅S.₂H₂O: 401.43

(6R,7R)-7-[(2R)-2-Amino-2-cyclohexa-1,4-dienylacetylamino]-3-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrate [51762-05-1, anhydride]

Cefroxadine Hydrate occurs as pale yellowish white to light yellow, crystalline particles or powder. It is very soluble in formic acid, slightly soluble in water and in methanol, and very slightly soluble in acetonitrile and in ethanol (95).

It dissolves in 0.001 mol/L hydrochloric acid TS and in dilute acetic acid.

Identification (1) Determine the absorption spectrum of a solution of Cefroxadine Hydrate in 0.001 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefroxadine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the 1H spectrum of a solution of Cefroxadine Hydrate in deuterated formic acid for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.27>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits three sharp single signals, A, B and C, at around δ 2.8 ppm, at around δ 4.1 ppm and at around δ 6.3 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 4:3:1.

Cefroxadine Hydrate

C₁₆H₁₉N₃O₅S.₂H₂O: 401.43

(6R,7R)-7-[(2R)-2-Amino-2-cyclohexa-1,4-dienylacetylamino]-3-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrate [51762-05-1, anhydride]

Cefroxadine Hydrate contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dehydrated basis. The potency of Cefroxadine Hydrate is expressed as mass (potency) of cefroxadine (C₁₆H₁₉N₃O₅S.₂H₂O: 401.43)

C₁₆H₁₉N₃O₅S.₂H₂O: 401.43

(6R,7R)-7-[(2R)-2-Amino-2-cyclohexa-1,4-dienylacetylamino]-3-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrate [51762-05-1, anhydride]
**System suitability**—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefroxadine obtained from 40 μL of this solution is equivalent to 7 to 13% of that obtained from 40 μL of the standard solution.

System performance: Dissolve 3 mg of Cefroxadine Hydrate and 15 mg of orcin in 100 mL of the mobile phase. When the procedure is run with 40 μL of this solution under the above operating conditions, orcin and cefroxadine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefroxadine is not more than 2.0%.

**Water**<2.48> Not less than 8.5% and not more than 12.0% (0.1 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Cefroxadine Hydrate and Cefroxadine RS, equivalent to about 50 mg (potency), dissolve each in a suitable amount of a mixture of dilute acetic acid and phosphoric acid (500:1), add exactly 5 mL of the internal standard solution and a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography<2.01> according to the following conditions, and calculate the ratios, Q7 and Q8, of the peak area of cefroxadine to that of the internal standard.

\[
\frac{Q_7}{Q_8} = M_5 \times \frac{S}{T} \times \frac{1000}{Q}
\]

\[
M_5: \text{Amount [mg (potency)] of Cefroxadine RS}
\]

\[
S, T: \text{Area of cefroxadine and internal standard, respectively}
\]

\[
Q: \text{Area of internal standard}
\]

**Internal standard solution**—Dissolve 1.6 g of vanillin in 5 mL of methanol, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 100 mL.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wave-length: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 50) and acetonitrile (97:3).

Flow rate: Adjust the flow rate so that the retention time of cefroxadine is about 10 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefroxadine and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of cefroxadine to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.
Cefroxadine for Syrup

シロップ用セフロキサジン

Cefroxadine for Syrup is a preparation for syrup, which is suspended before use. It contains not less than 93.0% and not more than 107.0% of the labeled potency of cefroxadine (C₁₆H₁₉N₃O₅S: 365.40).

**Method of preparation** Prepare as directed under Preparations for Syrups, with Cefroxadine Hydrate.

**Identification** Powder Cefroxadine for Syrup, if necessary. Weigh accurately an amount of Cefroxadine RS, equivalent to about 50 mg (potency), dissolve in a mixture of dilute acetic acid and phosphoric acid (500:1) to make 1 g in 10 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\times\)Q, and determine the absorbances, \(A_T\) and \(A_S\), at 267 nm.

**Dissolution rate (%) with respect to the labeled amount of cefroxadine (C₁₆H₁₉N₃O₅S)**

\[
\frac{M_S}{M_T} = \frac{A_T}{A_S} \times \frac{1}{C} \times 450
\]

\(M_S:\) Amount [mg (potency)] of Cefroxadine RS
\(M_T:\) Amount (g) of Cefroxadine for Syrup
\(C:\) Labeled amount [mg (potency)] of cefroxadine (C₁₆H₁₉N₃O₅S) in 1 g

**Assay** Powder Cefroxadine for Syrup, if necessary, weigh accurately a portion of the powder, equivalent to about 50 mg (potency) of Cefroxadine Hydrate, add 160 mL of a mixture of dilute acetic acid and phosphoric acid (500:1), shake well for 15 minutes, add exactly 5 mL of the internal standard solution, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 \(\mu\)m, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefroxadine RS, equivalent to about 50 mg (potency), dissolve in a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefroxadine Hydrate.

\[
M_S = M_5 \times \frac{Q_T}{Q_S} \times V/200
\]

\(M_5:\) Amount [mg (potency)] of Cefroxadine RS

**Internal standard solution—Dissolve 1.6 g of vanillin in 5 mL of methanol, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 100 mL.**

**Containers and storage** Containers—Tight containers.
Cefsulodin Sodium

セフスロジンナトリウム

C₂₂H₁₉N₄NaO₈S₂: 554.53

Cefsulodin Sodium contains not less than 900 μg (potency) and not more than 970 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefsulodin Sodium is expressed as mass (potency) of cefsulodin (C₂₂H₂₀N₄O₈S₂: 532.55).

**Description** Cefsulodin Sodium occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in water and in formamide, slightly soluble in methanol, and very slightly soluble in ethanol (95).

It is hygroscopic.

**Identification** (1) Determine the absorption spectrum of a solution of Cefsulodin Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefsulodin Sodium RS prepared in the same manner as sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefsulodin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefsulodin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the 'H spectrum of a solution of Cefsulodin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using sodium 3-trimethylsilylepropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound; it exhibits a multiple signal A between δ 7.3 ppm and δ 7.7 ppm, and double signals, B and C, at around δ 8.4 ppm and at around δ 9.1 ppm, respectively. The ratio of integrated intensity of these signals, A:B:C, is about 5:2:2.

(4) Cefsulodin Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

**Optical rotation** <2.49> [α]D: +16.5 – +20.0° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

**pH** <2.54> Dissolve 1.0 g of Cefsulodin Sodium in 10 mL of water: the pH of the solution is not less than 3.3 and not more than 4.8.

**Purity** (1) Clarity of solution—Dissolve 1.0 g of Cefsulodin Sodium in 10 mL of water: the solution is clear.

(2) Heavy metals <1.07>—To 1.0 g of Cefsulodin Sodium add 10 mL of a solution of magnesium nitrate hexahydrate in 10 mL of water: the solution is clear.

Determine the absorption spectrum of a solution of Cefsulodin Sodium RS prepared in heavy water for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a multiple signal A between δ 7.3 ppm and δ 7.7 ppm, and double signals, B and C, at around δ 8.4 ppm and at around δ 9.1 ppm, respectively. The ratio of integrated intensity of these signals, A:B:C, is about 5:2:2.

Measure the intensity of these signals, A:B:C, is about 5:2:2.

**Operating conditions**—Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (97:3).

Mobile phase B: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (92:8).

Flowing of the mobile phase: Change the mobile phase A to B at 14 minutes after the injection of sample.

Flow rate: Adjust the flow rate so that the retention time of cefsulodin is about 9 minutes.

Time span of measurement: About 4 times as long as the retention time of cefsulodin.

System suitability—

Test for required detection: Pipet 1 mL of the standard solution, add water to make exactly 10 mL. Confirm that the peak areas of isonicotinic acid amide and cefsulodin obtained from 10 μL of this solution are equivalent to 7 to 13% of those of isonicotinic acid amide and cefsulodin obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, isonicotinic acid amide and cefsulodin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefsulodin is not more than 1.0%.

Water <2.48> Not more than 5.0% (1 g, volumetric titration, direct titration, avoiding moisture absorption of the sample, using a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Cefsulodin Sodium and Cefsulodin Sodium RS, equivalent to about 0.1 g (potency), dissolve each in water to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of cefsulodin of each solution.

Amount [μg (potency)] of cefsulodin (C₂₂H₂₀N₄O₈S₂) = M₅ × A₁/A₅ × 1000

M₅: Amount [μg (potency)] of Cefsulodin Sodium RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (97:3).

Flow rate: Adjust the flow rate so that the retention time of cefsulodin is about 9 minutes.

System suitability—

System performance: Dissolve 40 mg of isonicotinic acid amide in 25 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, isonicotinic acid amide and cefsulodin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefsulodin is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Ceftazidime Hydrate

C₂₂H₂₂N₆O₇S₂·5H₂O: 636.65

Ceftazidime Hydrate contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Ceftazidime Hydrate is expressed as mass (potency) of ceftazidime (C₂₂H₂₀N₄O₈S₂: 546.58).

Description Ceftazidime Hydrate occurs as a white to light yellowish white crystalline powder.

It is slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (95%).

Identification (1) Determine the absorption spectrum of a solution of Ceftazidime Hydrate in phosphate buffer solution, pH 6.0 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ceftazidime RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ceftazidime Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ceftazidime RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.05 g of Ceftazidime Hydrate add 5 mg of dried sodium carbonate, and add 0.5 mL of heavy water for nuclear magnetic resonance spectroscopy to dissolve. Determine the ¹H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound:
it exhibits single signals, A and B, at around δ 1.5 ppm and at around δ 6.9 ppm, and a multiple signal C between δ 7.9 ppm and δ 9.2 ppm. The ratio of integrated intensity of each signal, A:B:C, is about 6:1:5.

**Optical rotation** <2.49> [α]D <25> 28°–34° (0.5 g calculated on the dried bases, phosphate buffer solution, pH 6.0, 100 mL, 100 mm).

**pH** <2.54> Dissolve 0.5 g of Ceftazidime Hydrate in 100 mL of water: the pH of the solution is between 3.0 and 4.0.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Ceftazidime Hydrate in 10 mL of a solution obtained by dissolving 5 g of anhydrous disodium hydrogenphosphate and 1 g of potassium dihydrogenphosphate in water to make 100 mL: the solution is clear, and its absorbance <2.24> at 420 nm is not more than 0.20.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ceftazidime Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 2 ppm).

(3) Arsenic <1.12>—Prepare the test solution with 1.0 g of Ceftazidime Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances (i) Trityl-α-butyl substance and α-butyl substance—Dissolve 0.10 g of Ceftazidime Hydrate in 2 mL of diluted disodium hydrogenphosphate TS (1 in 3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted disodium hydrogenphosphate TS (1 in 3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 2 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of n-butyl acetate, acetic acid (100), acetic acid buffer solution, pH 4.5 and 1-butanol (16:16:13:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots which appear upper in position than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(ii) Other related substances—Dissolve 20 mg of Ceftazidime Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak height, Hf and Hs, of pyridine of each solution: the amount of free pyridine is not more than 0.3%.

\[
\text{Amount (mg) of free pyridine} = M_s \times \frac{H_f}{H_s} \times \frac{1}{1000} \\
M_s: \text{Amount (mg) of pyridine}
\]

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.0 g of ammonium dihydrogenphosphate in 750 mL of water, adjust to pH 3.5 with phosphoric acid, and add water to make 870 mL. To this solution add 130 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftazidime is about 4 minutes.

Time span of measurement: About 3 times as long as the retention time of ceftazidime beginning after the solvent peak.

**System suitability**—
Test for required detection: Pipet 1 mL of the standard solution, add the mobile phase to make exactly 5 mL, and confirm that the peak area of ceftazidime obtained from 5 µL of this solution is equivalent to 15 to 25% of that of ceftazidime obtained from 5 µL of the standard solution.

System performance: Dissolve about 0.01 g each of Ceftazidime Hydrate and acetonitrile in 20 mL of the mobile phase. When the procedure is run with 5 µL of this solution under the above operating conditions, ceftazidime and acetonitrile are eluted in order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ceftazidime is not more than 2.0%.

(5) Free pyridine—Weigh accurately about 50 mg of Ceftazidime Hydrate, dissolve in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pyridine, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak height, Hf and Hs, of pyridine of each solution: the amount of free pyridine is not more than 0.3%.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.88 g of ammonium dihydrogenphosphate in 500 mL of water, add 300 mL of acetonitrile and water to make 1000 mL, and adjust to pH 7.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of pyridine is about 4 minutes.

**System suitability**—
Test for required detection: Confirm that the peak height of pyridine obtained from 10 µL of the standard solution is equivalent to 50% of the full scale.

System performance: Dissolve 5 mg of Ceftazidime Hydrate in 100 mL of a solution of pyridine in the mobile phase (1 in 20,000). When the procedure is run with 10 µL of this
solution under the above operating conditions, ceftazidime and pyridine are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the heights of pyridine is not more than 5.0%.

Loss on dryness <2.41> Not less than 13.0% and not more than 15.0% (0.1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Ceftazidime Hydrate and Ceftazidime RS, equivalent to about 0.1 g (potency), and dissolve each in 0.05 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL. Pipet 10 mL of each of these solutions, add exactly 5 mL of the internal standard solution, then add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 µL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of ceftazidime to that of the internal standard of each solution.

\[
\text{Amount [µg (potency)] of ceftazidime (C}_{22}\text{H}_{22}\text{N}_{6}\text{O}_{7}\text{S}_2} = M_S \times Q_T / Q_S \times 1000
\]

\( M_S \): Amount [mg (potency)] of Ceftazidime RS

Internal standard solution—A solution of dimedon in 0.05 mol/L phosphate buffer solution, pH 7.0 (11 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with hexasilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.26 g of anhydrous disodium hydrogenphosphate and 2.72 g of potassium dihydrogenphosphate in 980 mL of water, and add 20 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftazidime is about 4 minutes.

System suitability—

System performance: When the procedure is run with 5 µL of the standard solution under the above operating conditions, the internal standard and ceftazidime are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftazidime to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ceftazidime for Injection

注射用セフタジム

Ceftazidime for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of ceftazidime (C_{22}H_{22}N_{6}O_{7}S_{2}: 546.58).

Method of preparation Prepare as directed under Injections, with Ceftazidime Hydrate.

Description Ceftazidime for Injection is a white to pale yellowish white powder.

Identification Determine the absorption spectrum of a solution of Ceftazidime for Injection (1 in 100,000) in phosphate buffer solution, pH 6.0, as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 255 nm and 259 nm.

\[ \text{pH <2.54>} \] Dissolve an amount of Ceftazidime for Injection, equivalent to 1.0 g (potency) of Ceftazidime Hydrate according to the labeled amount, in 10 mL of water: the pH of this solution is 5.8 to 7.8.

Purity Clarity and color of solution—Dissolve 5 g of disodium hydrogen phosphate and 1 g of potassium dihydrogen phosphate in water to make 100 mL. In 10 mL of this solution dissolve an amount of Ceftazidime for Injection, equivalent to 1.0 g (potency) of Ceftazidime Hydrate according to the labeled amount: the solution is clear and colorless. Also, determine the absorption spectra of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 420 nm is not more than 0.3.

Loss on drying <2.41> Not more than 14.0% (0.1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 0.067 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filter method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Ceftazidime for Injection. Weigh accurately an amount of Ceftazidime Hydrate, equivalent to about 0.25 g (potency), and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0, to make exactly 250 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add more 0.05 mol/L phosphate buffer solution, pH 7.0, to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ceftazidime RS, equivalent to about 25 mg (potency), and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0, to make exactly 25 mL. Pipet 10 mL of this so-
solution, add exactly 5 mL of the internal standard solution, then add 0.05 mol/L phosphate buffer solution, pH 7.0, to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefazidime Hydrate.

Amount [mg (potency)] of cefazidime (C₁₂H₁₇N₅O₇S₂) = Mₛ × Qₛ/Qₛ × 10

Mₛ: Amount [mg(potency)] of Cefazidime RS

Internal standard solution—A solution of dimedon in 0.05 mol/L phosphate buffer solution, pH 7.0 (11 in 10,000).

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Cefteram Pivoxil

セフテラム ピボキシル

C₂₂H₂₇N₉O₇S₂: 593.64
2,2-Dimethylpropanoyloxymethyl (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-(5-methyl-2H-tetrazol-2-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

C₁₆H₁₇N₉O₅S₂: 479.49

Cefteram Pivoxil contains not less than 743 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefteram Pivoxil is expressed as mass (potency) of cefteram (C₁₆H₁₇N₉O₅S₂: 479.49).

Description Cefteram Pivoxil occurs as a white to pale yellowish white powder.

It is very soluble in acetonitrile, freely soluble in methanol, in ethanol (95) and in chloroform, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Cefteram Pivoxil in 0.05 mol/L hydrochloric acid-methanol TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the 1H spectrum of a solution of Cefteram Pivoxil in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.27>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals A, B and C, at around δ 1.2 ppm, at around δ 2.5 ppm and at around δ 4.0 ppm, respectively. The ratio of the integrated intensity of these signals, A:B:C, is about 3:1:1.

Optical rotation <2.49> [α]D: +35° to +43° (0.4 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Cefteram Pivoxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefteram Pivoxil according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Cefteram Pivoxil in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 with respect to cefteram pivoxil from the sample solution is not larger than 1.25 times the peak area of cefteram pivoxil from the standard solution, the area of the peak, having the relative retention time of about 0.1 is not larger than 1/4 times the peak area of cefteram pivoxil from the standard solution, and the total area of the peaks other than cefteram pivoxil is not more than 2.75 times the peak area of cefteram pivoxil from the standard solution. For the above calculation, use the area of the peak, having the relative retention time of about 0.1, after multiplying by its relative response factor, 0.74.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of cefteram pivoxil.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefteram pivoxil obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefteram pivoxil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefteram pivoxil is not more than 3.0%.

Water <2.49> Not more than 3.0% (0.3 g, coulometric titration).

Assay Weigh accurately an amount of Cefteram Pivoxil and Cefteram Pivoxil Mesylene Sulfonate RS, equivalent to about 40 mg (potency), dissolve each in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Qₛ, of the peak area of cefteram pivoxil to that of the internal standard.
Cefteram Pivoxil Fine Granules

Cefteram Pivoxil Fine Granules contain not less than 90.0% and not more than 110.0% of the labeled amount of cefteram (C₁₆H₁₇N₉O₅S₂: 479.49).

Method of preparation: Prepare as directed under Granules, with Cefteram Pivoxil.

Identification: Powder Cefteram Pivoxil Fine Granules. To a portion of the powder, equivalent to 0.1 g (potency) of Cefteram Pivoxil according to the labeled amount, add 20 mL of methanol, shake well, and filter. To 1 mL of the filtrate add 0.05 mol/L hydrochloric acid-methanol TS to make 500 mL, and determine the absorption spectrum as directed under Ultraviolet-Visible Spectrophotometry <2.24> it exhibits a maximum between 262 nm and 266 nm.

Purity: Related substances—Powder Cefteram Pivoxil Fine Granules, if necessary. To a portion, equivalent to 0.1 g (potency) of Cefteram Pivoxil according to the labeled amount, add diluted acetonitrile (1 in 2) to make 100 mL, disperse the particle with the aid of ultrasonic waves, then filter, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 with respect to cefteram pivoxil, is not larger than 1.75 times the peak area of cefteram pivoxil from the standard solution, the area of the peak, having the relative retention time of about 0.1, is not larger than 17/25 times the peak area of cefteram pivoxil from the standard solution, and the total area of the peaks other than cefteram pivoxil is not larger than 3.7 times the peak area of cefteram pivoxil from the standard solution. For this calculation, use the peak area for the peak having the relative retention time of about 0.1 after multiplying by its relative response factor, 0.74.

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Cefteram Pivoxil.

System suitability—
Proceed as directed in the system suitability in the Assay under Cefteram Pivoxil.

Water—Not more than 0.3% (0.1 g potency), coulometric titration.

Uniformity of dosage units—The Granules in single-unit container meet the requirement of the Mass variation test.

Dissolution—Being specified separately.

Particle size—It meets the requirements of Fine granules.

Assay: Powder Cefteram Pivoxil Fine Granules, if necessary, and use as the sample. Weigh accurately an amount of the sample, equivalent to about 0.3 g (potency) of Cefteram Pivoxil, add exactly 30 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 300 mL. Disperse the particle with the aid of ultrasonic waves, then filter, and use the filtrate as the sample solution. Separately, weigh accurately about 50 mg (potency) of Cefteram Pivoxil Mesitylene Sulfonate RS, dissolve in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of cefteram pivoxil to that of the internal standard.

\[
\frac{Q_1}{Q_2} = \frac{S_1}{S_2} \times \frac{F_1}{F_2}
\]

where, \(S_1\) and \(S_2\) are the peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 with respect to cefteram pivoxil, and the total area of the peaks other than cefteram pivoxil, respectively.

\[
\frac{M_s}{M_i} = \frac{Q_1}{Q_2} \times \frac{F_1}{F_2}
\]

\(M_s\): Amount [mg (potency)] of Cefteram Pivoxil (C₁₆H₁₇N₉O₅S₂) = \(M_s \times \frac{Q_1}{Q_2} \times 1000 \)

\(M_i\): Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate RS

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1:2) (1 in 1000).

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Cefteram Pivoxil.

System suitability—
Proceed as directed in the system suitability in the Assay under Cefteram Pivoxil.
Containers and storage  Containers—Tight containers.

Cefteram Pivoxil Tablets

セフテラム ピボキシル錠

Cefteram Pivoxil Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of cefteram (\(\text{C}_{16}\text{H}_{17}\text{N}_{9}\text{O}_{5}\text{S}_{2}: 479.49\)).

Method of preparation  Prepare as directed under Tablets, with Cefteram Pivoxil.

Identification  To a quantity of powdered Cefteram Pivoxil Tablets, equivalent to 0.1 g (potency) of Cefteram Pivoxil according to the labeled amount, add diluted acetonitrile (1 in 2) to make 100 mL. Disperse this solution with ultrasonic waves, filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefteram Pivoxil Mesitylene Sulfonate RS, equivalent to about 50 mg (potency), dissolve in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution, add diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefteram Pivoxil.

Amount [mg (potency)] of cefteram (\(\text{C}_{16}\text{H}_{17}\text{N}_{9}\text{O}_{5}\text{S}_{2}\))

\[ M_s \times \frac{Q_1}{Q_3} \times \frac{1}{50} \]

\( M_s \): Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate RS

Internal standard solution  A solution of methyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 1000).

Dissolution  When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Cefteram Pivoxil Tablets is not less than 75%.

Start the test with 1 tablet of Cefteram Pivoxil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains about 22 \(\mu\)g (potency) of Cefteram Pivoxil. Disperse this solution with ultrasonic waves, filter through a membrane filter with pore size of not exceeding 0.45 \(\mu\)m, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution.

Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.20>.

\[ \text{Absorbance} = A_T - A_S \]

For this calculation, use the area of the peak, having the relative retention time of about 0.1 with respect to cefteram pivoxil, obtained from the sample solution. The area of the peak is not larger than 1.75 times the peak area of cefteram pivoxil from the standard solution, and the area of the peak, having the relative retention time of about 0.1 is not larger than 17/25 times the peak area of cefteram pivoxil from the standard solution. Furthermore, the total area of the peaks other than cefteram pivoxil is not larger than 3.7 times the peak area of cefteram pivoxil from the standard solution. For this calculation, use the area of the peak, having the relative retention time of about 0.1 with respect to cefteram pivoxil, after multiplying by the relative response factor, 0.74.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Cefteram Pivoxil.

System suitability—

Proceed as directed in the system suitability in the Purity (3) under Cefteram Pivoxil.

Water  Not more than 4.0% (a quantity equivalent to 0.2 g potency) of powdered Cefteram Pivoxil Tablets, volumetric titration, direct titration).

Uniformity of dosage units  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cefteram Pivoxil Tablets add exactly 5 mL of the internal standard solution per 50 mg (potency) of Cefteram Pivoxil, and add diluted acetonitrile (1 in 2) to make \(V\) mL so that each mL contains about 1 mg (potency) of Cefteram Pivoxil. Disperse this solution with ultrasonic waves, filter through a membrane filter with pore size not exceeding 0.45 \(\mu\)m, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefteram Pivoxil Mesitylene Sulfonate RS, equivalent to about 50 mg (potency), dissolve in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution, add diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefteram Pivoxil.

Amount [mg (potency)] of cefteram (\(\text{C}_{16}\text{H}_{17}\text{N}_{9}\text{O}_{5}\text{S}_{2}\))

\[ M_t \times \frac{Q_1}{Q_3} \times \frac{1}{50} \]

\( M_t \): Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate RS

C: Labeled amount [mg (potency)] of cefteram (\(\text{C}_{16}\text{H}_{17}\text{N}_{9}\text{O}_{5}\text{S}_{2}\)) in 1 tablet

Assay  To a number of tablet of Cefteram Pivoxil Tablets, equivalent to about 1.0 g (potency) of Cefteram Pivoxil, add 120 mL of diluted acetonitrile (1 in 2), disperse with ultrasonic waves, and add diluted acetonitrile (1 in 2) to make exactly 200 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add diluted acetonitrile (1 in 2) to make 50 mL, filter through a membrane filter with pore size not exceeding 0.45 \(\mu\)m, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefteram Pivoxil Mesitylene Sulfonate RS, equivalent to about 50 mg (potency), dissolve in
20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution, add diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefteram Pivoxil.

Amount [mg (potency)] of cefteram (C16H17N9O5S2) = M5 × Qf/Q5 × 20

M5: Amount [mg (potency)] of Cefteram Pivoxil Mesylate

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 1000).

Containers and storage—Containers—Tight containers. Storage—Light-resistant.

Ceftibuten Hydrate

Ceftibuten Hydrate occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in N,N-dimethylformamide and in dimethyl sulfoxide, and practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification

(1) Determine the absorption spectrum of a solution of Cefteram Hydrate in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum; both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefteram Hydrate as directed in the paste method under same wavelengths.

(3) Determine the \(^1\)H spectrum of a solution of Cefteram Hydrate in deuterated dimethyl sulfoxide for nuclear magnetic resonance spectroscopy (1 in 30), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.24>: it exhibits double signals A and B, at around \(\delta\) 3.2 ppm and at around \(\delta\) 5.1 ppm, a quartet signal C, at around \(\delta\) 5.8 ppm, and a single signal D, at around \(\delta\) 6.3 ppm. The ratio of integrated intensity of each signal except the signal at around \(\delta\) 3.2 ppm, B:C:D is about 1:1:1.

Optical rotation <2.49>  \([\alpha]_D^2\) = +135° to +155° (0.3 g calculated on the anhydrous basis, 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0, 50 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cefteram Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Being specified separately.

Water <2.48> Not less than 8.0% and not more than 13.0% (0.2 g, volumetric titration, direct titration. Use a mixture of pyridine for water determination and ethylene glycol for water determination (5:1) instead of methanol for water determination).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay—Weigh accurately an amount of Cefteram Hydrate and Cefteram Hydrochloride RS, equivalent to about 10 mg (potency), dissolve each in 36 mL of 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0, and add exactly 4 mL of the internal standard solution, shake, and use these solutions as the sample solution and standard solution. Perform the test with 5 mL of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Qf and Qs, of the peak area of cefteram to that of the internal standard. Keep the sample solution and the standard solution at 5°C or below and use within 2 hours.

Amount [mg (potency)] of cefteram (C16H17N9O5S2) = M5 × Qf/Qs × 1000

M5: Amount [mg (potency)] of Cefteram Pivoxil Mesylate

Internal standard solution—A solution of methyl \(p\)-hydroxybenzoate in acetonitrile (3 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (7 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.005 mol/L \(n\)-decyl trimethylammonium bromide TS and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of cefteram is about 10 minutes.

System suitability—

System performance: Dissolve 5 mg of Cefteram Hydrate in 1 mol/L Hydrochloric acid TS to make 50 mL, and allow to stand for 4 hours at room temperature. To 10 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make 25 mL. When the procedure is run with 5 \(\mu\)L of this solution under the above operating conditions, trans-isomer and cefteram are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times
with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefditoren to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant, and not exceeding 5°C.

Ceftizoxime Sodium

セフチゾキシムナトリウム

C\textsubscript{13}H\textsubscript{12}N\textsubscript{5}NaO\textsubscript{5}S\textsubscript{2}: 405.38
Monosodium (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(methoxylimino)acetylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [68401-82-1]

Ceftizoxime Sodium contains not less than 925 μg (potency) and not more than 965 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ceftizoxime Sodium is expressed as mass (potency) of cefizoxime (C\textsubscript{13}H\textsubscript{13}N\textsubscript{5}O\textsubscript{5}S\textsubscript{2}: 383.40).

Description Ceftizoxime Sodium occurs as a white to light yellow, crystals or crystalline powder. It is very soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Ceftizoxime Sodium (1 in 63,000) as directed under Ultraviolet-visible Spectrophotometry <2,24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ceftizoxime Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the "H spectrum of a solution of Ceftizoxime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2,21>, using sodium 3-trimethylsilylpropionate-\textsubscript{d\textsubscript{4}} for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 4.0 ppm, a multiple signal B around δ 6.3 ppm, and a single signal C at around δ 7.0 ppm. The ratio of integrated intensity of each signal, A:B:C, is about 3:1:1.

(4) Ceftizoxime Sodium responds to the Qualitative Tests <1,09> (1) for sodium salt.

Optical rotation <2,49> [α]\textsubscript{D}\textsuperscript{20} +125 – +145° (0.25 g calculated on the anhydrous bases, water, 25 mL, 100 mm).

pH <2,54> Dissolve 1.0 g of Ceftizoxime Sodium in 10 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ceftizoxime Sodium in 10 mL of water: the solution is clear, and colorless to light yellow.

(2) Heavy metals <1,07>—Proceed with 2.0 g of Ceftizoxime Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1,11>—Prepare the test solution with 2.0 g of Ceftizoxime Sodium according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.11 g of Ceftizoxime Sodium in 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2,01> according to the following conditions, and determine the areas of each peak by the automatic integration method: each peak area other than cefizoxime is not more than 0.5% of the peak area of cefizoxime, and the total peak areas other than cefizoxime is not more than 1.0% of that of cefizoxime.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 2.31 g of disodium hydrogen-phosphate dodecahydrate and 1.42 g of citric acid monohydrate in 1000 mL of water, adjust to pH 3.6 with diluted phosphoric acid (1 in 10) or dilute sodium hydroxide TS. To 200 mL of this solution add 10 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefizoxime is about 12 minutes.

Time span of measurement: About 5 times as long as the retention time of cefizoxime beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 1 mL of the sample solution, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL, and use this solution as the solution for test for required detection. Pipet 1 mL of the solution, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and confirm that the peak area of cefizoxime obtained from 5 μL of this solution is equivalent to 7 to 13% of that of cefizoxime obtained from 5 μL of the solution for test for required detection.

System performance: Dissolve about 10 mg of Ceftizoxime RS in 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the solution for system suitability test. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefizoxime are not less than 4000 steps and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefizoxime is not more than 2.0%.

Water <2,48> Not more than 8.5% (0.4 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ceftizoxime Sodium and Ceftizoxime RS, equivalent to about 0.1 g (potency), and dissolve each in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 20 mL. Pipet 2 mL each of these solutions, add exactly 10 mL of the internal standard solution,
then add 0.1 mol/L phosphate buffer solution, pH 7.0 to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL of each of these solutions as directed under Liquid Chromatography <2.06> according to the following conditions, and calculate the ratios, \( Q_z \) and \( Q_b \), of the peak area of ceftriaxone to that of the internal standard of each solution.

\[
\text{Amount} [\text{μg (potency)}] \text{ of ceftriaxone (C}_{18}\text{H}_{18}\text{N}_8\text{O}_7\text{S}_3] = M_z \times Q_z / Q_b \times 1000
\]

\( M_z \): Amount [mg (potency)] of Ceftriaxone RS

**Internal standard solution**—A solution of 3-hydroxybenzoic acid in 0.1 mol/L phosphate buffer solution, pH 7.0 (3 in 500).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2.31 g of disodium hydrogenphosphate dodecahydrate and 1.42 g of citric acid monohydrate in 1000 mL of water, and adjust to pH 3.6 with diluted phosphoric acid (1 in 10) or dilute sodium hydroxide TS. To 450 mL of this solution add 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 4 minutes.

**System suitability**—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, ceftriaxone and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.0 and the symmetry factor of each peak is not more than 1.0%.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftriaxone to that of the internal standard is not more than 1.0%.

**Containers and storage**—
Containers—Tight containers.
Storage—Light-resistant.

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**Ceftriaxone Sodium Hydrate**

Ceftriaxone Sodium Hydrate contains not less than 905 μg (potency) and not more than 935 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ceftriaxone Sodium Hydrate is expressed as mass (potency) of ceftriaxone (C18H18N8O7S3: 554.58).

**Description**
Ceftriaxone Sodium Hydrate occurs as a white to yellowish white crystalline powder. It is freely soluble in water and in dimethylsulfoxide, sparingly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

**Identification** (1) Determine the absorption spectrum of a solution of Ceftriaxone Sodium Hydrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ceftriaxone Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the \(^1\)H spectrum of a solution of Ceftriaxone Sodium Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>:
It exhibits single signals, A, B, C and D, at around δ 3.5 ppm, at around δ 3.8 ppm, at around δ 6.7 ppm and at around δ 7.2 ppm, respectively. The ratio of integrated intensity of each signal, A: B: C: D, is about 3:3:1:2. When the signal at around δ 3.5 ppm overlaps with the signal of water, perform the measurement in the probe kept at about 50°C.

(3) Ceftriaxone Sodium Hydrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

**Optical rotation** <2.49> [α]D: -153 – -170° (50 mg calculated on the anhydrous basis, water, 2.5 mL, 20 mm).

**pH** <2.54> Dissolve 0.6 g of Ceftriaxone Sodium Hydrate in 5 mL of water: the pH of the solution is between 6.0 and 8.0.

**Purity** (1) Clarity and color of solution—Dissolve 0.6 g of Ceftriaxone Sodium Hydrate in 5 mL of water: the solution is clear and light yellow.
(2) Heavy metals $< 0.07$—Proceed with 1.0 g of Ceftriaxone Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic $< 0.1$—Prepare the test solution with 1.0 g of Ceftriaxone Sodium Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances 1—Dissolve 20 mg of Ceftriaxone Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography $< 2.01$ according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of the impurity 1 having the relative retention time of about 0.5 and the impurity 2 having the relative retention time of about 1.3 to ceftriaxone from the sample solution are not larger than the peak area of ceftriaxone from the standard solution. In this case, these peak areas for the impurity 1 and the impurity 2 are used after multiplying by each relative response factors 0.9 and 1.2, respectively.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilsanized silica gel for liquid chromatography (10 $\mu$m particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as the solution A. Separately, dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as the solution B. Dissolve 4.00 g of tetra-n-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, add 55 mL of the solution A, 5 mL of the solution B and 490 mL of water.
Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 7 minutes.
Time span of measurement: About 2 times as long as the retention time of ceftriaxone.

System suitability—
Test for required detectability: Measure exactly 5 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 200 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 100 mL. Confirm that the peak area of ceftriaxone obtained from 10 $\mu$L of this solution is equivalent to 0.9 to 1.1% of that from 10 $\mu$L of the solution for system suitability test.

System performance: Dissolve 10 mg of Ceftriaxone Sodium Hydrate in a mixture of water and acetonitrile for liquid chromatography (11:9) to make 5 mL, add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000), and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make 200 mL. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order, with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ceftriaxone is not more than 1.0%.

(5) Related substances 2—Dissolve 10 mg of Ceftriaxone Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile for liquid chromatography and water (23:11) to make exactly 100 mL, and use this solution as standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography $< 2.01$ according to the following conditions, and determine each peak area by the automatic integration method: the each peak area of the impurities which appear after the peak of ceftriaxone from the sample solution is not larger than the peak area of ceftriaxone from the standard solution, and the total peak area of these impurities is not larger than 2.5 times of the peak area from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilsanized silica gel for liquid chromatography (10 $\mu$m particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as the solution A. Separately, dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as the solution B. Dissolve 4.00 g of tetra-n-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, add 55 mL of the solution A, 5 mL of the solution B and 490 mL of water.
Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 3 minutes.
Time span of measurement: About 10 times as long as the retention time of ceftriaxone.

System suitability—
Test for required detectability: Measure exactly 5 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 100 mL. Confirm that the peak area of ceftriaxone obtained from 10 $\mu$L of this solution is equivalent to 0.9 to 1.1% of that from 10 $\mu$L of the solution for system suitability test.

System performance: Dissolve 10 mg of Ceftriaxone Sodium Hydrate in a mixture of acetonitrile for liquid chromatography and water (23:11) to make exactly 5 mL, add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000), and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make 5 mL, add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000), and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make 200 mL. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order, with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ceftriaxone is not more than 1.0%.
and add a mixture of acetonitrile for liquid chromatography and water (23:11) to make 200 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ceftriaxone is not more than 1.0%.

Water <2.48> Not less than 8.0% and not more than 11.0% (0.15 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ceftriaxone Sodium Hydrate and Ceftriaxone Sodium RS, equivalent to about 0.1 g (potency), dissolve each in a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 50 mL. Pipet 5 mL of each solution, add exactly 5 mL of the internal standard solution and a mixture of water and acetonitrile for liquid chromatography (11:9) to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL of each of these solutions as directed under Liquid Chromatography (11:9) (9 in 5000).

Internal standard solution—A solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as solution A. Dissolve 20.256 g of citric acid monohydrate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as solution B. Dissolve 4.00 g of tetra-n-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, and add 490 mL of water, 55 mL of solution A, and 5 mL of solution B.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ceftriaxone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftriaxone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Cefuroxime Axetil

セフロキシム アキセチル


Cefuroxime Axetil contains not less than 800 μg (potency) and not more than 850 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of acetone. The potency of Cefuroxime Axetil is expressed as mass (potency) of cefuroxime (C_{16}\text{H}_{16}\text{N}_{4}\text{O}_{8}\text{S}; 424.39).

Description Cefuroxime Axetil occurs as white to yellowish white, non-crystalline powder.

It is freely soluble in dimethylsulfoxide, soluble in methanol, sparingly soluble in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Cefuroxime Axetil in methanol (3 in 200, 000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefuroxime Axetil RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefuroxime Axetil as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefuroxime Axetil RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the 1H spectrum of a solution of Cefuroxime Axetil in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal or a pair of double signals A at around δ 2.25 ppm, a pair of single signals B at around δ 2.1 ppm, and a single signal C at around δ 3.9 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 1:1:1.

Optical rotation <2.49> [α]_{D}^{20}: +41 to +47° (0.5 g, methanol, 50 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of
Cefuroxime Axetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Put 1.0 g of Cefuroxime Axetil in a crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, then make gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming on a water bath, and perform the test using this solution as the test solution (not more than 2 ppm).

(3) Related substance—Dissolve 25 mg of Cefuroxime Axetil in 4 mL of methanol, add a solution of ammonium dihydrogen phosphate (23 in 1000) to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 40 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cefuroxime axetil obtained from the sample solution is not larger than 1.5 times the sum area of two peaks of cefuroxime axetil obtained from the standard solution, and the sum area of the peaks other than cefuroxime axetil is not larger than 4 times the sum area of two peaks of cefuroxime axetil from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the assay.

Time span of measurement: About 3 times as long as the retention time of the peak having the larger retention time of the two peaks of cefuroxime axetil beginning after the solvent peak.

**System suitability**—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add 4 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 10 mL. Confirm that the sum area of the two peaks of cefuroxime axetil obtained from 2 μL of this solution is equivalent to 7 to 13% of that obtained from 2 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the assay.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the sum area of the two peaks of cefuroxime axetil is not more than 2.0%.

(4) Acetone—Weigh accurately about 1 g of Cefuroxime Axetil, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of acetone, and add dimethylsulfoxide to make exactly 100 mL. Pipet 0.2 mL of this solution, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, determine each peak area by the automatic integration method, and calculate the ratios, Qt and Qs, of the peak area of acetone to that of the internal standard: the amount of acetone is not more than 1.3%.

Amount (%) of acetone = \( M_t/M_s \times Q_t/Q_s \times 1/5 \)

**Internal standard solution**—A solution of 1-propanol in dimethylsulfoxide (1 in 200).

**Operating conditions**—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography coated with a mixture of polyethylene glycol 600 for gas chromatography and polyethylene glycol 1500 for gas chromatography (1:1) in the ratio of 20% (125 – 150 μm in particle diameter).

Column temperature: A constant temperature of about 90°C.

Temperature of injection port: A constant temperature of about 115°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 4 minutes.

**System suitability**—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, acetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acetone to that of the internal standard is not more than 5.0%.

**Water** <2.48> Not more than 2.0% (0.4 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.2% (0.5 g).

**Isomer ratio** Perform the test with 10 μL of the sample solution obtained in the assay as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the area, \( A_s \), of the peak having the smaller retention time and the area, \( A_b \), of the peak having the bigger retention time of the two peaks of cefuroxime axetil: \( A_s/(A_s + A_b) \) is between 0.48 and 0.55.

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the assay.

**System suitability**—

System performance, and system repeatability: Proceed as directed in the system suitability in the assay.

**Assay** Weigh accurately an amount of Cefuroxime Axetil and Cefuroxime Axetil RS, equivalent to about 50 mg (potency), and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, 5 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make 50 mL, and use these solutions as the sample solution
and standard solution. Perform the test with 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the sum area of the two peaks of cefuroxime axetil to the peak area of the internal standard.

Amount [μg (potency)] of cefuroxime (C₁₆H₁₆N₄O₈S)  
\[ M = \frac{A}{Q₁/ Q₂} \times 1000 \]

M₂: Amount [mg (potency)] of Cefuroxime Axetil RS

**Internal standard solution**—A solution of acetanilide in methanol (27 in 5000).

**Operating conditions**—
- Detector: An ultraviolet absorption photometer (wavelength: 278 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with trimethylsilylized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of a solution of ammonium dihydrogen phosphate (23 in 1000) and methanol (5:3).
- Flow rate: Adjust the flow rate so that the retention time of the peak having the smaller retention time of the two peaks of cefuroxime axetil is about 8 minutes.

**System suitability**—
- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefuroxime axetil are eluted in this order with the resolution between the two peaks of cefuroxime axetil being not less than 1.5.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the sum area of the two peaks of cefuroxime axetil to the peak area of the internal standard is not more than 1.0%.

**Containers and storage**—Tight containers.
- Storage—Light-resistant.

**Cellacefate**

**Cellulose Acetate Phthalate**

セラセフェート

[9004-38-0]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (• •).

Cellacefate is a reaction product of phthalic anhydride and partially acetylated cellulose.

Cellacefate, calculated on the anhydrous and free acid-free basis, contains not less than 21.5% and not more than 26.0% of acetyl group (-COCH₃: 43.04), and not less than 30.0% and not more than 36.0% of carboxybenzoyl group (-COC₆H₄COOH: 149.12).

**Description**—Cellacefate occurs as a white powder or grain. It is freely soluble in acetone, and practically insoluble in water, in methanol and in ethanol (99.5).

**Identification**—Determine the infrared absorption spectrum of Cellacefate directly in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum of Cellacefate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Viscosity**—Weigh accurately a quantity of Cellacefate, equivalent to 15 g calculated on the anhydrous basis, dissolve in 85 g of a mixture of acetone and water (249: 1 in mass), and perform the test with this solution at 25 ± 0.2°C as directed in Method 1 to obtain the kinematic viscosity ν. Separately, determine the density, ρ, of Cellacefate as directed under Determination of Specific Gravity and Density <2.50>, and calculate the viscosity, η, as \[ η = ρ ν \] not less than 45 mPa·s and not more than 90 mPa·s.

**Purity**—
- (1) Heavy metals <1.07>—Proceed with 2.0 g of Cellacefate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
- (2) Free acids—Weigh accurately about 3 g of Cellacefate, put in a glass-stopped conical flask, add 100 mL of diluted methanol (1 in 2), stopper tightly, and filter after shaking for 2 hours. Wash both the flask and residue with two 10-mL portions each of diluted methanol (1 in 2), combine the washes to the filtrate, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform the blank determination with 120 mL of diluted methanol (1 in 2), and make any necessary correction.

\[ \text{Amount (％) of free acids} = 0.8306A/M \]

A: amount (mL) of 0.1 mol/L sodium hydroxide VS consumed  
M: amount (g) of the test sample, calculated on the anhydrous basis

The amount of free acids is not more than 3.0%, calculated as phthalic acid (C₈H₆O₄: 166.13).

**Water**—Not more than 5.0% (1 g, volumetric titration, direct titration, using a mixture of ethanol (99.5) and dichloromethane (3: 2) instead of methanol for Karl Fischer method).

**Residue on ignition**—Not more than 0.1% (1 g).

**Assay**—Weigh accurately about 1 g of Cellacefate, dissolve in 50 mL of a mixture of ethanol (95) and acetone (3: 2), and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

\[ \text{Content (％) of carboxybenzoyl group (C₆H₄O₂)} = \frac{1.491 \times A - 1.795 \times B}{100 - B} \times 100 \]

A: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed  
B: Amount (％) of free acids obtained in the Purity (2)
Free acids

\( M \): Amount (g) of the test sample, calculated on the anhydrous basis

(2) Acetyl group—Weigh accurately about 0.1 g of Cellulose, put in a glass-stoppered conical flask, add exactly 25 mL of 0.1 mol/L sodium hydroxide VS, and boil for 30 minutes under a reflux condenser. After cooling, add 5 drops of phenolphthalein TS, and titrate 2.50° with 0.1 mol/L hydrochloric acid VS. Perform a blank determination.

Content (%) of free acids and bound acetyl group (C\(_{2}H_{3}O\))
\[
A: \text{Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed, corrected by the blank determination}
\]
\[
M: \text{Amount (g) of the test sample, calculated on the anhydrous basis}
\]
Content (%) of acetyl group (C\(_{2}H_{3}O\))
\[
= 100 \times \left( \frac{P - 0.5182B}{100 - B} \right) - 0.5772C
\]

B: Amount (%) of free acids obtained in the Purity (2) Free acids
C: Content (%) of carboxybenzoyl group
P: Content (%) of free acids and bound acetyl group (C\(_{2}H_{3}O\))

Containers and storage—Containers—Tight containers.

Microcrystalline Cellulose

結晶セルロース

[9004-34-6, cellulose]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (• •).

Microcrystalline Cellulose is purified, partially depolymerized α-cellulose, obtained as a pulp from fibrous plant material, with mineral acids.

• The label indicates the mean degree of polymerization, loss on drying, and bulk density values with the range.

• Description Microcrystalline Cellulose occurs as a white crystalline powder having fluidity.

It is practically insoluble in water, in ethanol (95%) and in diethyl ether.

It swells with sodium hydroxide TS on heating.

Identification (1) Dissolve 20 g of zinc chloride and 6.5 g of potassium iodide in 10.5 mL of water, add 0.5 g of iodine, and shake for 15 minutes. Place about 10 mg of Microcrystalline Cellulose on a watch glass, and disperse in 2 mL of this solution: the substance develops a blue-violet color.

• (2) Sieve 20 g of Microcrystalline Cellulose for 5 minutes on an air-jet sieve equipped with a screen (No.391, 200 mm in inside diameter) having 38-μm openings. If more than 5% is retained on the screen, mix 30 g of Microcrystalline Cellulose with 270 mL of water; otherwise, mix 45 g with 255 mL of water. Perform the mixing for 5 minutes in a high-speed (18,000 revolutions per minute or more) power blender. Transfer 100 mL of the dispersion to a 100-mL graduated cylinder, and allow to stand for 3 hours: a white, opaque, bubble-free dispersion, which does not form a supernatant liquid at the surface, is obtained.

(3) Transfer 1.3 g of Microcrystalline Cellulose, accurately weighed, to a 125-mL conical flask, and add exactly 25 mL each of water and 1 mol/L cupriethylenediamine TS. Immediately purge the solution with nitrogen, insert the stopper, and shake on a suitable mechanical shaker to dissolve. Perform the test with a suitable amount of this solution, taken exactly, according to Method 1 under Viscosity Determination 2.53 by using a capillary viscometer having the viscosity constant (K) of approximately 0.03, at 25 ± 0.1°C, and determine the kinematic viscosity, \( v \). Separately, perform the test with a mixture of exactly 25 mL each of water and 1 mol/L cupriethylenediamine TS in the same manner as above, using a capillary viscometer having K of approximately 0.01, and determine the kinematic viscosity, \( v_{0} \).

Calculate the relative viscosity, \( \eta_{rel} \), of Microcrystalline Cellulose by the formula:

\[
\eta_{rel} = \frac{v}{v_{0}}
\]

Obtain the product, [\( \eta \)] of intrinsic viscosity [\( \eta \)(mL/g)] and concentration C (g/100 mL) from the value \( \eta_{rel} \) of the Table. When calculate the degree of polymerization, \( P \), by the following formula, \( P \) is not more than 350 • and within the labeled range.

\[
P = \left( \frac{95}{\eta} \right) C / M_{r}
\]

\( M_{r} \): Amount (g) of the sample, calculated on the dried basis

pH \(< 2.54\): Shake 5.0 g of Microcrystalline Cellulose with 40 mL of water for 20 minutes, and centrifuge: the pH of the supernatant liquid is between 5.0 and 7.5.

Purity • (1) Heavy metals \(< 0.07\)—Proceed with 2.0 g of Microcrystalline Cellulose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm). •

(2) Water-soluble substances—Shake 5.0 g of Microcrystalline Cellulose with 80 mL of water for 10 minutes, filter with the aid of vacuum through a filter paper for quantitative analysis (5C) into a vacuum flask. Evaporate the clear filtrate in a tared evaporating dish to dryness without charring, dry at 105°C for 1 hour, cool in a desiccator, and weigh: the difference between the mass of the residue and the...
600

Microcrystalline Cellulose / Official Monographs

mass obtained from a blank determination does not exceed
12.5 mg.
(3) Diethyl ether-soluble substances—Place 10.0 g of
Microcrystalline Cellulose in a column having an internal diameter of about 20 mm, and pass 50 mL of peroxide-free
diethyl ether through the column. Evaporate the eluate to
dryness in a previously dried and tared evaporation dish.

JP XVI

Dry the residue at 1059
C for 30 minutes, allow to cool in a
desiccator, and weigh: the difference between the mass of
the residue and the mass obtained from a blank determination does not exceed 5.0 mg.
Conductivity <2.51> Perform the test as directed in the
Conductivity Measurement with the supernatant liquid ob-

Table for Conversion of Relative Viscosity ( hrel) into the Product of Limiting Viscosity and Concentration ([h]C)
[ h]C
hrel

0.00

0.01

0.02

0.03

0.04

0.05

0.06

0.07

0.08

0.09

1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9

0.098
0.189
0.276
0.358
0.437
0.515
0.587
0.656
0.723

0.106
0.198
0.285
0.367
0.445
0.522
0.595
0.663
0.730

0.115
0.207
0.293
0.375
0.453
0.529
0.602
0.670
0.736

0.125
0.216
0.302
0.383
0.460
0.536
0.608
0.677
0.743

0.134
0.225
0.310
0.391
0.468
0.544
0.615
0.683
0.749

0.143
0.233
0.318
0.399
0.476
0.551
0.622
0.690
0.756

0.152
0.242
0.326
0.407
0.484
0.558
0.629
0.697
0.762

0.161
0.250
0.334
0.414
0.491
0.566
0.636
0.704
0.769

0.170
0.259
0.342
0.422
0.499
0.573
0.642
0.710
0.775

0.180
0.268
0.350
0.430
0.507
0.580
0.649
0.717
0.782

2.0
2.1
2.2
2.3
2.4
2.5
2.6
2.7
2.8
2.9

0.788
0.852
0.912
0.971
1.028
1.083
1.137
1.190
1.240
1.290

0.795
0.858
0.918
0.976
1.033
1.089
1.142
1.195
1.245
1.295

0.802
0.864
0.924
0.983
1.039
1.094
1.147
1.200
1.250
1.300

0.809
0.870
0.929
0.988
1.044
1.100
1.153
1.205
1.255
1.305

0.815
0.876
0.935
0.994
1.050
1.105
1.158
1.210
1.260
1.310

0.821
0.882
0.941
1.000
1.056
1.111
1.163
1.215
1.265
1.314

0.827
0.888
0.948
1.006
1.061
1.116
1.169
1.220
1.270
1.319

0.833
0.894
0.953
1.011
1.067
1.121
1.174
1.225
1.275
1.324

0.840
0.900
0.959
1.017
1.072
1.126
1.179
1.230
1.280
1.329

0.846
0.906
0.965
1.022
1.078
1.131
1.184
1.235
1.285
1.333

3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9

1.338
1.386
1.432
1.477
1.521
1.562
1.604
1.646
1.687
1.727

1.343
1.390
1.436
1.482
1.525
1.566
1.608
1.650
1.691
1.731

1.348
1.395
1.441
1.486
1.529
1.570
1.612
1.654
1.695
1.735

1.352
1.400
1.446
1.491
1.533
1.575
1.617
1.658
1.700
1.739

1.357
1.405
1.450
1.496
1.537
1.579
1.621
1.662
1.704
1.742

1.362
1.409
1.455
1.500
1.542
1.583
1.625
1.666
1.708
1.746

1.367
1.414
1.459
1.504
1.546
1.587
1.629
1.671
1.712
1.750

1.371
1.418
1.464
1.508
1.550
1.591
1.633
1.675
1.715
1.754

1.376
1.423
1.468
1.513
1.554
1.595
1.637
1.679
1.719
1.758

1.381
1.427
1.473
1.517
1.558
1.600
1.642
1.683
1.723
1.762

4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9

1.765
1.804
1.841
1.878
1.914
1.950
1.986
2.020
2.053
2.087

1.769
1.808
1.845
1.882
1.918
1.954
1.989
2.023
2.057
2.090

1.773
1.811
1.848
1.885
1.921
1.957
1.993
2.027
2.060
2.093

1.777
1.815
1.852
1.889
1.925
1.961
1.996
2.030
2.063
2.097

1.781
1.819
1.856
1.893
1.929
1.964
2.000
2.033
2.067
2.100

1.785
1.822
1.859
1.896
1.932
1.968
2.003
2.037
2.070
2.103

1.789
1.826
1.863
1.900
1.936
1.971
2.007
2.040
2.073
2.107

1.792
1.830
1.867
1.904
1.939
1.975
2.010
2.043
2.077
2.110

1.796
1.833
1.870
1.907
1.943
1.979
2.013
2.047
2.080
2.113

1.800
1.837
1.874
1.911
1.946
1.982
2.017
2.050
2.083
2.116

5.0
5.1
5.2
5.3
5.4
5.5
5.6
5.7
5.8
5.9

2.119
2.151
2.183
2.212
2.243
2.273
2.303
2.332
2.361
2.390

2.122
2.154
2.186
2.215
2.246
2.276
2.306
2.335
2.364
2.393

2.125
2.158
2.190
2.218
2.249
2.279
2.309
2.338
2.367
2.396

2.129
2.160
2.192
2.221
2.252
2.282
2.312
2.341
2.370
2.400

2.132
2.164
2.195
2.224
2.255
2.285
2.315
2.344
2.373
2.403

2.135
2.167
2.197
2.227
2.258
2.288
2.318
2.347
2.376
2.405

2.139
2.170
2.200
2.230
2.261
2.291
2.320
2.350
2.379
2.408

2.142
2.173
2.203
2.233
2.264
2.294
2.324
2.353
2.382
2.411

2.145
2.176
2.206
2.236
2.267
2.297
2.326
2.355
2.384
2.414

2.148
2.180
2.209
2.240
2.270
2.300
2.329
2.358
2.387
2.417

6.0

2.419

2.422

2.425

2.428

2.431

2.433

2.436

2.439

2.442

2.444


tained in the pH as the sample solution, and determine the conductivity at 25 ± 0.1°C. Determine in the same way the conductivity of water used for the preparation of the sample solution: the difference between these conductivities is not more than 75 μS cm⁻¹.

**Loss on drying** Not more than 7.0% and within a range as specified on the label (1 g, 105°C, 3 hours).

**Residue on ignition** Not more than 0.1% (2 g).

**Bulk density** (i) Apparatus—Use a volumeter shown in the figure. Put a No.8.6 sieve (2000 μm) on the top of the volumeter. A funnel is mounted over a baffle box, having four glass baffle plates inside which the sample powder slides as it passes. At the bottom of the baffle box is a funnel that collects the powder, and allows it to pour into a sample.
receiving cup mounted directly below it.

(ii) Procedure—Weigh accurately the mass of a brass or stainless steel cup, which has a capacity of 25.0 ± 0.05 mL and an inside diameter of 30.0 ± 2.0 mm, and put the cup directly below the funnel of the volumeter. Slowly pour Microcrystalline Cellulose 5.1 cm height from the upper part of the powder funnel through the sieve, at a rate suitable to prevent clogging, until the cup overflows. If the clogging occurs, take out the sieve. Level the excess powder with the aid of a slide glass, weigh the filled cup, and weigh accurately the content of the cup, and then calculate the bulk density by the following expression: the bulk density is within the labeled specification.

\[
\text{Bulk density (g/cm}^3\text{)} = \frac{A}{25}
\]

\(A: \text{Measured mass (g) of the content of the cup}\)

*Microbial limit* \(<4.0\)—The acceptance criteria of TAMC and TYMC are \(10^3\) CFU/g and \(10^2\) CFU/g, respectively. *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.

*Containers and storage* Containers—Tight containers.

**Powdered Cellulose**

粉末セルロース

[9004-34-6, Cellulose]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (*) and ( ).

Powdered Cellulose is a purified, mechanically disintegrated alpha cellulose obtained as a pulp, *after partial hydrolysis as occasion demands*, from fibrous plant materials.

The label indicates the mean degree of polymerization value with a range.

*Description* Powdered Cellulose occurs as a white powder.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 20 g of zinc chloride and 6.5 g of potassium iodide in 10.5 mL of water, add 0.5 g of iodine, and shake for 15 minutes. Place about 10 mg of Powdered Cellulose on a watch glass, and disperse in 2 mL of this solution: the substance develops a blue-violet color.

(2) Mix 30 g of Powdered Cellulose with 270 mL of water in a high-speed (18,000 revolutions per minute or more) blender for 5 minutes, transfer 100 mL of the dispersion to a 100-mL graduated cylinder, and allow to stand for 1 hour: a supernatant liquid appears above the layer of the cellulose.

(3) Transfer 0.25 g of Powdered Cellulose, accurately weighed, to a 125-mL conical flask, add exactly 25 mL of each of water and 1 mol/L cupriethylenediamine TS, and proceed as directed in the Identification (3) under Microcrystalline Cellulose. The mean degree of polymerization, \( \bar{P} \), is not less than 440 and is within the labeled specification.

**Celmoleukin (Genetical Recombination)**

セルモロイキン（遺伝子組換え）

\[
\text{Celmoleukin} \leftrightarrow \text{Celmoleukin (Genetical Recombination)}
\]

\[
\text{C}_{693}\text{H}_{1118}\text{N}_{178}\text{O}_{203}\text{S}_{7}: 15415.82
\]

The desired product of Celmoleukin (Genetical Recombination) is a protein consisting of 133 amino acid residues manufactured by *E. coli* through expression of human interleukin-2 cDNA. It is a solution having a T-lymphocyte activating effect. It contains not less than 0.5 and not more than 1.5
mg of protein per mL, and 1 mg of this protein contains potency not less than 8.0 × 10^6 units.

**Description** Celmoleukin (Genetical Recombination) occurs as a colorless, clear liquid.

**Identification (1)** To 1 mL of Celmoleukin (Genetical Recombination) add 0.05 mL of diluted copper (II) sulfate TS (1 in 10), shake, add 0.9 g of potassium hydroxide, and shake. When 0.3 mL of ethanol (99:5) is added to this solution and shaken, the ethanol layer exhibits a violet color.

(2) Based on the results of the Assay (1), place an amount of Celmoleukin (Genetical Recombination) equivalent to about 50 μg of protein in two hydrolysis tubes, and evaporate to dryness under vacuum. To one of the tubes add 100 μL of a mixture of diluted hydrochloric acid (59 → 125), mercapto acetic acid and phenol (100:10:1), and shake. Place this hydrolysis tube in a vial and humidify the inside of the vial with 200 μL of the mixture of diluted hydrochloric acid (59 → 125), mercapto acetic acid and phenol (100:10:1). Replace the vial interior with inert gas or reduce the pressure, and heat for 24 hours at about 115°C. After drying under vacuum, dissolve in 0.5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution (1). To the other hydrolysis tube, add 100 μL of ice cold performic acid, oxidize for 1.5 hours on ice, add 50 μL of hydrobromic acid, and dry under vacuum. Add 200 μL of water, repeat the dry under vacuum procedure two more times, place the hydrolysis tube in a vial, and humidify the inside of the vial with 200 μL of diluted hydrochloric acid (59 → 125). Replace the vial interior with inert gas or reduce the pressure, and heat for 24 hours at about 115°C. After drying under vacuum, dissolve in 0.5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution (2). Separately, accurately weigh 60 mg of L-aspartic acid, 100 mg of L-glutamic acid, 17 mg of L-alanine, 23 mg of L-methionine, 21 mg of L-tyrosine, 24 mg of L-histidine hydrochloride monohydrate, 58 mg of L-threonine, 22 mg of L-proline, 14 mg of L-cystine, 45 mg of L-isoleucine, 37 mg of L-phenylalanine, 32 mg of L-arginine hydrochloride, 32 mg of L-serine, 6 mg of glycine, 18 mg of L-valine, 109 mg of L-leucine, 76 mg of L-lysine hydrochloride, and 8 mg of L-tryptophan, add 0.1 mol/L hydrochloric acid TS to dissolve to make 500 mL, add 40 μL of this solution to two hydrolysis tubes, and evaporate to dryness under vacuum to make the standard solutions calculate the molar number of the amino acids contained in 1 mL of the sample solutions. Further-

**Operating conditions—**

**System suitability**—

System performance: Add 2 μL of 2-mercaptoethanol to 100 μL of celmoleukin for liquid chromatography, leave for 2 hours at 37°C, and then run this solution under the above conditions. Under these conditions, celmoleukin and its reduced form are eluted in this order with the resolution between these peaks being not less than 1.5.

(4) Add 100 μL of protein digestive enzyme TS to 100 μL of Celmoleukin (Genetical Recombination), shake, leave standing for 18 to 24 hours at 37°C, and then add 2 μL of 2-mercaptoethanol. Leave for a further 30 minutes at 37°C, and add 5 μL of trifluoroacetic acid solution (1 in 10). This is the sample solution. Separately, process celmoleukin for liquid chromatography using the same method. This is the standard solution. Perform the test using 50 μL of each both the sample and standard solutions as directed under Liquid Chromatography to compare the chromatograms obtained from the sample and standard solutions, the retention times for the sample and standard solutions are identical, and the peak heights are similar.

**Flow:** Adjust so that the retention time of celmoleukin is about 70 minutes.
moleukin to hole B. Add 50 μL of culture medium for celmoleukin to another hole (hole C). After shaking the microtest plate, warm for 30 minutes to 2 hours at 37°C in air containing 5% carbon dioxide. Next, add to each hole 50 μL of culture medium for celmoleukin containing the interleukin-2 dependent mouse natural killer cells NKC3 and culture for 16 to 24 hours at 37°C. Add 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide TS, culture for 4 to 6 hours at 37°C, and add sodium lauryl sulfate TS and leave for 24 to 48 hours. When the absorbance at 590 nm of the solution in each hole is measured as directed under Ultraviolet-visible Spectrophotometry (<2.24>, the difference in absorbance between the solutions from holes A and C is not more than 3% of the difference in absorbance between the solutions from holes B and C.

**pH 2.54–4.5–5.5**

**Purity (1)** Host cell-derived protein—Prepare a sample solution by the accurate two times stepwise dilution of Celmoleukin (Genetical Recombination) with phosphate buffer-sodium chloride TS (hereinafter referred to as PBS) containing bovine serum. Prepare a series of 5 standard solutions by accurately diluting E. coli protein (hereinafter referred to as ECP) over a range of 0.25 to 6 ng per mL with PBS containing bovine serum. Pipet 100 μL of goat anti-ECP antibody TS into each hole of a flat-bottomed microtest plate, leave for 16 to 24 hours at 4°C, and then remove the liquid. Wash three times with PBS, add 200 μL of PBS containing bovine serum albumin, leave for at least 3 hours at room temperature, and then wash three more times with PBS. Pipet 100 μL of the sample solution and each standard solution into each hole, leave for 16 to 24 hours at 4°C, and then wash 5 times with PBS. Add 100 μL of peroxidase-labeled rabbit anti-ECP antibody Fab’ TS, leave for at least 4 hours at room temperature, and wash 5 times with PBS. Next, add 100 μL of substrate buffer for celmoleukin, allow to react for 5 to 25 minutes at room temperature in a dark place, and then add 100 μL of diluted sulfuric acid (3 in 25). Measure the absorbances of these flat-bottomed microtest plates by Ultraviolet-visible Spectrophotometry (<2.24> at a wavelength of 492 nm. Separately, using 100 μL of PBS containing bovine serum, perform a blank test using the same method and correct. Determine the absorbance of each standard solution, prepare a calibration curve, calculate the amount of ECP per mL of the sample solution, and multiply by the sample solution dilution factor. When determining the concentration of ECP per mg of protein in the sample solution, there is not more than 0.02% (0.2 μg/mg of protein).

(2) Polymers—Dilute (at least 4 steps) the sample solution prepared in the Identification (3) with buffer solution for celmoleukin so that the protein content is within the range of about 2 to 32 μg per mL to prepare a series of standard solutions. Pipet 20 μL of the sample solution or each of the standard solutions into the stacking gel well, and perform vertical uncoupled buffer SDS-polyacrylamide gel electrophoresis followed by immersion in Coomassie staining TS. Each electrophoretic band is stained blue. Next, determine the peak area of the electrophoretic bands obtained from each standard solution using a densitometer and calculate the protein content using the calibration curve mentioned above. When determining the polymer proteins derived from celmoleukin, other than celmoleukin monomer, the amount is not more than 2% in relation to the total protein.

(3) Related substances—Perform the test with 10 μL each of Celmoleukin (Genetical Recombination) and 0.01 mol/L acetic acid buffer solution, pH 5.0, as directed under Liquid Chromatography (<2.07> under the following conditions, and measure the area of each peak by an automatic integration method. When the amount of related substances other than celmoleukin is calculated by the area percent method, the total amount is not more than 5%.

**Operating conditions—**


Column: Stainless steel tube with an inside diameter of 4 mm and a length of 30 cm packed with octadecylsilanized silica gel for liquid chromatography (particle size: 5 μm).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A solution of trifluoroacetic acid in a mixture of acetic acid and water (3:2) (1 in 1000).

Mobile phase B: A solution of trifluoroacetic acid in a mixture of acetic acid and water (13:7) (1 in 1000).

Mobile phase flow: The concentration gradient is controlled by changing the ratio of mobile phases A and B as shown in the table below.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 60</td>
<td>70 → 10</td>
<td>30 → 90</td>
</tr>
</tbody>
</table>

Flow: Adjust so that the retention time of celmoleukin is about 50 minutes.

Time span of measurement: About 1.3 times as long as the retention time of celmoleukin beginning after the solvent peak.

**System suitability—**

Test for required detectability: Measure exactly 0.5 mL of Celmoleukin (Genetical Recombination), and add 0.01 mol/L acetic acid buffer solution, pH 5.0, to make exactly 50 mL. Confirm that the celmoleukin peak area obtained from 10 μL of this solution is 0.9 to 1.1% of the peak area obtained from 10 μL of Celmoleukin (Genetical Recombination).

System performance: Add 2 μL of 2-mercaptoethanol to 100 μL of Celmoleukin (Genetical Recombination), leave for 2 hours at 37°C, and then run this solution under the above conditions. Celmoleukin and its reduced form are eluted in this order with the resolution between these peaks being not less than 3.0.

**Ammonium acetate** Measure exactly 0.1 mL of Celmoleukin (Genetical Recombination), and add water to make exactly 10 mL. This is the sample solution. Separately, accurately weigh about 0.1 g of ammonium chloride, and add water to make exactly 100 mL. Measure exactly 5 mL of this solution, and add water to make exactly 100 mL. This is the standard stock solution. Measure exactly 3 mL of the standard stock solution, and add water to make exactly 50 mL. This is the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography (<2.07> according to the following conditions. When determining the area of the
ammonium ion peak $A_1$ and $A_S$, Celmoleukin (Genetical Recombination) contains from 0.28 to 0.49 mg of ammonium acetate per mL.

Amount (mg) of ammonium acetate (CH₃COONH₄) per mL = $A_1/A_S \times M_S \times 0.003 \times 1.441$

$M_S$: Amount (mg) of ammonium chloride

0.003: Dilution correction coefficient

1.4410: Molecular weight conversion coefficient for converting ammonium chloride to ammonium acetate

Operating conditions—

Detector: Electronic conductivity detector.

Column: Resin column with an inside diameter of 5 mm and a length of 25 cm packed with weakly acidic ion exchange resin for liquid chromatography (particle size: 5.5 μm).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Diluted 0.1 mol/L methanesulfonic acid TS (3 in 10).

Flow: Adjust the flow so that the retention time of ammonium is about 8 minutes.

System suitability—

System performance: Measure exactly 1 mL of Standard Sodium Stock Solution and 0.2 mL of Standard Potassium Stock Solution, and then add water to make exactly 100 mL. Measure exactly 5 mL of this solution and 3 mL of Standard Ammonium Solution, and then add water to make exactly 50 mL. When 25 μL of this solution is run under the above conditions, sodium, ammonium and potassium are eluted in this order with the resolution between the peaks of sodium and ammonium being not less than 3.0.

System repeatability: When the test is repeated 5 times with 25 μL of the standard solution under the above conditions, the relative standard deviation of the ammonium peak area is not more than 10%.

Bacterial endotoxins <4.06> Less than 100 EU/mL.

Sterility <4.06> Perform the test according to the Direct method: it meets the requirement. In the test, add 0.5 mL of Celmoleukin (Genetical Recombination) to 8 test tubes and 1.0 mL of Celmoleukin (Genetical Recombination) to 8 test tubes containing 15 mL of thiglycyl acid I for sterility test, as well as 1.0 mL of Celmoleukin (Genetical Recombination) to 8 test tubes containing 15 mL of soybean-casein digest medium.

Assay (1) Total protein content—Measure accurately 1 mL of Celmoleukin (Genetical Recombination) and add water to make exactly 10 mL. This is the sample solution. Separately, weigh accurately about 50 mg of bovine serum albumin for assay in water to prepare standard dilution solutions of 50, 100, and 150 μg/mL. Measure exactly 1 mL of the sample solution and each standard dilution solution, add exactly 2.5 mL of alkaline copper TS for protein content determination, shake, and leave for 15 minutes. Next, add exactly 2.5 mL of water and 0.5 mL of dilute Folin’s TS, and leave for 30 minutes at 37°C. Measure the absorbances of these solutions at 750 nm as directed under Ultraviolet-visible Spectrophotometry <2,24>, using 1 mL of water processed in the same way as control. Using the calibration curve prepared from the absorbance of the standard dilution solution, calculate the protein content of Celmoleukin (Genetical Recombination).

(2) Specific activity—Measure exactly 0.1 mL of Celmoleukin (Genetical Recombination) and add exactly 0.9 mL of culture medium for clemoleukin to make the sample solution. Separately, take one Interleukin-2 RS and add exactly 1 mL of water to dissolve. This is the standard solution. Accurately serially dilute the sample and standard solutions in two-fold steps with culture medium for clemoleukin, and add equal volumes of interleukin-2 dependent mouse natural killer NKC3 cells to the serially diluted solutions. The control solution is a mixture of equal volumes of interleukin-2 dependent mouse natural killer NKC3 and culture medium for clemoleukin. Incubate these solutions for 16 to 24 hours at 37°C. Following this, add a volume of 3(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide TS that is 1/5 that of the volume of culture medium for clemoleukin, incubate for 4 to 6 hours at 37°C, add a volume of sodium lauryl sulfate TS equivalent to the volume of the culture medium for clemoleukin, and leave for 24 to 48 hours. After eluting the blue-colored pigment generated, perform the test on these solutions as directed under Ultraviolet-visible Spectrophotometry <2,24>, and measure the absorbance at 590 nm. Taking the absorbance obtained when 1000 to 2000 units of clemoleukin per mL are added as 100% and the absorbance of the control solution as 0%, determine the dilution factor (A) of the Interleukin-2 RS that shows an absorbance of 50% and dilution factor of Celmoleukin (Genetical Recombination) (B). Multiply the B/A value by the unit number of the Interleukin-2 RS to calculate the biological activity of 1 mL of Celmoleukin (Genetical Recombination). Calculate the ratio of biological activity in relation to protein content determined in the total protein content test.

Containers and storage Containers—Sterilized, tight containers.

Storage—Store at −20°C or lower.

Cetanol セタノール

Cetanol is a mixture of solid alcohols, and consists chiefly of C₁₆H₃₁O: 242.44.

Description Cetanol occurs as unctuous, white flakes, granules, or masses. It has a faint, characteristic odor. It is tasteless.

It is very soluble in pyridine, freely soluble in ethanol (95), in ethanol (99.5) and in diethyl ether, very slightly soluble in acetic anhydride, and practically insoluble in water.

Melting point <1.13> 47–53°C Prepare the sample according to Method 2, then attach tightly a capillary tube to the bottom of the thermometer by means of a rubber band or by any suitable means, and make the bottom of the capillary tube equal in position to the lower end of the thermometer. Insert this thermometer into a test tube 17 mm in inside diameter and about 170 mm in height, fasten the thermometer with cork stopper so that the lower end of the thermometer is about 25 mm distant from the bottom of the test tube. Suspend the test tube in a beaker containing water, and heat the beaker with constant stirring until the temperature rises
Cetirizine Hydrochloride

C₂₁H₂₅ClN₂O₃·2HCl: 461.81
2-((2-(4-[[RS]-4-Chlorophenyl][phenyl]methyl)piperazin-1-yl)ethoxy)acetic acid dihydrochloride

Cetirizine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C₂₁H₂₅ClN₂O₃·2HCl.

Description Cetirizine Hydrochloride occurs as a white crystalline powder.

It is very soluble in water, and slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Cetirizine Hydrochloride (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Cetirizine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the infrared absorption spectrum of Cetirizine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25S>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Cetirizine Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cetirizine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Cetirizine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than cetirizine obtained from the sample solution is not larger than the peak area of cetirizine from the standard solution. Furthermore, the total area of the peaks other than cetirizine is not larger than 2.5 times the peak area of cetirizine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and diluted 0.5 mol/L sulfuric acid TS (2 in 25) (47:3).

Flow rate: Adjust the flow rate so that the retention time of cetirizine is about 9 minutes.

Time span of measurement: About 3 times as long as the retention time of cetirizine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cetirizine obtained from 10 μL of this solution is equivalent to 35 to 65% of that from 10 μL of the standard solution.

System performance: Dissolve 20 mg of Cetirizine Hydrochloride in the mobile phase to make 100 mL. To 5 mL of this solution, add 3 mL of a solution of aminopyrine in the mobile phase (1 in 2500), and adjust the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cetirizine and aminopyrine are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cetirizine is not more than 2.0%.

Loss on drying <2.4f> Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.4f> Not more than 0.2% (1g).

Assay Weigh accurately about 0.1 g of Cetirizine Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetone and water (7:3), and titrate <2.5d> to the second equivalence point with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 15.39 mg of C₂₁H₂₅ClN₂O₃·2HCl
Containers and storage  Containers—Well-closed containers.

Cetirizine Hydrochloride Tablets

Cetirizine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cetirizine hydrochloride (C21H25ClN2O3·2HCl: 461.81).

Method of preparation  Prepare as directed under Tablets, with Cetirizine Hydrochloride.

Identification  To a quantity of powdered Cetirizine Hydrochloride Tablets, equivalent to 10 mg of Cetirizine Hydrochloride according to the labeled amount, add about 70 mL of 0.1 mol/L hydrochloric acid TS, shake, add 0.1 mol/L hydrochloric acid TS to make 100 mL, and filter. To 4 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.2.4): it exhibits a maximum between 230 nm and 234 nm.

Uniformity of dosage units  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Cetirizine Hydrochloride Tablets, add 4V/5 mL of sodium 1-heptanesulfonate solution (1 in 5000) adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, shake, add ultrasonic waves for 20 minutes, adjust the volume to exactly V mL, by adding sodium 1-heptanesulfonate solution (1 in 5000) adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, so that each mL contains about 0.2 mg of cetirizine hydrochloride (C21H25ClN2O3·2HCl), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 2 mL of the internal standard solution, add acetonitrile to make exactly 10 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
M_5 = M_s \times \frac{Q_t}{Q_s} \times \frac{S}{100}
\]

Amount (mg) of cetirizine hydrochloride
(C21H25ClN2O3·2HCl)

M5: Amount (mg) of cetirizine hydrochloride for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 1000).

Operating conditions—

System suitability—

Mobile phase: A mixture of a solution of sodium 1-heptanesulfonate (1 in 2900) and acetonitrile (29:21), adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS.

Flow rate: Adjust the flow rate so that the retention time of cetirizine is about 5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, cetirizine and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cetirizine to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Well-closed containers.
Cetraxate Hydrochloride

セトラキサート塩酸塩

\[ \text{Cetrazate Hydrochloride} \]

\[
\text{C}_{17}\text{H}_{23}\text{NO}_4\text{HCl}: 341.83
\]

3-\{4-\{4-\{2,4,6-\text{Triaminophenyl}\}propionic acid\}\}monohydrochloride

[27724-96-5]

Cetraxate Hydrochloride, when dried, contains not less than 98.5% of \( \text{C}_{17}\text{H}_{23}\text{NO}_4\text{HCl} \).

**Description**

Cetraxate Hydrochloride occurs as white crystals or crystalline powder.

It is soluble in methanol, sparingly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 236°C (with decomposition).

**Identification**

(1) Determine the absorption spectrum of a solution of Cetraxate Hydrochloride in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.5 g of Cetraxate Hydrochloride in 5 mL of a mixture of water and 2-propanol (1:1) by warming, cool to below 25°C. Filter, dry the formed crystals in vacuum for 4 hours, and further dry at 105°C for 1 hour. Determine the infrared absorption spectrum of the dried matter as directed in the potassium chloride disk method under Infrared Spectroscopy <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Cetraxate Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

**Purity**

(1) Heavy metals <1.07>—Proceed with 2.0 g of Cetraxate Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cetraxate Hydrochloride according to Method 3, and perform the test with a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 2 ppm).

(3) cis Isomer—Dissolve 0.10 g of Cetraxate Hydrochloride in 10 mL of water, and use this solution as the sample solution. To exactly 5 mL of the sample solution add water to make exactly 100 mL. To exactly 2 mL of this solution add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak which has a retention time 1.3 to 1.6 times that of cetraxate from the sample solution is not larger than the peak area of cetraxate from the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Adjust the pH of a mixture of water, methanol, and 0.5 mol/L ammonium acetate TS (15:10:4) to 6.0 with acetic acid (31).

Flow rate: Adjust the flow rate so that the retention time of cetraxate is about 10 minutes.

**System suitability**

System performance: Dissolve 0.02 g of Cetraxate Hydrochloride and 0.01 g of phenol in 100 mL of water. To 2 mL of this solution add water to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cetraxate and phenol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cetraxate is not more than 2.0%.

(4) 3-(\{p-Hydroxyphenyl\}\)propionic acid—To 0.10 g of Cetraxate Hydrochloride add exactly 2 mL of the internal standard solution and methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of 3-(\{p-hydroxyphenyl\}\)propionic acid in methanol to make exactly 100 mL. To exactly 2 mL of this solution add exactly 2 mL of the internal standard solution and methanol to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of 3-(\{p-hydroxyphenyl\}\)propionic acid to that of the internal standard: \( Q_T \) is not larger than \( Q_S \).

**Internal standard solution**

A solution of caffeine in methanol (1 in 4000).

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of a mixture of water, methanol, and 0.5 mol/L ammonium acetate TS (15:5:2) to 5.5 with acetic acid (31).

Flow rate: Adjust the flow rate so that the retention time of 3-(\{p-hydroxyphenyl\}\)propionic acid is about 7 minutes.

**System suitability**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, 3-(\{p-hydroxyphenyl\}\)propionic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 3-(\{p-hydroxyphenyl\}\)propionic acid to that
of the internal standard is not more than 1.0%.

(5) Related substances—Dissolve 0.10 g of Cetraxate Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 µL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and acetic acid (100:20:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 90°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Cetraxate Hydrochloride, previously dried, dissolve in 100 mL of water, and adjust the pH of this solution to between 7.0 and 7.5 with dilute sodium hydroxide TS. To this solution add 10 mL of chloroform, previously dried, dissolve in 100 mL of water, and make the test with this solution. Prepare the control solution as follows: To 0.10 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of methanol, 10 mL of dilute nitric acid and water to make 50 mL (not more than 0.1%).

Each mL of 0.1 mol/L sodium hydroxide VS = 34.18 mg of C₂₄H₄₀O₄·3·H₂O·HCl

**Containers and storage** Containers—Tight containers.

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**Chenodeoxycholic Acid**

ケノデオキシコール酸

C₂₄H₄₀O₄: 392.57
3α,7α-Dihydroxy-5β-cholan-24-oic acid

[778-25-9]

Chenodeoxycholic Acid, when dried, contains not less than 98.0% and not more than 101.0% of C₂₄H₄₀O₄.

**Description** Chenodeoxycholic Acid occurs as white, crystals, crystalline powder or powder. It is freely soluble in methanol and in ethanol (99.5), soluble in acetone, and practically insoluble in water.

**Identification** Determine the infrared absorption spectrum of Chenodeoxycholic Acid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [α]D²⁰: +11.0 – +13.0° (after drying, 0.4 g, ethanol (99.5), 20 mL, 100 nm).

**Melting point** <2.60> 164 – 169°C

**Purity**

(1) Chloride <1.02>—Dissolve 0.36 g of Chenodeoxycholic Acid in 30 mL of methanol, add 10 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution as follows: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of methanol, 10 mL of dilute nitric acid and water to make 50 mL (not more than 0.1%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Chenodeoxycholic Acid according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Barium—To 2.0 g of Chenodeoxycholic Acid add 100 mL of water, and boil for 2 minutes. To this solution add 2 mL of hydrochloric acid, boil for 2 minutes, filter after cooling, and wash the filter with water until to get 100 mL of the filtrate. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is appeared.

(4) Related substances—Dissolve 0.20 g of Chenodeoxycholic Acid in a mixture of acetone and water (9:1) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of lithocholic acid for thin-layer chromatography in the mixture of acetone and water (9:1) to make exactly 10 mL. Pipet 2 mL of this solution, add the mixture of acetone and water (9:1) to exactly 100 mL, and use this solution as the standard solution (1). Separately, dissolve 10 mg of ursodeoxycholic acid in the mixture of acetone and water (9:1) to make exactly 100 mL, and use this solution as the standard solution (2). Separately, dissolve 10 mg of cholic acid for thin-layer chromatography in the mixture of acetone and water (9:1) to make exactly 100 mL, and use this solution as the standard solution (3). Pipet 1 mL of the sample solution, and add the mixture of acetone and water (9:1) to make exactly 20 mL. Pipet 0.5 mL, 1 mL, 2 mL, 3 mL and 5 mL of this solution, add the mixture of acetone and water (9:1) to each of them to make exactly 50 mL, and designate these solutions as standard solution A, standard solution B, standard solution C, standard solution D and standard solution E, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 µL of each of the sample solution, standard solutions (1), (2), (3) and standard solutions A, B, C, D and E on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 4-methyl-2-pentanone, toluene and formic acid (16:6:1) to a distance of about 15 cm, air-dry the plate, and further dry at 120°C for 30 minutes. Immediately, spray evenly a solution of phosphomolybdic acid n-hydrate in ethanol (95) (1 in 5) on the plate, and heat at 120°C for 2 to 3 minutes: the spot corresponding to the spot with the standard solution (1) is not more intense than the spot with the standard solution (1), the spot corresponding to the spot with the standard solution (2) is not more intense than the spot with the standard solution (2), and the spot corresponding to the spot with the standard solution (3) is not more intense than the spot with the standard solution (3).

As compared to the spots with the standard solutions A, B, C, D and E, the spots other than the principal spot and other than the spots mentioned above are not more intense than the spot with the standard solution E, and the total
Chloral Hydrate / Official Monographs

amount of them is not more than 1.5%.

Loss on drying <2.41> Not more than 1.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Chenodeoxycholic Acid, previously dried, dissolve in 40 mL of ethanol (95) and 20 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 165.4 mg of C_2H_3Cl_3O_2

Containers and storage Containers—Tight containers.

Chloral Hydrate

Chloramphenicol

C_11H_12Cl_2N_2O_5: 323.13
2,2-Dichloro-N-{[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide
[56-75-7]

Chloramphenicol contains not less than 980 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Chloramphenicol is expressed as mass (potency) of chloramphenicol (C_11H_12Cl_2N_2O_5).

Description Chloramphenicol occurs as white to yellowish white, crystals or crystalline powder. It is freely soluble in methanol and in ethanol (99.5), and slightly soluble in water.

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chloramphenicol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chloramphenicol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Chloramphenicol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D^20 + 18.5 – + 21.5° (1.25 g, ethanol (99.5), 25 mL, 100 mm).

Melting point <2.60> 150 – 155°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Chloramphenicol according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 25 ppm).

(2) Arsenic <1.1D>—Prepare the test solution with 2.0 g of Chloramphenicol according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Chloramphenicol in 10 mL of methanol, and use this solution as the
sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.03>.

These solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of chloroform, methanol and acetic acid (100) (79:14:7) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot on the original obtained from the sample solution are not more intense than the spot from the standard solution (1), and the total amount of these spots is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Chloramphenicol and Chloramphenicol RS, equivalent to about 0.1 g (potency), dissolve each in 20 mL of methanol, and add water to make exactly 100 mL. Pipet 20 mL each of these solutions, and add water to make exactly 100 mL. Pipet 10 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, $A_1$ and $A_2$, at 278 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount [µg (potency)] of C11H12Cl2N2O5

$$M_g = M_5 	imes A_1/A_2 	imes 1000$$

$M_5$: Amount [mg (potency)] of Chloramphenicol RS

Containers and storage Containers—Tight containers.

Chloramphenicol Palmitate

Chloramphenicol Palmitate contains not less than 558 µg (potency) and not more than 587 µg (potency) per mg, calculated on the dried basis. The potency of Chloramphenicol Palmitate is expressed as mass (potency) of chloramphenicol (C11H12Cl2N2O5: 323.13).

Description Chloramphenicol Palmitate occurs as a white to grayish white, crystalline powder. It is freely soluble in acetone, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Chloramphenicol Palmitate in ethanol (99.5) (1 in 33,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chloramphenicol Palmitate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Chloramphenicol Palmitate and Chloramphenicol Palmitate RS in 1 mL of acetone, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone and cyclohexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same Rf value as the spot from the standard solution.

Optical rotation <2.49> $[\alpha]_2^2$: $+21 - +25^\circ$ (1 g calculated on the dried basis, ethanol (99.5), 20 mL, 100 mm).

Melting point <2.60> 91 - 96°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Chloramphenicol Palmitate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Chloramphenicol Palmitate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Chloramphenicol Palmitate in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. The test should be performed within 30 minutes after the sample solution and standard solution are prepared. Determine each peak area by the automatic integration method: the total area of the peaks other than the peak of chloramphenicol palmitate from the sample solution is not larger than 3.5 times the peak area of chloramphenicol palmitate from the standard solution. For this calculation, use the peak areas for chloramphenicol, having the relative retention time of about 0.5 with respect to chloramphenicol palmitate, and for chloramphenicol dipalmitate, having the relative retention time of about 5.0 with respect to chloramphenicol palmitate, after multiplying by their relative response factors, 0.5 and 1.4, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Methanol.
Flow rate: Adjust the flow rate so that the retention time of chloramphenicol palmitate is about 5 minutes.

Time span of measurement: About 6 times as long as the retention time of chloramphenicol palmitate.

System suitability—
Test for required detectability: Dissolve 50 mg of Chloramphenicol Palmitate in 50 mL of methanol. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add methanol to make exactly 50 mL. Confirm that the peak area of chloramphenicol palmitate obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates of the peak of chloramphenicol palmitate is not less than 5000.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of chloramphenicol palmitate is not more than 1.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Chloramphenicol Palmitate and Chloramphenicol Palmitate RS, equivalent to about 37 mg (potency), dissolve each in 40 mL of methanol and exactly 1 mL of acetic acid (100), and add methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, and exactly 1 mL of acetic acid (100), and add methanol to make about 37 mg (potency), dissolve each in 40 mL of methanol.

Chloramphenicol Sodium Succinate
クロラムフェニコールコハク酸エステルナトリウム

C₁₅H₁₅Cl₂N₂NaO₈: 445.18
Monosodium (2R,3R)-2-(dichloroacetyl)amino-3-hydroxy-3-(4-nitrophenyl)propan-1-yl succinate [982-57-0]

Chloramphenicol Sodium Succinate contains not less than 711 μg (potency) per mg, calculated on the anhydrous basis. The potency of Chloramphenicol Sodium Succinate is expressed as mass (potency) of chloramphenicol (C₁₁H₁₂Cl₂N₂O₅: 323.13).

Description Chloramphenicol Sodium Succinate occurs as white to yellowish white, crystals or crystalline powder.

It is very soluble in water, and freely soluble in methanol and in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Chloramphenicol Sodium Succinate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chloramphenicol Sodium Succinate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Chloramphenicol Sodium Succinate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> [α]D₉₀: + 5 – + 8° (1.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.4 g of Chloramphenicol Sodium Succinate in 5 mL of water is between 6.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Chloramphenicol Sodium Succinate in 10 mL of water: the solution is clear and colorless to yellowish.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Chloramphenicol Sodium Succinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.10>—Prepare the test solution with 1.0 g of Chloramphenicol Sodium Succinate according to Method 1, and perform the test (not more than 2 ppm).
**Chlordiazepoxide**

クロルジアゼポキシド

C₁₆H₁₄ClN₃O: 299.75
7-Chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-4-oxide
[58-25-3]

Chlordiazepoxide, when dried, contains not less than 98.5% of C₁₆H₁₄ClN₃O.

**Description** Chlordiazepoxide occurs as white to light yellow crystals or crystalline powder.

It is freely soluble in acetate acid (100), sparingly soluble in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually affected by light.

Melting point: about 240°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Chlordiazepoxide in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of Chlordiazepoxide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the infrared absorption spectra of Chlordiazepoxide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Chlordiazepoxide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Proceed with Chlordiazepoxide as directed under Flame Coloration Test <1.40> (2), and perform the test: a green color develops.

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Chlordiazepoxide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.20 g of Chlordiazepoxide in exactly 10 mL of a mixture of methanol and ammonia TS (97:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and ammonia TS (97:3) to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with the these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 μL of the sample solution and 5 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ethanol (99:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1). Spray evenly a solution of sodium nitrite in 1 mol/L hydrochloric acid TS (1 in 100) on the plate, allow to stand for 1 minute, and spray evenly N-(1-naphthyl)-N'-diethylthelyenediamine oxalate-acetone TS on the plate: the spots from the sample solution are not more intense than the spots from the standard solution (2).

**Loss on drying** <2.49> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.6 g of Chlordiazepoxide, previously dried, and dissolve in 50 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the supernatant liquid changes from purple through blue-purple to blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 29.98 mg of C₁₆H₁₄ClN₃O

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Chlordiazepoxide Powder**

クロルジアゼポキシド散

Chlordiazepoxide Powder contains not less than 93.0% and not more than 107.0% of the labeled amount of chlordiazepoxide (C₁₆H₁₄ClN₃O: 299.75).
Method of preparation  Prepare as directed under Granules or Powders, with Chlordiazepoxide.

Identification (1) Weigh a portion of Chlordiazepoxide Powder, equivalent to 0.01 g of Chlordiazepoxide according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 5 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry. If it exhibits maxima between 244 nm and 248 nm and between 306 nm and 310 nm, and a minimum between 288 nm and 292 nm.

(2) Weigh a portion of Chlordiazepoxide Powder, equivalent to 0.02 g of Chlordiazepoxide according to the labeled amount, add 10 mL of methanol, shake for 5 minutes, then filter by suction through a glass filter (G4), evaporate the filtrate with the aid of a current of air to dryness, and dry the residue in vacuum at 60°C for 1 hour. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry. It exhibits absorption at the wave numbers of about 1625 cm⁻¹, 1465 cm⁻¹, 1265 cm⁻¹, 850 cm⁻¹ and 765 cm⁻¹.

Purity  Conduct this procedure without exposure to daylight, using light-resistant vessels. To a portion of Chlordiazepoxide Powder, equivalent to 50 mg of Chlordiazepoxide according to the labeled amount, add exactly 5 mL of a mixture of methanol and ammonia TS (97:3), shake, and centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 50 mg of Chlordiazepoxide RS in a mixture of methanol and ammonia TS (97:3) to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 5.0 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 25 μL of the sample solution and 10 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Purity (2) under Chlordiazepoxide.

Assay  Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of Chlordiazepoxide Powder, equivalent to about 0.1 g of chlordiazepoxide (C₁₅H₁₄ClN₃O), transfer to a glass-stoppered flask, wet with exactly 10 mL of water, add exactly 90 mL of methanol, stopper, shake vigorously for 15 minutes, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Chlordiazepoxide RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, and dissolve in exactly 10 mL of water and 90 mL of methanol. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q₁ and Q₀, of the peak area of chlordiazepoxide to that of the internal standard.

Amount (mg) of chlordiazepoxide (C₁₅H₁₄ClN₃O) = Mₛ × Q₁/Q₀

Mₛ: Amount (mg) of Chlordiazepoxide RS

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of methanol and 0.02 mol/L ammonium dihydrogen phosphate TS (7:3).
Flow rate: Adjust the flow rate so that the retention time of chlordiazepoxide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, chlordiazepoxide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of chlordiazepoxide to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Light-resistant.

Chlordiazepoxide Tablets

クロルジアゼポキシド錠

Chlordiazepoxide Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of chlordiazepoxide (C₁₅H₁₄ClN₃O: 299.75).

Method of preparation  Prepare as directed under Tablets, with Chlordiazepoxide.

Identification (1) Weigh a portion of powdered Chlordiazepoxide Tablets, equivalent to 0.01 g of Chlordiazepoxide according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 5 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry. If it exhibits maxima between 244 nm and 248 nm and between 306 nm and 310 nm, and a minimum between 288 nm and 292 nm.

(2) Weigh a portion of powdered Chlordiazepoxide Tablets, equivalent to 0.01 g of Chlordiazepoxide according to the labeled amount, add 10 mL of diethyl ether, shake vigorously, and centrifuge. Evaporate 5 mL of the supernatant liquid by warming on a water bath to dryness. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry. It exhibits absorption at the wave numbers of about 1625 cm⁻¹, 1465 cm⁻¹, 1265 cm⁻¹, 850 cm⁻¹ and 765 cm⁻¹.
Purity  Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. To a portion of powdered Chlordiazepoxide Tablets, equivalent to 50 mg of Chlordiazepoxide according to the labeled amount, add exactly 5 mL of a mixture of methanol and ammonia TS (97:3), shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 50 mg of Chlordiazepoxide RS in a mixture of methanol and ammonia TS (97:3) to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 5.0 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 25 μL of the sample solution and 10 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Purity (2) under Chlordiazepoxide.

Uniformity of dosage units 6.02 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Chlordiazepoxide Tablets add 1 mL of water, shake to disintegrate the tablet, then add 20 mL of methanol, shake, add methanol to make exactly 25 mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 5 mL of the filtrate, take exactly V mL of the subsequent filtrate equivalent to about 2 mg of chlordiazepoxide (C₁₆H₁₄ClN₃O), transfer to a glass-stoppered flask, add 10 mL of water, and shake well to disintegrate. Add 60 mL of methanol, shake well, add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Chlordiazepoxide RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, dissolve in 1 mL of water and a suitable amount of methanol, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.08 according to the following conditions, and calculate the ratios, Q₁ and Q₅, of the peak area of chlordiazepoxide to that of the internal standard.

\[
M₅ = \frac{M_s \times Q_5}{Q_s \times V} 
\]

M₅: Amount (mg) of Chlordiazepoxide RS

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Dissolution 6.10 When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Chlordiazepoxide Tablets is not less than 70%.

Start the test with 1 tablet of Chlordiazepoxide Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the dissolution medium to make exactly V mL so that each mL contains about 3.7 μg of chlordiazepoxide (C₁₆H₁₄ClN₃O) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 12 mg of chlordiazepoxide for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, and dissolve in the dissolution medium to make exactly 200 mL. Pipet 3 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A₄ and A₅, of the sample solution and standard solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry 2.24.

Dissolution rate (%) with respect to the labeled amount of chlordiazepoxide (C₁₆H₁₄ClN₃O)

\[
M_s = M \times \frac{A_4}{A_5} \times \frac{V}{V} \times \frac{1}{C} \times \frac{27}{2} 
\]

Mₕ: Amount (mg) of chlordiazepoxide for assay.

C: Labeled amount (mg) of chlordiazepoxide (C₁₆H₁₄ClN₃O) in 1 tablet.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of Chlordiazepoxide Tablets, equivalent to about 0.1 g of chlordiazepoxide (C₁₆H₁₄ClN₃O), transfer to a glass-stoppered flask, add 10 mL of water, and shake well to disintegrate. Add 60 mL of methanol, shake well, add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Chlordiazepoxide RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, dissolve in 1 mL of water and a suitable amount of methanol, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.08 according to the following conditions, and calculate the ratios, Q₁ and Q₅, of the peak area of chlordiazepoxide to that of the internal standard.

\[
M₅ = \frac{M_s \times Q_5}{Q_s \times 10} 
\]

M₅: Amount (mg) of Chlordiazepoxide RS

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.02 mol/L ammonium dihydrogen phosphate TS (7:3).

Flow rate: Adjust the flow rate so that the retention time of chlordiazepoxide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, chlordiazepoxide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of chlordiazepoxide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Chlorhexidine Gluconate Solution

クロルヘキシジングルコン酸塩液

Chlorhexidine Gluconate Solution is a solution of digluconate of chlorhexidine.

It contains not less than 19.0 w/v% and not more than 21.0 w/v% of chlorhexidine gluconate (C22H30Cl2N10.2C6H12O7: 897.76).

Description Chlorhexidine Gluconate Solution is a clear, colorless or pale yellow liquid. It is odorless, and has a bitter taste.

It is miscible with water and with acetic acid (100). 1 mL of Chlorhexidine Gluconate Solution is miscible with not more than 5 mL of ethanol (99.5) and with not more than 3 mL of acetone. By further addition of each of these solvents, a white turbidity is formed.

It is gradually colored by light.

Specific gravity d20 1.06 - 1.07

Identification (1) To 0.05 mL of Chlorhexidine Gluconate Solution add 5 mL of methanol, 1 mL of bromine TS and 1 mL of 8 mol/L sodium hydroxide TS: a deep red color is produced.

(2) To 0.5 mL of Chlorhexidine Gluconate Solution add 10 mL of water and 0.5 mL of copper (II) sulfate TS: a white precipitate is formed. Heat to boiling: the precipitate changes to light purple.

(3) To 10 mL of Chlorhexidine Gluconate Solution add 5 mL of water, cool on ice, and add 5 mL of sodium hydroxide TS dropwise with stirring: a white precipitate is formed. Collect the precipitate by filtration, wash with water, recrystallize from diluted ethanol (95) (7 in 10), and dry at 105°C for 30 minutes: the crystals thus obtained melt <2.60> between 130°C and 134°C.

(4) Neutralize the filtrate obtained in (3) with 5 mol/L hydrochloric acid TS. To 5 mL of this solution add 0.65 mL of acetic acid (100) and 1 mL of freshly distilled phenylhydrazine, and heat on a water bath for 30 minutes. After cooling, scratch the inner wall of the vessel with a glass rod to induce crystallization. Collect the crystals, dissolve in 10 mL of hot water, add a small amount of activated charcoal, and filter. Cool the filtrate, scratch the inner side of the vessel, collect the formed crystals, and dry: the crystals thus obtained melt <2.60> at about 195°C (with decomposition).

pH <2.5> To 5.0 mL of Chlorhexidine Gluconate Solution add water to make 100 mL: the pH of the solution is between 5.5 and 7.0.

Purity 4-Chloroaniline—To 2.0 mL of Chlorhexidine Gluconate Solution add water to make exactly 100 mL. Pipet 5 mL of the solution, and add 20 mL of water and 5 mL of 1 mol/L hydrochloric acid TS. Add 0.3 mL of sodium nitrite TS, shake, and allow to stand for 2 minutes. Add 4 mL of ammonium amidosulfate TS, and then allow to stand for 1 minute. Add 5 mL of N-(1-naphthyl)-N'-diethylethlenediamine oxalate-acetone TS, allow to stand for 10 minutes, add 1 mL of ethanol (95), and then add water to make 50 mL: the color of the solution is not more intense than the following control solution.

Control solution: Dissolve 20 mg of 4-chloroaniline in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of the solution, and add water to make exactly 100 mL. Pipet 5 mL of the solution, add 20 mL of water and 5 mL of 1 mol/L hydrochloric acid TS, and proceed as directed for the preparation of the sample solution.

Residue on ignition <2.4> Not more than 0.1% (2 g, after evaporation).

Assay Pipet 2 mL of Chlorhexidine Gluconate Solution, evaporate to dryness on a water bath, dissolve the residue in 60 mL of acetic acid for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 22.44 mg of C22H30Cl2N10.2C6H12O7

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Chlorhexidine Hydrochloride

クロルヘキシジン塩酸塩

Chlorhexidine Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is soluble in formic acid, slightly soluble in methanol and in warm methanol, and practically insoluble in water, in ethanol (95) and in diethyl ether. It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Chlorhexidine Hydrochloride in 50 mL of methanol by warming, and add 1 mL of bromine TS and 1 mL of 8 mol/L sodium hydroxide TS: a deep red color is produced.

(2) Dissolve 0.3 g of Chlorhexidine Hydrochloride in 10 mL of 6 mol/L hydrochloric acid TS, cool in ice, and add 10 mL of 8 mol/L sodium hydroxide TS dropwise with stirring: a white precipitate is produced. Collect the precipitate, wash with water, recrystallize from diluted ethanol (95) (7 in 10), and dry at 105°C for 30 minutes: the crystals so obtained melt <2.60> between 130°C and 134°C.

(3) Dissolve 0.1 g of Chlorhexidine Hydrochloride in 50 mL of dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Chlorhexidine Hydrochloride according to Method 2, and
perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

2. Arsenic (1.11)—To 1.0 g of Chlorhexidine Hydrochloride in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol (95) to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming on a water bath, use this solution as the test solution, and perform the test (not more than 2 ppm).

3. p-Chloroaniline—Dissolve 0.10 g of Chlorhexidine Hydrochloride in 2 mL of formic acid, and add 15 mL of 1 mol/L hydrochloric acid TS and 20 mL of water immediately. Add 0.3 mL of sodium nitrite TS, shake, and allow to stand for 2 minutes. Add 4 mL of ammonium amidosulfate TS, and then allow to stand for 1 minute. Add 5 mL of N-(1-naphthyl)-N’-diethylthelyenediamine oxalate-acetone TS, allow to stand for 10 minutes, and add 1 mL of ethanol (95) and water to make 50 mL: the solution has no more color than the following control solution.

Control solution: Dissolve 20 mg of 4-chloroaniline in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of the solution, and add water to make exactly 100 mL. To 2.0 mL of the solution add 2 mL of formic acid, 15 mL of 1 mol/L hydrochloric acid TS and 20 mL of water, and proceed in the same manner.

Loss on drying (2.41)—Not more than 2.0% (1 g, 130°C, 2 hours).

Residue on ignition (2.44)—Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Chlorhexidine Hydrochloride, previously dried, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.46 mg of C22H30Cl2N10.2HCl

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Chlorinated Lime

サラシ粉

Chlorinated Lime contains not less than 30.0% of available chlorine (Cl: 35.45).

Description Chlorinated Lime occurs as a white powder. It has a chlorine-like odor.

It dissolves partially in water. The solution changes red litmus paper to blue, then gradually decolorizes.

Identification (1) To Chlorinated Lime add dilute hydrochloric acid: a gas, which has the odor of chlorine, evolves, and the gas changes moistened starch-potassium iodide paper to blue.

2. Shake 1 g of Chlorinated Lime with 10 mL of water, and filter: the filtrate responds to the Qualitative Tests (1.00) (2) and (3) for calcium salt.

Assay Weigh accurately about 5 g of Chlorinated Lime, transfer to a mortar, and triturate thoroughly with 50 mL of water. Transfer to a 500-mL volumetric flask with the aid of water, and add water to make 500 mL. Mix well, immediately take exactly 50 mL of the mixture in an iodine flask, add 10 mL of potassium iodide TS and 10 mL of dilute hydrochloric acid, and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 3.545 mg of Cl

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Chlormadinone Acetate

クロルマジノン酢酸エステル

C23H29ClO4· 404.93
6-Chloro-3,20-dioxopregna-4,6-dien-17-yl acetate
[302-22-7]

Chlormadinone Acetate, when dried, contains not less than 98.0% of C23H25ClO4.

Description Chlormadinone Acetate occurs as white to light yellow crystals or crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in acetonitrile, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 2 mg of Chlormadinone Acetate in 1 mL of ethanol (95), and add 1 mL of 1,3-dinitrobenzene TS and 1 mL of a solution of potassium hydroxide (1 in 5): a red-purple color develops.

2. To 0.05 g of Chlormadinone Acetate add 2 mL of potassium hydroxide-ethanol TS, and boil on a water bath for 5 minutes. After cooling, add 2 mL of diluted sulfuric acid (2 in 7), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

3. Determine the infrared absorption spectrum of Chlormadinone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Chlormadinone Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

4. Perform the test with Chlormadinone Acetate as directed under Flame Coloration Test (1.04) (2): a green color appears.

Optical rotation (2.49) [α]D: −10.0° to −14.0° (after drying, 0.2 g, acetonitrile, 10 mL, 100 mm).

Melting point (2.60) 211–215°C
Purity (1) Heavy metals \(<1.07\>— Proceed with 1.0 g of Chlormadinone Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \(<1.17\>— Prepare the test solution with 1.0 g of Chlormadinone Acetate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 20 mg of Chlormadinone Acetate in 10 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\> according to the following conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the peak of chlormadinone acetate from the sample solution is not larger than the peak area of chlormadinone acetate from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 236 nm).
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadeysilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: A mixture of acetonitrile and water (13:7).
Flow rate: Adjust the flow rate so that the retention time of chlormadinone acetate is about 10 minutes.
Time span of measurement: About 1.5 times as long as the retention time of chlormadinone acetate beginning after the solvent peak.
System suitability—
Test for required detection: To exactly 5 mL of the standard solution add acetonitrile to make exactly 50 mL. Confirm that the peak area of chlormadinone acetate obtained from 10 \(\mu\)L of this solution is equivalent to 7 to 13% of that of chlormadinone acetate obtained from 10 \(\mu\)L of the standard solution.
System performance: Dissolve 8 mg of Chlormadinone Acetate and 2 mg of butyl parahydroxybenzoate in 100 mL of acetonitrile. When the procedure is run with 10 \(\mu\)L of this solution under the above operating conditions, butyl parahydroxybenzoate and chlormadinone acetate are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlormadinone acetate is not more than 1.0%.

Loss on drying \(<2.41\> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).
Residue on ignition \(<2.44\> Not more than 0.1% (0.5 g).
Assay Weigh accurately about 20 mg each of Chlormadinone Acetate and Chlormadinone Acetate RS, previously dried, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 5 mL each of these solutions, to each add ethanol (95) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>, and determine the absorbances, \(A_T\) and \(A_S\), at 285 nm.

\[
\text{Amount (mg) of C}_2\text{H}_9\text{ClO}_4 = M_S \times \frac{A_T}{A_S}
\]

\(M_S\): Amount (mg) of Chlormadinone Acetate RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Chlorobutanol

\(\text{クロロブタノール}\)

\(\text{CH}_3\text{Cl}_3\text{H}_7\text{O}\): 177.46
1,1,1-Trichloro-2-methylpropan-2-ol
[57-15-8]

Chlorobutanol contains not less than 98.0% of \(\text{C}_3\text{H}_7\text{Cl}_3\text{O}\), calculated on the anhydrous basis.

Description Chlorobutanol occurs as colorless or white crystals. It has a camphoraceous odor. It is very soluble in methanol, in ethanol (95) and in diethyl ether, and slightly soluble in water. It slowly volatilizes in air. Melting point: not lower than about 76°C.

Identification (1) To 5 mL of a solution of Chlorobutanol (1 in 200) add 1 mL of sodium hydroxide TS, then slowly add 3 mL of iodine TS: a yellow precipitate is produced and the odor of iodoform is perceptible.

(2) To 0.1 g of Chlorobutanol add 5 mL of sodium hydroxide TS, shake well the mixture, add 3 to 4 drops of aniline, and warm gently: the disagreeable odor of phenyl isocyanide (poisonous) is perceptible.

Purity (1) Acidity—Shake thoroughly 0.10 g of the powder of Chlorobutanol with 5 mL of water: the solution is neutral.

(2) Chloride \(<1.07\>— Dissolve 0.5 g of Chlorobutanol in 25 mL of dilute ethanol, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS by adding 25 mL of dilute ethanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.071%).

Water \(<2.45\> Not more than 6.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition \(<2.44\> Not more than 0.1% (1 g).

Assay Transfer about 0.1 g of Chlorobutanol, accurately weighed, to a 200-mL conical flask, and dissolve in 10 mL of ethanol (95). Add 10 mL of sodium hydroxide TS, boil under a reflux condenser for 10 minutes, cool, add 40 mL of dilute nitric acid and exactly 25 mL of 0.1 mol/L silver nitrate VS, and shake well. Add 3 mL of nitrobenzene, and shake vigorously until the precipitate is coagulated. Titrate \(<2.50\> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III))
Chlorphenesin Carbamate

**Description** Chlorphenesin Carbamate occurs as white crystals or a crystalline powder. It is freely soluble in methanol, in ethanol (95) and in pyridine, and slightly soluble in water. A solution of Chlorphenesin Carbamate in ethanol (95) (1 in 20) shows no optical rotation.

**Identification**

1. Determine the absorption spectrum of a solution of Chlorphenesin Carbamate in ethanol (95) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
2. Determine the infrared absorption spectrum of Chlorphenesin Carbamate, as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
3. Perform the test with Chlorphenesin Carbamate as directed under Flame Coloration Test (1.04) (2): a green color appears.

**Melting point** 2.60 88 – 91°C

**Purity**

1. Heavy metals (1.07)—Dissolve 2.0 g of Chlorphenesin Carbamate in 20 mL of ethanol (95), and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 20 mL of ethanol (95), 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).
2. Arsenic (1.17)—Prepare the test solution with 1.0 g of Chlorphenesin Carbamate according to Method 3, and perform the test (not more than 2 ppm).
3. Chlorphenesin-2-carbamate—Dissolve 0.10 g of Chlorphenesin Carbamate in 20 mL of a mixture of hexane for liquid chromatography and 2-propanol (7:3), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography (2.27) according to the following conditions. Determine the peak area, A, of chlorphenesin carbamate and the peak area, A, of chlorphenesin-2-carbamate by the automatic integration method: the ratio, A/A , is not more than 0.007.

**Operating conditions**

- Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
- Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: A mixture of hexane for liquid chromatography, 2-propanol and acetic acid (100) (700:300:1).
- Flow rate: Adjust the flow rate so that the retention time of chlorphenesin carbamate is about 9 minutes.

**System suitability**

- Test for required detection: Pipet 1 mL of the sample solution, add a mixture of hexane for liquid chromatography and 2-propanol (7:3) to make exactly 100 mL, and use this solution as the solution for system suitability test. To exactly 5 mL of the solution for system suitability test add the mixture of hexane for liquid chromatography and 2-propanol (7:3) to make exactly 10 mL. Confirm that the peak area of chlorphenesin carbamate obtained from 10 μL of this solution is equivalent to 40 to 60% of that of chlorphenesin carbamate obtained from 10 μL of the solution for system suitability test.
- System performance: Dissolve 0.1 g of Chlorphenesin Carbamate in 50 mL of methanol. To 25 mL of this solution add 25 mL of dilute sodium hydroxide TS, and warm at 60°C for 20 minutes. To 20 mL of this solution add 5 mL of 1 mol/L hydrochloric acid TS, shake well with 20 mL of ethyl acetate, and allow to stand to separate the upper layer. When the procedure is run with 10 μL of this layer under the above operating conditions, chlorphenesin, chlorphenesin carbamate and chlorphenesin-2-carbamate are eluted in this order, with the relative retention times of chlorphenesin and chlorphenesin-2-carbamate with respect to chlorphenesin carbamate being about 0.7 and about 1.2, respectively, and with the resolution between the peaks of chlorphenesin and chlorphenesin carbamate being not less than 2.0.
- System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of chlorphenesin carbamate is not more than 2.0%.

(4) Related substances—Dissolve 0.10 g of Chlorphenesin Carbamate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 20 mL. Pipet 2 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.07). Spot 50 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (17:2:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 20 minutes: the spot other than the principal spot from the sample solution is not more than one, and it is not more intense than the spot from the standard solution.
Chlorphenesin Carbamate Tablets

Chlorphenesin Carbamate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of chlorphenesin carbamate (C_{10}H_{12}ClNO_{4}: 245.66).

Method of preparation Prepare as directed under Tablets, with Chlorphenesin Carbamate.

Identification To a quantity of powdered Chlorphenesin Carbamate Tablets, equivalent to 0.15 g of Chlorphenesin Carbamate according to the labeled amount, add 60 mL of ethanol (95), treat with ultrasonic waves, and add ethanol (95) to make 100 mL. Centrifuge 20 mL of this solution, add ethanol (95) to 1 mL of the supernatant liquid to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 226 nm and 230 nm, between 279 nm and 283 nm, and between 286 nm and 290 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Chlorphenesin Carbamate Tablets add 10 mL of water to disintegrate the tablet, add 70 mL of a mixture of water and methanol (1:1), treat with ultrasonic waves for 15 minutes with occasional stirring, then add the mixture of water and methanol (1:1) to make exactly 100 mL. Centrifuge this solution, pipet V mL of the supernatant liquid equivalent to about 2.5 mg of chlorphenesin carbamate (C_{10}H_{12}ClNO_{4}), add the mixture of water and methanol (1:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of chlorphenesin carbamate for assay, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 2 mL of this solution, add the mixture of water and methanol (1:1) to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances at 280 nm, A_{s} and A_{y}, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Amount (mg) of chlorphenesin carbamate (C}_{10}\text{H}_{12}\text{ClNO}_{4}\] = \(M_{5} \times A_{S} / A_{y} \times 1/V \times 5\)

\(M_{5}\): Amount (mg) of chlorphenesin carbamate for assay

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Chlorphenesin Carbamate Tablets is not less than 85%.

Start the test with 1 tablet of Chlorphenesin Carbamate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains about 0.14 mg of chlorphenesin carbamate (C_{10}H_{12}ClNO_{4}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of chlorphenesin carbamate for assay, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, dissolve in 1 mL of methanol, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, \(A_{s}\) and \(A_{S}\), at 278 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Dissolution rate (\%)} = \frac{M_{5} \times A_{S} / A_{y} \times 1/V \times 450}{C}\]

\(M_{5}\): Amount (mg) of chlorphenesin carbamate for assay

C: Labeled amount (mg) of chlorphenesin carbamate (C_{10}H_{12}ClNO_{4}) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Chlorphenesin Carbamate Tablets, and powder them in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 0.25 g of chlorphenesin carbamate (C_{10}H_{12}ClNO_{4}), add 30 mL of ethyl acetate, disperse using ultrasonic waves, then add ethyl acetate to make exactly 50 mL. Centrifuge 20 mL of this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add ethyl acetate to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of chlorphenesin carbamate for assay, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in ethyl acetate to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, then add ethyl acetate to make 20 mL, and use this solution as the standard solution. Perform the test with 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, \(Q_{T}\) and \(Q_{S}\), of the peak area of chlorphenesin carbamate to that of the internal standard.

\[
\text{Amount (mg) of chlorphenesin carbamate (C}_{10}\text{H}_{12}\text{ClNO}_{4}\] = \(M_{5} \times Q_{T} / Q_{S} \times 5/2\)

\(M_{5}\): Amount (mg) of chlorphenesin carbamate for assay

Internal standard solution—A solution of ethenzamide in...
ethyl acetate (1 in 400).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of hexane for liquid chromatography, 2-propanol and acetic acid (100:700:300:1).
Flow rate: Adjust the flow rate so that the retention time of chlorphenesin carbamate is about 9 minutes.

System suitability—
System performance: Proceed as directed in the system suitability in the Purity (3) under Chlorphenesin Carbamate.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorphenesin carbamate to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Chlorpheniramine and Calcium Powder
クロフェニラミン・カルシウム散

Chlorpheniramine and Calcium Powder contains not less than 0.27% and not more than 0.33% of chlorpheniramine maleate (C_{16}H_{19}ClN_{2}·C_{4}H_{4}O_{4}: 390.86).

Method of preparation
Chlorpheniramine Maleate 3 g
Dibasic Calcium Phosphate Hydrate 800 g
Starch, Lactose Hydrate, or their mixture a sufficient quantity

Prepare as directed under Powders, with the above ingredients.

Description Chlorpheniramine and Calcium Powder occurs as a white powder.

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 263 nm and 267 nm (chlorpheniramine maleate).

(2) To 0.5 g of Chlorpheniramine and Calcium Powder add 10 mL of dilute hydrochloric acid, shake well, and filter: the filtrate responds to the Qualitative Tests <1.09> (3) for calcium salt.

(3) To 0.5 g of Chlorpheniramine and Calcium Powder add 10 mL of dilute nitric acid, shake well, and filter: the filtrate responds to the Qualitative tests <1.09> (2) for phosphate.

(4) Shake 1 g of Chlorpheniramine and Calcium Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g of Chlorpheniramine Maleate RS in 17 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.02>. Spot 10 μL each of the sample solution and standard solution on the plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm. Air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and from the standard solution show the same RT value.

Spray evenly Dragendorff’s TS for spraying upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal an orange color.

Assay Weigh accurately about 0.5 g of Chlorpheniramine and Calcium Powder, transfer to a 30-mL glass-stoppered centrifuge tube, add 20 mL of 0.05 mol/L sulfuric acid VS, shake for 5 minutes, centrifuge, and collect the supernatant liquid. Add 20 mL of 0.05 mol/L sulfuric acid VS to the residue, and proceed twice in the same manner mentioned above. Transfer all the supernatant liquid to a 200-mL separator, add 30 mL of diethyl ether, shake, and allow to stand for 5 minutes. Filter the water layer through dry filter paper into another separator. Extract the diethyl ether layer with two 10-mL portions of 0.05 mol/L sulfuric acid VS, filter the extracts into the preceding separator containing the water layer. Wash the filter paper with 5 mL of 0.05 mol/L sulfuric acid VS, combine the washings with the water layer in the preceding separator, and add 10 mL of ammonia TS. Extract with two 50-mL portions of diethyl ether, combine the diethyl ether layer, wash with 20 mL of water, and extract the diethyl ether layer with two 20-mL and 5-mL portions of 0.25 mol/L sulfuric acid VS. Combine all the extracts, add 0.25 mol/L sulfuric acid VS to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve about 75 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours and accurately weighed, in 10 mL of 0.05 mol/L sulfuric acid VS, and add 0.05 mol/L sulfuric acid VS to make exactly 100 mL. Pipet 2 mL of the solution into a 200-mL separator, add 58 mL of 0.05 mol/L sulfuric acid VS and 30 mL of diethyl ether, and shake. Proceed in the same manner as the sample solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 265 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 0.25 mol/L sulfuric acid VS as the blank.

Amount (mg) of chlorpheniramine maleate (C_{16}H_{19}ClN_{2}·C_{4}H_{4}O_{4})

\[ M_S = M_f \times A_T / A_S \times 1 / 50 \]

M_S: Amount (mg) of Chlorpheniramine Maleate RS

Containers and storage Containers—Well-closed containers.
Chlorpheniramine Maleate
クロルフェニラミンメチル酸塩

C₁₆H₁₉ClN₂.C₄H₄O₄: 390.86
(3RS)-3-(4-Chlorophenyl)-N,N-dimethyl-3-pyridin-2-ylpropylamine monomaleate
[113-92-8]

Chlorpheniramine Maleate, when dried, contains not less than 98.0% and not more than 101.0% of dl-chlorpheniramine maleate (C₁₆H₁₉ClN₂.C₆H₄O₄).

Description Chlorpheniramine Maleate occurs as white, fine crystals.

It is very soluble in acetic acid (100), freely soluble in water and in methanol, and soluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

A solution of Chlorpheniramine Maleate (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Chlorpheniramine Maleate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.2.A, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chlorpheniramine Maleate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chlorpheniramine Maleate, previously dried, as directed in the paste method under Infrared Spectrophotometry 2.2.B, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Chlorpheniramine Maleate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.10 g of Chlorpheniramine Maleate in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 56 mg of maleic acid in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Liquid Chromatography 2.0.F according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than maleic acid and chlorpheniramine obtained with the sample solution is not larger than 2/3 the peak area of chlorpheniramine with the standard solution, and the total area of the peaks other than maleic acid and chlorpheniramine is not larger than the peak area of chlorpheniramine with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 8.57 g of ammonium dihydrogen phosphate and 1 mL of phosphoric acid in water to make 1000 mL. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 11 minutes.

Time span of measurement: About 4 times as long as the retention time of chlorpheniramine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2.5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of chlorpheniramine obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of chlorpheniramine are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlorpheniramine is not more than 4.0%.

Loss on drying 2.4.F Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition 2.4.G Not more than 0.1% (1 g).

Assay Dissolve about 0.4 g of Chlorpheniramine Maleate, previously dried and accurately weighed, in 20 mL of acetic acid (100). Titrate 2.5.D with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through
Chlorpheniramine Maleate Injection

Chlorpheniramine Maleate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of dl-chlorpheniramine maleate (C₁₆H₁₉ClN₂.C₄H₄O₄: 390.86).

Method of preparation
Prepare as directed under Injections, with Chlorpheniramine Maleate.

Description
Chlorpheniramine Maleate Injection is a clear, colorless liquid.

pH: 4.5 - 7.0

Identification
Take a volume of Chlorpheniramine Maleate Injection, equivalent to 25 mg of Chlorpheniramine Maleate according to the labeled amount, add 5 mL of dilute sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 10 mL of water, shake with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath at 50°C under a reduced pressure, and determine the infrared absorption spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry (2.25); it exhibits absorption at the wave numbers of about 2940 cm⁻¹, 2810 cm⁻¹, 2770 cm⁻¹, 1589 cm⁻¹, 1491 cm⁻¹, 1470 cm⁻¹, 1434 cm⁻¹, 1091 cm⁻¹ and 1015 cm⁻¹.

Bacterial endotoxins (4.01)
Less than 8.8 EU/mg.

Extractable volume (6.05)
It meets the requirement.

Foreign insoluble matter (6.06)
Perform the test according to Method 1; it meets the requirement.

Insoluble particulate matter (6.07)
Perform the test according to Method 1; it meets the requirement.

Sterility (4.06)
Perform the test according to the Membrane filtration method; it meets the requirement.

Assay
Transfer an exactly measured volume of Chlorpheniramine Maleate Injection, equivalent to about 3 mg of chlorpheniramine maleate (C₁₆H₁₉ClN₂.C₄H₄O₄), to a 100-mL separator, add 20 mL of water and 2 mL of sodium hydroxide TS, and extract with two 50-mL portions of diethyl ether. Determine the absorbances A₁ and A₂ of the sample solution and standard solution at a wavelength of the maximum absorbance at about 265 nm as directed under Ultraviolet-visible Spectrophotometry (2.24).

\[
\text{Amount (mg) of chlorpheniramine maleate} = M_S \times \frac{A_1}{A_2} \times \frac{1}{10}
\]

Containers and storage
Containers—Tight containers. Storage—Light-resistant.

Chlorpheniramine Maleate Powder

Chlorpheniramine Maleate Powder contains not less than 93.0% and not more than 107.0% of the labeled amount of dl-chlorpheniramine maleate (C₁₆H₁₉ClN₂.C₄H₄O₄: 390.86).

Method of preparation
Prepare as directed under Granules or Powders, with Chlorpheniramine Maleate.

Identification
Weigh a portion of Chlorpheniramine Maleate Powder, equivalent to 50 mg of Chlorpheniramine Maleate according to the labeled amount, shake with 40 mL of 0.1 mol/L hydrochloric acid TS, and filter. Transfer the filtrate to a separator, and wash with 40 mL of hexane. Add 10 mL of sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 5 mL of water. Centrifuge, if necessary, shake the hexane extract with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath at about 50°C under reduced pressure, and determine the infrared absorption spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry (2.25); it exhibits absorption at the wave number of about 2940 cm⁻¹, 2810 cm⁻¹, 2770 cm⁻¹, 1589 cm⁻¹, 1491 cm⁻¹, 1470 cm⁻¹, 1434 cm⁻¹, 1091 cm⁻¹ and 1015 cm⁻¹.

Bacterial endotoxins

Less than 8.8 EU/mg.

Extractable volume
It meets the requirement.

Foreign insoluble matter
Perform the test according to Method 1; it meets the requirement.

Insoluble particulate matter
Perform the test according to Method 1; it meets the requirement.

Sterility
Perform the test according to the Membrane filtration method; it meets the requirement.

Assay
Weigh accurately an amount of Chlorpheniramine Maleate Powder, equivalent to about 4 mg of chlorpheniramine maleate (C₁₆H₁₉ClN₂.C₄H₄O₄), add 70 mL of the internal standard solution, shake for 15 minutes, then add the internal standard solution to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution, transfer to a 100-mL separator, add 2 mL of sodium hydroxide TS, and extract with two 50-mL portions of diethyl ether. Proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Determine the absorbances A₁ and A₂ of the sample solution and standard solution at a wavelength of the maximum absorbance at about 265 nm as directed under Ultraviolet-visible Spectrophotometry (2.24).

\[
\text{Amount (mg) of chlorpheniramine maleate} = M_S \times \frac{A_1}{A_2} \times \frac{1}{10}
\]

Containers and storage
Containers—Hermetic containers. Storage—Light-resistant.
that of the internal standard.

\[
\text{Amount (mg) of chlorpheniramine maleate} \\
(C_{16}H_{19}ClN_{2}.C_{4}H_{4}O_{4}) = M_s \times \frac{Q_1}{Q_s} \times \frac{1}{5}
\]

\(M_s\): Amount (mg) of Chlorpheniramine Maleate RS

**Internal standard solution**—To 7 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 1000) add water to make 1000 mL.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 265 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilaized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in 900 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 8 minutes.

**System suitability**—
System performance: When the procedure is run with 30 \(\mu\)L of the standard solution under the above operating conditions, chlorpheniramine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.
System repeatability: When the test is repeated 6 times with 30 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpheniramine to that of the internal standard is not more than 1.0%.

**Containers and storage**
Containers—Tight containers.

### Chlorpheniramine Maleate Tablets

クロルフェニラミン マレイン酸塩錠

Chlorpheniramine Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of dl-chlorpheniramine maleate \((C_{16}H_{19}ClN_{2}.C_{4}H_{4}O_{4})\) in tablet

**Method of preparation**
Prepare as directed under Tablets, with Chlorpheniramine Maleate.

**Identification**
Weigh a portion of powdered Chlorpheniramine Maleate Tablets, equivalent to 50 mg of Chlorpheniramine Maleate according to the labeled amount, shake with 40 mL of 0.1 mol/L hydrochloric acid TS, and filter. Transfer the filtrate to a separator, and wash with 40 mL of hexane. Add 10 mL of sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 5 mL of water. Centrifuge, if necessary, shake the hexane extract with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath at about 50°C under a reduced pressure, and determine the infrared absorption spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry \(<2.25>\); it exhibits absorption at the wave numbers of about 2940 cm\(^{-1}\), 2810 cm\(^{-1}\), 2770 cm\(^{-1}\), 1589 cm\(^{-1}\), 1491 cm\(^{-1}\), 1470 cm\(^{-1}\), 1434 cm\(^{-1}\), 1091 cm\(^{-1}\) and 1015 cm\(^{-1}\).

**Uniformity of dosage unit** \(<6.02>\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.
To 1 tablet of Chlorpheniramine Maleate Tablets add 10 mL of water, shake to disintegrate the tablet, then add water to make exactly \(V\) mL of a solution containing about 80 \(\mu\)g of chlorpheniramine maleate \((C_{16}H_{19}ClN_{2}.C_{4}H_{4}O_{4})\) per mL, and filter through a membrane filter with pore size of not more than 0.5 \(\mu\)m. Pipet 5 mL of the filtrate, add exactly 2.5 mL of the internal standard solution, add water to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 25 mL of the internal standard solution, add water to make 100 mL, and use this solution as the standard solution. Perform the test with 30 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the conditions described in the Assay, and calculate the ratios, \(Q_1\) and \(Q_2\), of the peak area of chlorpheniramine to that of the internal standard.

\[
\text{Amount (mg) of chlorpheniramine maleate} \\
(C_{16}H_{19}ClN_{2}.C_{4}H_{4}O_{4}) = M_s \times \frac{Q_1}{Q_s} \times \frac{V}{250}
\]

\(M_s\): Amount (mg) of Chlorpheniramine Maleate RS

**Internal standard solution**—To 7 mL of a solution of methyl parahydroxybenzoate (1 in 250) add water to make 1000 mL.

**Dissolution** \(<6.10D>\) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Chlorpheniramine Maleate Tablets is not less than 75%.
Start the test with 1 tablet of Chlorpheniramine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains about 4.4 \(\mu\)g of chlorpheniramine maleate \((C_{16}H_{19}ClN_{2}.C_{4}H_{4}O_{4})\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\), and determine the peak areas, \(A_T\) and \(A_S\), of chlorpheniramine of both solutions.

Dissolution rate (%) with respect to the labeled amount of chlorpheniramine maleate \((C_{16}H_{19}ClN_{2}.C_{4}H_{4}O_{4})\)

\[
= \frac{M_s \times \frac{1}{5} \times \frac{1}{V} \times \frac{1}{C} \times 18}{C_{\text{table}}} \times 100
\]

\(M_s\): Amount (mg) of Chlorpheniramine Maleate RS

**C**: Labeled amount (mg) of chlorpheniramine maleate \((C_{16}H_{19}ClN_{2}.C_{4}H_{4}O_{4})\) in 1 tablet
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 265 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in 900 mL of water, add 10 mL of acetic acid (100), and add water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 8 minutes.

System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of chlorpheniramine are not less than 2000 and not more than 2.5, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlorpheniramine is not more than 2.0%.

Assay
Weigh accurately the mass of not less than 20 Chlorpheniramine Maleate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 4 mg of chlorpheniramine maleate (C₁₆H₁₉ClN₂·C₄H₄O₄), add 70 mL of the internal standard solution to make exactly 100 mL, filter through a membrane filter with pore size of not more than 0.5 μm, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and add the internal standard prepared to make exactly 100 mL. Pipet 20 mL of this solution, and use this solution as the standard solution. Perform the test with 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.2D) according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of chlorpheniramine to that of the internal standard.

\[
M_S = \frac{Q_1}{Q_2} \times M_s \times \frac{1}{5}
\]

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 8 minutes.

System suitability—
System performance: When the procedure is run with 30 μL of the standard solution under the above operating conditions, the internal standard and chlorpheniramine are eluted in this order with the resolution between these peaks being not less than 2.0.
System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpheniramine to that of the internal standard is not more than 1.0%.

Containers and storage
Containers—Tight containers.

\[\text{d-Chlorpheniramine Maleate}\]

\[d-\text{クロルフェニラミンマレイン酸塩}\]

\[
\text{C}_{16}\text{H}_{16}\text{ClN}_2\cdot\text{C}_9\text{H}_6\text{O}_4; \quad 390.86
\]

(3S)-3-(4-Chlorophenyl)-N,N-dimethyl-3-pyridin-2-ylpropylamine monomaleate

[2438-32-6]

\[\text{d-Chlorpheniramine Maleate, when dried, contains not less than 99.0% and not more than 101.0% of C}_{16}\text{H}_{19}\text{ClN}_2\cdot\text{C}_9\text{H}_6\text{O}_4.\]

Description
\[\text{d-Chlorpheniramine Maleate occurs as a white, crystalline powder.}\]

It is very soluble in water, in methanol and in acetic acid (100), and freely soluble in \(N, N\)-dimethylformamide and in ethanol (99.5). It dissolves in dilute hydrochloric acid.

Identification
(1) Determine the absorption spectrum of a solution of \textit{d-Chlorpheniramine Maleate} in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry (2.2D), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of \textit{d-Chlorpheniramine Maleate}, previously dried, as directed in the paste method under Infrared Spectrophotometry (2.2D), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 0.10 g of \textit{d-Chlorpheniramine Maleate} in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 56 mg of maleic acid in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.2D). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, methanol, acetic acid (100) and water.
Chlorpromazine Hydrochloride / Official Monographs

(70:20:7:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot among two of the spots obtained with the sample solution shows the same intense to the spot with the standard solution, and its Rf value is about 0.4.

Optical rotation \( \angle \sigma^\circ; +39.5 - +43.0^\circ \) (after drying, 0.5 g, \( N,N \)-dimethylformamide, 10 mL, 100 nm).

pH \( \angle 2.5 \rightarrow \) Dissolve 1.0 g of \( \text{d-Chlorpheniramine Maleate} \) in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.0 and 5.0.

Melting point \( \angle 2.60 \) 111 - 115°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of \( \text{d-Chlorpheniramine Maleate} \) in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals \( \angle 1.07 \rightarrow \) Proceed with 1.0 g of \( \text{d-Chlorpheniramine Maleate} \) according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of \( \text{d-Chlorpheniramine Maleate} \) in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \( \angle 2.01 \) according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than maleic acid and \( \text{d-Chlorpheniramine} \) obtained with the sample solution is not larger than 2/3 times the peak area of \( \text{d-Chlorpheniramine} \) with the standard solution, and the total area of these peaks is not larger than the peak area of \( \text{d-Chlorpheniramine} \) with the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wave-length: 225 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 \( \mu \text{m} \) in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 8.57 g of ammonium dihydrogen phosphate and 1 mL of phosphoric acid in water to make 1000 mL. To 800 mL of this solution add 200 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of \( \text{d-Chlorpheniramine} \) is about 11 minutes.
Time span of measurement: About 4 times as long as the retention time of \( \text{d-Chlorpheniramine} \) beginning after the solvent peak.

System suitability—
Test for required detectability: To exactly 2.5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of \( \text{d-Chlorpheniramine} \) obtained with 20 \( \mu L \) of this solution is equivalent to 7 to 13% of that with 20 \( \mu L \) of the standard solution.
System performance: When the procedure is run with 20 \( \mu L \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of \( \text{d-Chlorpheniramine} \) are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of \( \text{d-Chlorpheniramine} \) is not more than 4.0%.

Loss on drying \( \angle 2.41 \) Not more than 0.5% (1 g, 65°C, 4 hours).

Residue on ignition \( \angle 2.44 \) Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of \( \text{d-Chlorpheniramine Maleate} \), previously dried, and dissolve in 20 mL of acetic acid (100). Titrate \( \angle 2.50 \) with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 19.54 mg of \( C_{16}H_{19}ClN_2C_4H_4O_4 \).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlorpromazine Hydrochloride

クロルプロマジン塩酸塩

\( \text{C}_{17}\text{H}_{19}\text{ClN}_2\text{S.HCl} \): 355.33
3-(2-Chloro-10\( \text{H} \)-phenothiazin-10-yl)-\( N,N \)-dimethylpropylamine monohydrochloride [69-09-0]

Chlorpromazine Hydrochloride, when dried, contains not less than 99.0% of \( \text{C}_{17}\text{H}_{19}\text{ClN}_2\text{S.HCl} \).

Description Chlorpromazine Hydrochloride occurs as a white to pale yellow, crystalline powder. It is odorless, or has a faint, characteristic odor.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is gradually colored by light.

Identification (1) To 5 mL of a solution of Chlorpromazine Hydrochloride (1 in 1000) add 1 drop of iron (III) chloride TS: a red color develops.

(2) Dissolve 0.1 g of Chlorpromazine Hydrochloride in 20 mL of water and 3 drops of dilute hydrochloric acid, add 10 mL of 2,4,6-trinitrophenol TS, and allow to stand for 5 hours. Collect the resulting precipitate, wash with water, recrystallize from a small portion of acetone, and dry at 105°C for 1 hour: the crystals so obtained melt \( \angle 2.60 \) between 175°C and 179°C.

(3) Dissolve 0.5 g of Chlorpromazine Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and heat on a water bath for 5 minutes. Cool, filter, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualita-
Chlorpromazine Hydrochloride Injection

クロルプロマジン塩酸塩注射液

Chlorpromazine Hydrochloride Injection is an aqueous solution for injection. It contains not less than 95.0% and not more than 105.0% of the labeled amount of chlorpromazine hydrochloride (C₁₇H₁₉ClN₂S.HCl: 355.33).

Method of preparation Prepare as directed under Injections, with Chlorpromazine Hydrochloride.

Identification (1) Proceed with a volume of Chlorpromazine Hydrochloride Injection, equivalent to 5 mg of Chlorpromazine Hydrochloride according to the labeled amount, as directed in the Identification (1) under Chlorpromazine Hydrochloride.

(2) Proceed with a volume of Chlorpromazine Hydrochloride Injection, equivalent to 0.1 g of Chlorpromazine Hydrochloride according to the labeled amount, as directed in the Identification (2) under Chlorpromazine Hydrochloride.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Chlorpromazine Hydrochloride Tablets

クロルプロマジン塩酸塩錠

Chlorpromazine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of chlorpromazine hydrochloride (C₁₇H₁₉ClN₂S.HCl: 355.33).

Method of preparation Prepare as directed under Tablets, with Chlorpromazine Hydrochloride.

Identification (1) Shake a quantity of powdered Chlorpromazine Hydrochloride Tablets, equivalent to 0.2 g of Chlorpromazine Hydrochloride according to the labeled amount, with 40 mL of 0.1 mol/L hydrochloric acid TS, and filter. To 1 mL of the filtrate add 4 mL of water and 1 drop of iron (III) chloride TS: a red color develops.

(2) To 20 mL of the filtrate obtained in (1) add 10 mL of 2,4,6-trinitrophenol TS dropwise, and proceed as directed in the Identification (2) under Chlorpromazine Hydrochloride.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedures using light-resistant vessels. To 1
tablet of Chlorpromazine Hydrochloride Tablets add an amount of a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) so that each mL contains about 0.83 mg of chlorpromazine hydrochloride \((C_{17}H_{19}ClN_2S.HCl)\), treat with the ultrasonic waves for 5 minutes, then shake vigorously for 20 minutes, and add the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly \(V\) mL so that each mL contains about 0.45 mg of chlorpromazine hydrochloride \((C_{17}H_{19}ClN_2S.HCl)\). Filter through a membrane filter with a pore size not exceeding 0.8 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 25 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of chlorpromazine hydrochloride} \quad (C_{17}H_{19}ClN_2S.HCl) = M_S \times Q_T / Q_s \times V / 50
\]

\(M_S\): Amount (mg) of chlorpromazine hydrochloride for assay

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 4500).

**Dissolution**<6.10> When the test is performed at 75 revolutions per minute according to the Paddle method, use 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Chlorpromazine Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Chlorpromazine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet the subsequent \(V\) mL, add the dissolution medium to make exactly \(V\) mL so that each mL contains about 5.6 \(\mu\)g of chlorpromazine hydrochloride \((C_{17}H_{19}ClN_2S.HCl)\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 90 mg of chlorpromazine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 25 mL, and use this solution as the standard solution. Perform the test with 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2,01> according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_s\), of the peak area of chlorpromazine to that of the internal standard.

\[
\text{Amount (mg) of chlorpromazine hydrochloride} \quad (C_{17}H_{19}ClN_2S.HCl) = M_S \times Q_T / Q_s \times 2
\]

\(M_S\): Amount (mg) of chlorpromazine hydrochloride for assay

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 4500).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 256 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile (27:13).
Flow rate: Adjust the flow rate so that the retention time of chlorpromazine is about 15 minutes.

**System suitability**—
System performance: When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, the internal standard and chlorpromazine are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpromazine to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.
Storage—Light-resistant.
Chlorpropamide

クロルプロパミド

\[ \text{C}_{10}\text{H}_{13}\text{ClN}_{2}\text{O}_{3}\text{S}: 276.74} \]

4-Chloro-N-(propylcarbamoyl)benzenesulfonamide [94-20-2]

Chlorpropamide, when dried, contains not less than 98.0% of \( \text{C}_{10}\text{H}_{13}\text{ClN}_{2}\text{O}_{3}\text{S} \).

**Description** Chlorpropamide occurs as white, crystals or crystalline powder.

It is freely soluble in methanol and in acetone, soluble in ethanol (95), and slightly soluble in diethyl ether, and practically insoluble in water.

**Identification** (1) Dissolve 0.08 g of Chlorpropamide in 50 mL of methanol. To 1 mL of the solution add 0.01 mol/L hydrochloric acid TS to make 200 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the infrared absorption spectrum of Chlorpropamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.03). Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, 3-methyl-1-butanol, methanol and ammonia solution (28) (15:10:5:1) to a distance of about 10 cm, and air-dry the plate. After drying the plate at 100°C for 1 hour, spray evenly sodium hypochlorite TS on the plate, and air-dry for 15 minutes. Then spray evenly potassium iodide-starch TS on the plate: the spot from the sample solution equivalent to the spot from the standard solution (2) is not more intense than the spot from the standard solution (2), and the spots other than the spot mentioned above and other than the principal spot is not more intense than the spot from the standard solution (1).

**Loss on drying** (2.41) Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** (2.44) Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.5 g of Chlorpropamide, previously dried, dissolve in 30 mL of neutralized ethanol, and add 20 mL of water. Titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

\[ \text{Each mL of 0.1 mol/L sodium hydroxide VS} = 27.67 \text{mg of C}_{10}\text{H}_{13}\text{ClN}_{2}\text{O}_{3}\text{S} \]

**Containers and storage** Containers—Well-closed containers.

Chlorpropamide Tablets

クロルプロパミド錠

Chlorpropamide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of chlorpropamide (\( \text{C}_{10}\text{H}_{13}\text{ClN}_{2}\text{O}_{3}\text{S}: 276.74 \)).

**Method of preparation** Prepare as directed under Tablets, with Chlorpropamide.

**Identification** Take a quantity of powdered Chlorpropamide Tablets, equivalent to 0.08 g of Chlorpropamide according to the labeled amount, add 50 mL of methanol, shake, and filter. To 1 mL of the filtrate add 0.01 mol/L hydrochloric acid TS to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits a maximum between 231 nm and 235 nm.

**Uniformity of dosage units** (6.02) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Chlorpropamide Tablets add 75 mL of the mobile phase, treat with the ultrasonic waves for 20 minutes with occasional strong shaking, then add the mobile phase to make exactly 100 mL so that each mL contains about 2.5 mg of Chlorpropamide. Centrifuge the solution, pipet 2 mL of the supernatant liquid, and add the mobile phase to make exactly
100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
M_S: \text{Amount (mg) of chlorpropamide for assay}
\]

\[
\text{Amount (mg) of chlorpropamide (C_{10}H_{13}ClN_2O_3S)} = M_S \times A_I/A_S \times V/V_{20}
\]

**Dissolution**

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Chlorpropamide Tablets is not less than 70%.

Start the test with 1 tablet of Chlorpropamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent 20 mL of the filtrate, add the dissolution medium to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.05 g of chlorpropamide for assay, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_I \) and \( A_S \), of the sample solution and standard solution at 232 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Dissolution rate } (\%) = \frac{M_S \times A_I/A_S \times V/V_{20}}{0.05} \times 100
\]

**Assay**

Weigh accurately and powder not less than 20 Chlorpropamide Tablets. Weigh accurately a quantity of the powder, equivalent to about 50 mg of chlorpropamide (C_{10}H_{13}ClN_2O_3S), add 75 mL of the mobile phase, shake for 10 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of chlorpropamide for assay, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the peak areas, \( A_I \) and \( A_S \), of chlorpropamide of the sample solution and standard solution.

\[
\text{Amount (mg) of chlorpropamide (C_{10}H_{13}ClN_2O_3S)} = M_S \times A_I/A_S \times V/V_{20}
\]

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecysililated silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100) and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of chlorpropamide is about 5 minutes.

**System suitability—**

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of chlorpropamide are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlorpropamide is not more than 1.5%.

**Containers and storage**

Containers—Well-closed containers.

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**Cholera Vaccine**

コレラワクチン

Cholera Vaccine is a liquid for injection containing inactivated Vibrio cholerae of the Ogawa and Inaba strains.

Monotypic products may be manufactured, if necessary.

It conforms to the requirements of Cholera Vaccine in the Minimum Requirements for Biological Products.

**Description**

Cholera Vaccine is a white-turbid liquid.

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**Cholecalciferol**

Vitamin D3

コレカルシフェロール

Cholecalciferol occurs as white crystals. It is odorless.

\[ \text{C}_{27}\text{H}_{44}\text{O}: 384.64 \]  
\[ (35,5Z,7E)-9,10-\text{Secocholesta}-5,7,10(19)-\text{trien}-3-\text{ol} \]  
\[ \{67-97-0\} \]

Cholecalciferol contains not less than 97.0% and not more than 103.0% of C_{27}H_{44}O.
It is freely soluble in ethanol (95), in chloroform, in diethyl ether and in isooctane, and practically insoluble in water.

Melting point: 84 – 88°C. Transfer Cholecalciferol to a capillary tube, and dry for 3 hours in a desiccator (in vacuum at a pressure not exceeding 2.67 kPa). Immediately fireseal the capillary tube, put it in a bath fluid, previously heated to a temperature about 10°C below the expected melting point, and heat at a rate of rise of about 3°C per minute, and read the melting point.

Identification (1) Dissolve 0.5 mg of Cholecalciferol in 5 mL of chloroform, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake: a red color is produced, and rapidly changes through purple and blue to green.

(2) Determine the infrared absorption spectrum of Cholecalciferol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cholecalciferol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance $\angle 2.24 \angle E_{1\text{cm} \text{mL}}^{10\text{mg}}$ (265 nm): 450 – 490 (10 mg, ethanol (95), 1000 mL).

Optical rotation $\angle 2.49 \angle [\alpha]_{D}^{20} + 103 + 112\degree$ (50 mg, ethanol (95), 10 mL, 100 mm). Prepare the solution without delay, using Cholecalciferol from a container opened not longer than 30 minutes, previously, and determine the rotation within 30 minutes after the solution has been prepared.

Purity 7-Dehydrocholesterol—Dissolve 10 mg of Cholecalciferol in 2.0 mL of diluted ethanol (95) (9 in 10), add a solution prepared by dissolving 20 mg of digitonin in 2.0 mL of diluted ethanol (95) (9 in 10), and allow the mixture to stand for 18 hours: no precipitate is formed.

Assay Proceed with the operation avoiding contact with air or other oxidizing agents and using light-resistant containers. Dissolve separately about 30 mg each of Cholecalciferol and Cholecalciferol RS, accurately weighed, in isooctane to make exactly 50 mL. Pipet 10 mL each of these solutions, add 3 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the standard solution and the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography $\angle 2.0 \angle$ according to the following conditions, and calculate the ratios, $Q_{T}$ and $Q_{S}$, of the peak area of cholecalciferol to that of the internal standard.

Amount (mg) of $C_{27}H_{46}O = M_{S} \times Q_{T}/Q_{S}$

$M_{S}$: Amount (mg) of Cholecalciferol RS

Internal standard solution—A solution of dimethyl phthalate in isooctane (1 in 100).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column about 4 mm in inside diameter and 10 to 30 cm in length, packed with silica gel for liquid chromatography (5 to 10 μm in particle diameter).
Column temperature: Ordinary temperature.
Mobile phase: A mixture of hexane and n-amylalcohol (997:3).

Flow rate: Adjust the flow rate so that the retention time of cholecalciferol is about 25 minutes.

Selection of column: Dissolve 15 mg of Cholecalciferol RS in 25 mL of isooctane. Transfer this solution to a flask, heat under a reflux condenser in an oil bath for 2 hours, and cool to room temperature rapidly. Transfer this solution to a quartz test tube, and irradiate under a short-wave lamp (main wavelength: 254 nm) and a long-wave lamp (main wavelength: 365 nm) for 3 hours. To this solution add the mobile phase to make 50 mL. Proceed with 10 μL of this solution under the above operating conditions. Use a column with the ratios of the retention time of previtamin D$_{3}$, trans-vitamin D$_{3}$ and tachysterol$_{3}$ to that of cholecalciferol being about 0.5, about 0.6 and about 1.1, respectively, and with resolution between previtamin D$_{3}$ and trans-vitamin D$_{3}$, and that between cholecalciferol and tachysterol$_{3}$ being not less than 1.0.

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

Cholesterol

**Cholecalciferol**

*C_{27}H_{46}O: 386.65 Cholest-5-en-3β-ol [57-88-5]

**Description**

Cholesterol occurs as white to pale yellow crystals or granules. It is odorless, or has a slight odor. It is tasteless.

It is freely soluble in chloroform and in diethyl ether, soluble in 1,4-dioxane, sparingly soluble in ethanol (99.5), and practically insoluble in water.

It gradually changes to a yellow to light yellow-brown color by light.

Identification (1) Dissolve 0.01 g of Cholesterol in 1 mL of chloroform, add 1 mL of sulfuric acid, and shake: a red color develops in the isooctane layer, and the sulfuric acid layer shows a green fluorescence.

(2) Dissolve 5 mg of Cholesterol in 2 mL of chloroform, add 1 mL of acetic anhydride and 1 drop of sulfuric acid, and shake: a red color is produced, and it changes to green through blue.

Optical rotation $\angle 2.49 \angle [\alpha]_{D}^{20} = -34 – -38\degree$ (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point $\angle 2.60 \angle 147 – 150\degree$

**Purity**

(1) Clarity of solution—Place 0.5 g of Cholesterol in a glass-stoppered flask, dissolve in 50 mL of warm ethanol (95), and allow to stand at room temperature for 2 hours: no turbidity or deposit is produced.

(2) Acidity—Place 1.0 g of Cholesterol in a flask, dis-
solve in 10 mL of diethyl ether, add 10.0 mL of 0.1 mol/L sodium hydroxide VS, and shake for 1 minute. Expel the diethyl ether, and boil for 5 minutes. Cool, add 10 mL of water, and titrate with 0.05 mol/L sulfuric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

The volume of 0.1 mol/L sodium hydroxide VS consumed is not more than 0.30 mL.

**Loss on drying** <2.4> Not more than 0.30% (1 g, in vacuum, 60°C, 4 hours).

**Residue on ignition** <2.4> Not more than 0.1% (1 g).

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Cibenzoline Succinate**

シベンゾリンコハク酸塩

![Cibenzoline Succinate](https://example.com/cibenzoline_succinatestructure.png)

\[ \text{C}_{18}\text{H}_{18}\text{N}_{2}\cdot\text{C}_{4}\text{H}_{6}\text{O}_{4} \cdot 380.44 \]

\[ 2\cdot[(1\text{RS})\cdot2\text{-Diphenylcyclopropan-1-yl}]\cdot4,5\text{-dihydro-1H-imidazole monosuccinate} \]

[100678-32-8]

Cibenzoline Succinate, when dried, contains not less than 98.5% and not more than 101.0% of \( \text{C}_{18}\text{H}_{18}\text{N}_{2}\cdot\text{C}_{4}\text{H}_{6}\text{O}_{4} \).

**Description** Cibenzoline Succinate occurs as a white crystalline powder.

It is freely soluble in methanol and in acetic acid (100), and sparingly soluble in water and in ethanol (99.5).

A solution of Cibenzoline Succinate in methanol (1 in 10) shows no optical rotation.

**Identification**

1. Determine the absorption spectrum of a solution of Cibenzoline Succinate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Cibenzoline Succinate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

3. Shake 0.4 g of Cibenzoline Succinate with 2.5 mL of sodium hydroxide TS and 5 mL of ethyl acetate, allow to stand, and to 1 mL of the water layer so obtained add 0.5 mL of 1 mol/L hydrochloric acid TS and 0.5 mL of iron (III) chloride TS: a blown precipitate is formed.

**Melting point** <2.60> 163 – 167°C

**pH** <2.54> Dissolve 0.2 g of Cibenzoline Succinate in 10 mL of water: the pH of this solution is between 4.0 and 6.0.

**Purity**

1. **Clarity and color of solution**—A solution obtained by dissolving 0.2 g of Cibenzoline Succinate in 10 mL of water is clear and colorless.

2. **Heavy metals** <1.07>—Proceed with 1.0 g of Cibenzoline Succinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

3. **Arsenic** <1.17>—Prepare the test solution with 1.0 g of Cibenzoline Succinate according to Method 3, using a solution of magnesium nitrate hexahydrate in ethanol (95%) (1 in 25), and perform the test (not more than 2 ppm).

4. **Related substances**—Dissolve 0.10 g of Cibenzoline Succinate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL and 2 mL of this solution, and add 0.5 mL of 0.1 mol/L perchloric acid VS to each. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, and ammonia solution (28:20:3:2) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution (1). Allow the plate to stand for 30 minutes in iodine vapor: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution (1), and the spot, which is more intense than the spot with the standard solution (2), is not more than two.

**Loss on drying** <2.4> Not more than 0.3% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.4> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Cibenzoline Succinate, previously dried, dissolved in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from violet to blue-green through blue (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 38.04 mg of \( \text{C}_{18}\text{H}_{18}\text{N}_{2}\cdot\text{C}_{4}\text{H}_{6}\text{O}_{4} \).

**Containers and storage** Containers—Tight containers.

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**Cibenzoline Succinate Tablets**

シベンゾリンコハク酸塩錠

Cibenzoline Succinate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cibenzoline succinate (\( \text{C}_{18}\text{H}_{18}\text{N}_{2}\cdot\text{C}_{4}\text{H}_{6}\text{O}_{4} \cdot 380.44 \)).

**Method of preparation** Prepare as directed under Tablets, with Cibenzoline Succinate.

**Identification** To a quantity of powdered Cibenzoline Suc-
cinate Tablets, equivalent to 50 mg of Cibenzoline Succinate according to the labeled amount, add 100 mL of water, shake for 10 minutes, and centrifuge. To 2 mL of the supernatant liquid add water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry. It exhibits a maximum between 221 nm and 225 nm.

**Uniformity of dosage units** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cibenzoline Succinate Tablets add a suitable amount of water so that each mL contains about 10 mg of cibenzoline succinate (C₁₈H₁₈N₂.C₄H₆O₄), and allow standing for 10 minutes while occasional shaking. To this solution add methanol so that each mL contains about 2 mg of cibenzoline succinate (C₁₈H₁₈N₂.C₄H₆O₄), add exactly 1 mL of the internal standard solution per 10 mg of cibenzoline succinate (C₁₈H₁₂N₂.C₄H₆O₄), then add methanol so that each mL contains about 1 mg of cibenzoline succinate (C₁₈H₁₂N₂.C₄H₆O₄). Centrifuge the solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

**Dissolution** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cibenzoline Succinate Tablets is not less than 80%.

Start the test with 1 tablet of Cibenzoline Succinate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 11 μg of cibenzoline succinate (C₁₈H₁₂N₂.C₄H₆O₄) according to the labeled amount, and use this solution as the sample solution. Separate, weigh accurately about 28 mg of cibenzoline succinate for assay, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₅, of the peak area of cibenzoline to that of the internal standard. System repeatability: When the test is repeated 6 times under the above operating conditions, cibenzoline and the internal standard are eluted in this order with the resolution between these peaks being not less than 6. System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cibenzoline and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (3 μm in particle diameter).
Mobile phase: 2.67 g of sodium di-2-ethylhexyl sulfosuccinate in 2000 mL of a mixture of water, acetonitrile and diluted phosphoric acid (1 in 10) (1000:1000:1).
Flow rate: Adjust the flow rate so that the retention time of cibenzoline is about 3 minutes.

**System suitability**—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cibenzoline and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

**Containers and storage**—
Containers—Tight containers.

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### Assay

Weigh accurately not less than 20 Cibenzoline Succinate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of cibenzoline succinate (C₁₈H₁₂N₂.C₄H₆O₄), add 10 mL of water, shake, and add 40 mL of methanol and exactly 10 mL of the internal standard solution. Shake for 20 minutes, add methanol to make 100 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.1 g of cibenzoline succinate for assay, previously dried at 105°C for 2 hours, add 10 mL of water and 40 mL of methanol to dissolve, then add exactly 10 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography. The ratio of the peak area of cibenzoline succinate tablets is not less than 0.1 g of cibenzoline succinate tablets, equivalent to 50 mg of Cibenzoline Succinate Tablets, according to the labeled amount. The area of cibenzoline to that of the internal standard is not more than 1.0%.

**Internal standard solution**—Dissolve 0.1 g of 2-ethylhexyl parahydroxybenzoate in methanol to make 100 mL.

**Amount (mg) of cibenzoline succinate (C₁₈H₁₂N₂.C₄H₆O₄) in tablet**

\[
M = M_C \times \frac{Q_C}{Q_S} \times \frac{C}{100}
\]

**M**: Amount (mg) of cibenzoline succinate for assay

**C**: Labeled amount (mg) of cibenzoline succinate (C₁₈H₁₂N₂.C₄H₆O₄) in 1 tablet

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (3 μm in particle diameter).
Mobile phase: 2.67 g of sodium di-2-ethylhexyl sulfosuccinate in 2000 mL of a mixture of water, acetonitrile and diluted phosphoric acid (1 in 10) (1000:1000:1).
Flow rate: Adjust the flow rate so that the retention time of cibenzoline is about 3 minutes.

**System suitability**—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cibenzoline and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

**Containers and storage**—
Containers—Tight containers.
Ciclacillin

Description
Ciclacillin occurs as white to light yellowish white crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, and practically insoluble in acetonitrile and in ethanol (95).

Identification
Determine the infrared absorption spectrum of Ciclacillin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ciclacillin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 850 mL of this solution add 150 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of ciclacillin is about 4 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ciclacillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of ciclacillin to that of the internal standard is not more than 1.0%.

Containers and storage
Containers—Tight containers.

Ciclosporin

Ciclosporin A

Description
Ciclosporin occurs as a white powder.

It is very soluble in acetonitrile, in methanol and in ethanol (95), freely soluble in diethyl ether, and practically insoluble in water.

Identification
Determine the infrared absorption spectrum of Ciclosporin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ciclosporin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 850 mL of this solution add 150 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of ciclacillin is about 4 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ciclacillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of ciclacillin to that of the internal standard is not more than 1.0%.

Containers and storage
Containers—Tight containers.

Ciclosporin

Ciclosporin A

C62H111N11O12: 1202.61
[39865-13-3]

Ciclosporin contains not less than 98.5% and not more than 101.5% of C62H111N11O12, calculated on the dried basis.

Description
Ciclosporin occurs as a white powder.

It is very soluble in acetonitrile, in methanol and in ethanol (95), freely soluble in diethyl ether, and practically insoluble in water.

Identification
Determine the infrared absorption spectrum of Ciclosporin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ciclosporin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 850 mL of this solution add 150 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of ciclacillin is about 4 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ciclacillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of ciclacillin to that of the internal standard is not more than 1.0%.

Containers and storage
Containers—Tight containers.
method under Infrared Spectrophotometry <2,25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ciclosporin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2,49> \([\alpha]_D^{25} = -185 \text{ to } -193^\circ\) (0.1 g calculated on the dried basis, methanol, 20 mL, 100 mm).

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of Ciclosporin in 10 mL of ethanol (95); the solution is clear, and has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To exactly 3.0 mL of Iron (III) Chloride CS and exactly 0.8 mL of Cobalt (II) Chloride CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

Control solution (2): To exactly 3.0 mL of Iron (III) Chloride CS, exactly 1.3 mL of Cobalt (II) Chloride CS and exactly 0.5 mL of Copper (II) Sulfate CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

Control solution (3): To exactly 0.5 mL of Iron (III) Chloride CS and exactly 1.0 mL of Cobalt (II) Chloride CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Heavy metals <1,07>—Proceed with 1.0 g of Ciclosporin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 2 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2,01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak of ciclosporin is not more than 1.5 times the peak area of ciclosporin obtained from 20 mL of the standard solution. The total area of all peaks other than the ciclosporin from the sample solution is not more than 7/10 times the peak area of ciclosporin from the standard solution, and the total area of all peaks other than the ciclosporin is not larger than 1.5 times the peak area of ciclosporin from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of ciclosporin beginning after the solvent peak.

**System suitability**

Test for required detection: To exactly 2 mL of the standard solution add a mixture of water and acetonitrile (1:1) to make exactly 20 mL. Confirm that the peak area of ciclosporin obtained from 20 \(\mu\)L of this solution is equivalent to 7 to 13% of that of ciclosporin obtained from 20 \(\mu\)L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ciclosporin is not more than 3.0%.

**Loss on drying** <2,49> Not more than 2.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 60°C, 3 hours).

**Residue on ignition** <2,44> Not more than 0.1% (0.5 g).
Cilastatin Sodium

シラスタチナトリウム

\[
C_{16}H_{25}N_2NaO_5S: 380.43 \\
\]

Cilastatin Sodium contains not less than 98.0% and not more than 101.0% of \( C_{16}H_{25}N_2NaO_5S \), calculated on the anhydrous basis and corrected on the amount of the residual solvent.

**Description** Cilastatin Sodium occurs as a white to pale yellowish white powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

**Identification (1)** Determine the infrared absorption spectrum of Cilastatin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\)\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Cilastatin Sodium (1 in 10) responds to the Qualitative Tests \(<1.09\) for sodium salt.

**Optical rotation** \(<2.49\> \quad [\alpha]_D^{20} +41.5 - +44.5^\circ \quad (0.1 \text{ g}, \text{calculated on the anhydrous basis and corrected on the amount of the residual solvent}, \text{a solution of hydrochloric acid in methanol (9 in 1000), 10 mL, 100 mL}).

**pH** \(<2.54\> \quad \text{The pH of a solution prepared by dissolving 1.0 g of Cilastatin Sodium in 100 mL of water is between 6.5 and 7.5.}

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Cilastatin Sodium in 100 mL of water: the solution is clear and the solution has no more color than the following control solution.

Control solution: To a mixture of 2.4 mL of Iron (III) Chloride CS and 0.6 mL of Cobalt (II) Chloride CS add water to make 10 mL, pipet 5 mL of this solution, and add water to make exactly 100 mL.

(2) Heavy metals \(<1.07\>—\text{Proceed with 1.0 g of Cilastatin Sodium according to Method 2, and perform the test. After carbonization, add 0.5 mL of sulfuric acid instead of nitric acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).}

(3) Arsenic \(<1.10\>—\text{To 2.0 g of Cilastatin Sodium add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat carefully until white fumes are evolved. After cooling, heat with two 2-mL portions of nitric acid, then heat with several 2-mL portions of hydrogen peroxide (30) until a colorless or pale yellow solution is obtained. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution: it shows no more color than the following color standard.}

Color standard: Prepare a solution according to the above procedure without using Cilastatin Sodium, add exactly 2 mL of Standard Arsenic Solution, and perform the test in the same manner as the test solution (not more than 1 ppm).

(4) Related substances—Dissolve about 40 mg of Cilastatin Sodium in 25 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07\) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cilastatin from the sample solution is not larger than 1/6 times the peak area of cilastatin from the standard solution, and the total area of the peaks other than the peak of cilastatin is not larger than the peak area of cilastatin from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.5 mm in inner diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 \( \mu \text{m} \) in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (7:3).

Mobile phase B: Diluted phosphoric acid (1 in 1000).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>15 → 100</td>
<td>85 → 0</td>
</tr>
<tr>
<td>30 – 40</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Flow rate: 2.0 mL per minute.

Time span of measurement: 40 minutes.

**System suitability**—

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 30 mL. Confirm that the peak area of cilastatin obtained with 20 \( \mu \text{L} \) of this solution is equivalent to 2.3 to 4.5% of that obtained with 20 \( \mu \text{L} \) of the standard solution.

System performance: When the procedure is run with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the retention time of cilastatin is about 20 minutes, and the number of theoretical plates and the symmetry factor of the peak of cilastatin are not less than 10,000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 3 times with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilastatin is not more than 2.0%.

(5) Residual solvents \(<2.46\>—\text{Weigh accurately about 0.2 g of Cilastatin Sodium, add exactly 2 mL of the internal fluid to a vessel, and stir to dissolve. Add water to make 50 mL, and analyze by liquid chromatography (2.46) under the following conditions:}

- Mobile phase A: A mixture of phosphoric acid (1 in 10) and acetonitrile (7:3).
- Mobile phase B: Phosphoric acid (1 in 1000).
- Column: A stainless steel column 4.5 mm in inner diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 \( \mu \text{m} \) in particle diameter).
- Flow rate: 2.0 mL per minute.
- Time span of measurement: 40 minutes.
- Time after injection of sample: 0 – 30 minutes.
- System suitability:
  - Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 30 mL. Confirm that the peak area of cilastatin obtained with 20 \( \mu \text{L} \) of this solution is equivalent to 2.3 to 4.5% of that obtained with 20 \( \mu \text{L} \) of the standard solution.
  - System performance: When the procedure is run with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the retention time of cilastatin is about 20 minutes, and the number of theoretical plates and the symmetry factor of the peak of cilastatin are not less than 10,000 and not more than 2.5, respectively.
  - System repeatability: When the test is repeated 3 times with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilastatin is not more than 2.0%.
standard solution, dissolve in water to make 10 mL, and use this solution as the sample solution. Separately, measure exactly 2 mL of acetone, 0.5 mL of methanol and 0.5 mL of mesityl oxide, and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, add water to make 10 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Calculate the ratios of the peak areas of acetone, methanol and mesityl oxide and to the peak area of the internal standard are not more than 4.0 and not more than 0.4%, respectively. Not more than 50 ppm. Not more than 20 ppm.

Internal standard solution—To 0.5 mL of 1-propanol add water to make 1000 mL.

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3.2 mm in inside diameter and 2.1 m in length, packed with teflon for gas chromatography (250 – 420 μm) coated with polyethylene glycol 20 M for gas chromatography at the ratio of 10%.
Column temperature: A constant temperature of about 70°C.
Carrier gas: Helium.
Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 5 minutes.
Time span of measurement: About 3 times as long as the retention time of the internal standard.

System suitability—
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the relative standard deviations of the ratio of the peak area of acetone, methanol and mesityl oxide to that of the internal standard are not more than 4.0%, respectively.

Water <2.48> Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.3 g of Cilastatin Sodium, dissolve in 30 mL of methanol, add 5 mL of water, and adjust to pH 3.0 with 0.1 mol/L hydrochloric acid TS. Titrate <2.50> with 0.1 mol/L sodium hydroxide VS from the first equivalence point to the third equivalence point (potentiometric titration).

Cilazapril Hydrate
シラザプリル水和物

C₂₂H₃₁N₃O₅.H₂O: 435.51
(15,95)-(1S)-9-[(1S)-(1-Ethoxycarbonyl-3-phenylpropyl)amino]-10-o xoctahydro-6H-pyridazino[1,2-a][1,2]diazepine-1-carboxylic acid monohydrate [92077-78-6] Cilazapril Hydrate contains not less than 98.5% and not more than 101.0% of cilazapril (C₂₂H₃₁N₃O₅: 417.50), calculated on the anhydrous basis.

Description Cilazapril Hydrate occurs as white to yellowish white crystals or crystalline powder.
It is very soluble in methanol, freely soluble in ethanol (99.5) and in acetic acid (100), and slightly soluble in water. It gradually turns yellow on exposure to light. Melting point: about 101°C (with decomposition).

Identification (1) To 4 mL of a solution of Cilazapril Hydrate (1 in 1000) add 2 mL of Dragendorff’s TS: an orange precipitate is produced.
(2) Determine the infrared absorption spectrum of Cilazapril Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]₂₀°: −53 – −58° (0.2 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) Chloride <1.03>—Perform the test using 1.0 g of Cilazapril Hydrate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).
(2) Sulfate <1.14>—Dissolve 1.0 g of Cilazapril Hydrate in 40 mL of water and 1.5 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).
(3) Heavy metals <1.07>—Proceed with 1.0 g of Cilazapril Hydrate according to Method 4, and perform the test. However, use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 8). Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(4) Related substances—Dissolve 0.10 g of Cilazapril Hydrate in 20 mL of methanol, and use this solution as the
Cilazapril Tablets / Official Monographs

The Cilazapril Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of cilazapril (C\(_{22}\)H\(_{21}\)N\(_3\)O\(_5\)) (417.50).

**Method of preparation**
Prepare as directed under Tablets, with Cilazapril Hydrate.

**Identification**
To a quantity of powdered Cilazapril Tablets, equivalent to 2 mg of cilazapril (C\(_{22}\)H\(_{21}\)N\(_3\)O\(_5\)) according to the labeled amount, add 2 mL of a mixture of acetonitrile and ethyl acetate (3:1), shake, and centrifuge. Pipet 4 mL of the supernatant liquid, add exactly 1 mL of the internal standard solution, add the mixture of water and acetonitrile (7:3) to make 10 mL, and use this solution as the standard solution. Separately, weigh accurately about 26 mg of Cilazapril for assay (separately determine the water \(<\, 2.4\text{mg}\) in the same manner as Cilazapril Hydrate), and dissolve in the mixture of water and acetonitrile (7:3) to make 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 100 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography (2.02). Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cilazapril Tablets add 5 mL of a mixture of water and acetonitrile (7:3), shake well until disintegration, add the mixture of water and acetonitrile (7:3) to make exactly 10 mL, and shake for 2 hours, and immediately examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the sample and standard solutions are dark brown and they show the same \(R_f\) value.

**Uniformity of dosage units**
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Internal standard solution—A solution of dimethyl phthalate in a mixture of water and acetonitrile (7:3) (1 in 12,500).

**Operating conditions**
Proceed as directed in the operating conditions in the Assay.

**System suitability**
System performance: When the procedure is run with 100 \(\mu\)L of the standard solution under the above conditions, cilazapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 100 \(\mu\)L of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of cilazapril to that of the internal standard is not more than 2.0%.

**Dissolution**
When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cilazapril Tablets is not less than 85%.

Start the test with 1 tablet of Cilazapril Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \(\mu\)m. Establish the first 10 mL of the filtrate, pipet \(V\, mL\) of the subsequent filtrate, and add water to make exactly \(V + 10\, mL\) so that each mL contains...
about 0.28 µg of cilazapril (C₂₂H₃₁N₃O₅) according to the labeled amount. Pipet 10 mL of the solution, add exactly 5 mL of acetonitrile, and use this solution as the sample solution. Separately, weigh accurately about 29 mg of cilazapril for assay (separately determine the water in the same manner as Cilazapril Hydrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of the solution, add water to make exactly 100 mL. Then, pipet 2 mL of this solution, add water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of acetonitrile, and use this solution as the standard solution. Perform the test with exactly 100 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of cilazapril to that of the internal standard.

$$M_5: \text{Amount (mg) of cilazapril for assay, calculated on the anhydrous basis}$$

C: Labeled amount (mg) of cilazapril (C₂₂H₃₁N₃O₅) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To a solution consisting of 180 mL of tetrahydrofuran for liquid chromatography, 120 mL of acetonitrile for liquid chromatography and 3 mL of triethylamine add water to make 1000 mL, and adjust the pH to 2.5 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of cilazapril is about 10 minutes.

System suitability—

System performance: When the procedure is run with 50 µL of the standard solution under the above conditions, cilazapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 50 µL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of cilazapril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cilostazol

シロスタゾール

$$C_{20}H_{27}N_5O_2: 369.46$$

6-[4-{1-Cyclohexyl-1H-tetrazol-5-yl]butyloxy}-3,4-dihydroquinolin-2(1H)-one [73963-72-1]

Cilostazol, when dried, contains not less than 98.5% and not more than 101.5% of C₂₀H₂₇N₅O₂.

Description Cilostazol occurs as white to pale yellowish white, crystals or crystalline powder.
It is slightly soluble in methanol, in ethanol (99.5) and in acetonitrile, and practically insoluble in water.

**Identification (1)** Determine the absorption spectrum of a solution of Cilostazol in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 224, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cilostazol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cilostazol as directed in the potassium bromide disk method under Infrared Spectrophotometry 225, and compare the spectrum with the Reference Spectrum or the spectrum of Cilostazol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** 260 - 162°C

**Purity (1)** Heavy metals 207—Proceed with 2.0 g of Cilostazol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 25 mg of Cilostazol in 25 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 100 mL. Pipet 10 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 207 according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cilostazol obtained with the sample solution is not larger than 7/10 times the peak area of cilostazol with the standard solution, and the total area of the peaks other than the peak of cilostazol is not larger than 1.2 times the peak area of cilostazol with the standard solution.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol, ethyl acetate and methanol (10:7:3).

Flow rate: Adjust the flow rate so that the retention time of cilostazol is about 7 minutes.

Time span of measurement: About 3 times as long as the retention time of cilostazol beginning after the solvent peak.

**System suitability—**
Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of cilostazol obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: Pipet 1 mL of the sample solution, add 1 mL of a solution prepared by dissolving 5 mg of 3,4-dihydro-6-hydroxy-2(1H)-quinolinone in 10 mL of acetonitrile and acetonitrile to make exactly 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, 3,4-dihydro-6-hydroxy-2(1H)-quinolinone and cilostazol are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilostazol is not more than 2.0%.

**Loss on drying** 247—Not more than 0.1% (1 g, 105°C, 2 hours).

**Residue on ignition** 247—Not more than 0.1% (1 g).

**Assay** Weigh accurately about 50 mg each of Cilostazol and Cilostazol RS, previously dried, dissolve each in a suitable amount of methanol, add exactly 5 mL of the internal standard solution and methanol to make 50 mL. To 1 mL each of these solutions add methanol to make 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 207 according to the following conditions, and calculate the ratios, Qf and Qs, of the peak area of cilostazol to that of the internal standard.

Amount (mg) of C20H27N5O2 = M5 × Qf/Qs

M5: Amount (mg) of Cilostazol RS

**Internal standard solution**—A solution of benzophenone in methanol (1 in 250).

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and methanol (10:9:1).

Flow rate: Adjust the flow rate so that the retention time of cilostazol is about 9 minutes.

**System suitability—**
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cilostazol and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilostazol to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

### Cilostazol Tablets

シロスタゾール錠

Cilostazol Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cilostazol (C20H27N5O2: 369.46).
**Method of preparation** Prepare as directed under Tablets, with Cilostazol.

**Identification** Mix well an amount of powdered Cilostazol Tablets, equivalent to 50 mg of Cilostazol according to the labeled amount, with 10 mL of acetone, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of Cilostazol RS in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 6 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, acetonitrile, methanol and formic acid (75:25:5:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate: the principal spot with the sample solution and the spot with the standard solution are orange in color and have the same Rf value.

**Uniformity of dosage units**<6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cilostazol Tablets add 2 mL of water to disintegrate the tablet, add the internal standard solution exactly 5 mL for a 50-mg tablet and exactly 10 mL for a 100-mg tablet, and add methanol to make 50 mL. Shake for 10 minutes for the 50-mg tablet and for 20 minutes for the 100-mg tablet. To 1 mL of the solution add methanol to make 10 mL for the 50-mg tablet and 20 mL for the 100-mg tablet, filter through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of cilostazol (C}_{20}\text{H}_{27}\text{N}_{5}\text{O}_{2}) = M_s \times Q_t / Q_s \times C / 50
\]

\[M_s: \text{Amount (mg) of Cilostazol RS}
\]

\[C: \text{Labeled amount (mg) of cilostazol (C}_{20}\text{H}_{27}\text{N}_{5}\text{O}_{2}) \text{ in 1 tablet}
\]

**Internal standard solution**—A solution of benzophenone in methanol (1 in 250).

**Dissolution**<6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution of sodium lauryl sulfate (3 in 1000) as the dissolution medium, the dissolution rates of a 50-mg tablet and a 100-mg tablet in 45 minutes and a 100-mg tablet in 60 minutes are not less than 75% and not less than 70%, respectively.

Start the test with 1 tablet of Cilostazol Tablets, withdraw not less than 20 mL of the medium at the specified time after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the dissolution medium to make exactly V mL so that each mL contains about 5.6 μg of Cilostazol (C_{20}H_{27}N_{5}O_{2}) according to the labeled amount, and use this solution as the standard solution. Separately, weigh accurately about 28 mg of Cilostazol RS, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_s and A_c, of the sample solution and standard solution at 257 nm as directed under Ultraviolet-visible Spectrophotometry <2.20> using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of cilostazol (C_{20}H_{27}N_{5}O_{2})

\[M_s = M_s \times A_s / A_c \times V / V \times 1 / C \times 18 \]

\[M_s: \text{Amount (mg) of Cilostazol RS}
\]

\[C: \text{Labeled amount (mg) of cilostazol (C}_{20}\text{H}_{27}\text{N}_{5}\text{O}_{2}) \text{ in 1 tablet}
\]

**Assay** Weigh accurately not less than 20 Cilostazol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of Cilostazol (C_{20}H_{27}N_{5}O_{2}), add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and shake well for 10 minutes. To 1 mL of this solution add methanol to make 10 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 50 mg of Cilostazol RS, previously dried at 105°C for 2 hours, dissolve in a suitable amount of methanol, and add exactly 5 mL of the internal standard solution, and add methanol to make 50 mL. To 1 mL of this solution add methanol to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q_t and Q_s, of the peak area of cilostazol to that of the internal standard.

\[\text{Amount (mg) of cilostazol (C}_{20}\text{H}_{27}\text{N}_{5}\text{O}_{2}) = M_s \times Q_t / Q_s \]

\[M_s: \text{Amount (mg) of Cilostazol RS}
\]

**Internal standard solution**—A solution of benzophenone in methanol (1 in 250).

**Operating conditions**—
Proceed as directed in the operating conditions in the Assay under Cilostazol.

**System suitability**—
System performance: Proceed as directed in the system suitability in the Assay under Cilostazol.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilostazol to that of the internal standard is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

**Cimetidine**

シメチジン

\[\text{C}_{10}\text{H}_{16}\text{N}_{6}\text{S}: 252.34 \]

2-Cyano-1-methyl-3-[2-[(5-methyl-1H-imidazol-4-yl)methylsulfanyl]ethyl]guanidine [51481-61-9]

Cimetidine, when dried, contains not less than 99.0% of C_{10}H_{16}N_{6}S.

**Description** Cimetidine occurs as a white crystalline pow-
**Cinoxacin**

**Identification (1)** To 0.1 mL of a solution of Cimetidine in ethanol (95) (1 in 100) add 5 mL of citric acid-acetic anhydride TS, and heat in a water bath for 15 minutes: a red-purple color develops.

(2) Determine the infrared absorption spectrum of Cimetidine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**pH** $<2.54>$ Dissolve 0.5 g of Cimetidine in 50 mL of freshly boiled and cooled water, shake for 5 minutes and filter: the pH of the filtrate is between 9.0 and 10.5.

**Melting point** $<2.60>$ 140 – 144°C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Cimetidine in 10 mL of methanol: the solution is clear and colorless to pale yellow in color.

(2) Heavy metals $<1.07>$—Proceed with 2.0 g of Cimetidine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic $<1.11>$—Dissolve 1.0 g of Cimetidine in 5 mL of dilute hydrochloric acid, and perform the test with this solution (not more than 2 ppm).

(4) Related substances—Dissolve 0.5 g of Cimetidine in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$, spot 4 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (14:4:1) to a distance of about 15 cm, air-dry the plate, and then dry at 80°C for 30 minutes. Allow the plate to stand in iodine vapor for 45 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** $<2.41>$ Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** $<2.44>$ Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.24 g of Cimetidine, previously dried, dissolve in 75 mL of acetic acid (100), and titrate $<2.50>$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 25.23 mg of C$_{10}$H$_{10}$N$_{2}$S

**Containers and storage** Containers—Well-closed containers. Storage—Light-resistant.

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**Cinoxacin**

シノキサシン

C$_{12}$H$_{10}$N$_{2}$O$_{5}$: 262.22

5-Ethyl-8-oxo-5,8-dihydro[1,3]dioxolo[4,5-g]cinnoline-7-carboxylic acid

Cinoxacin, when dried, contains not less than 98.0% and not more than 101.0% of C$_{12}$H$_{10}$N$_{2}$O$_{5}$.

**Description** Cinoxacin occurs as a white to pale yellow crystalline powder. It is odorless or has a slight, characteristic odor. It has a bitter taste.

It is slightly soluble in N,N-dimethylformamide and in acetone, very slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in sodium hydroxide TS.

**Purity (1)** Sulfate $<1.14>$—Dissolve 0.20 g of Cinoxacin in 10 mL of dilute sodium hydroxide TS, and add water to make 100 mL. To 1 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $<2.24>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave lengths.

(2) Determine the infrared absorption spectrum of Cinoxacin as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Related substances**—Dissolve 10 mg of Cinoxacin in 10 mL of dilute sodium hydroxide TS, add 20 mL of 0.1 mol/L hydrochloric acid TS, shake, filter, and add water to the filtrate to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.005 mol/L sulfuric acid VS by adding 10 mL of dilute sodium hydroxide TS, 20 mL of 0.1 mol/L hydrochloric acid TS, and water to make 50 mL (not more than 0.048%).

(2) Heavy metals $<1.07>$—Proceed with 1.0 g of Cinoxacin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Cinoxacin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, water and ammonia solution (28) (14:4:1) to a distance of...
about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

(4) Residual solvent—Being specified separately.

**Loss on drying** 2.44
Not more than 0.5% (1 g, 105°C, 1 hour).

**Residue on ignition** 2.44
Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.4 g of Cinoxacin, previously dried, add 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and dissolve by warming. After cooling, titrate with 0.1 mol/L perchloric acid VS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24>, and determine the absorbances, \(A_T\) and \(A_S\), at 351 nm.

\[
\text{Amount (mg) of cinoxacin (C}_{12}\text{H}_{10}\text{N}_{2}\text{O}_{5}) = M_S \times A_T/A_S \times V/V_0
\]

\(M_S\): Amount (mg) of cinoxacin for assay
\(C\): Labeled amount (mg) of cinoxacin (C\(_{12}\)H\(_{10}\)N\(_2\)O\(_5\)) in 1 capsule

**Dissolution** 6.1D
When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd solution for dissolution test as the dissolution medium, the dissolution rate in 90 minutes of Cinoxacin Capsules is not less than 70%.

Start the test with 1 capsule of Cinoxacin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add the dissolution medium to make exactly \(V\) mL so that each mL contains about 11 μg of cinoxacin (C\(_{12}\)H\(_{10}\)N\(_2\)O\(_5\)) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of cinoxacin for assay, previously dried at 105°C for 1 hour, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24>, and determine the absorbances, \(A_T\) and \(A_S\), at 351 nm.

\[
\text{Dissolution rate (%)} = \frac{M_S \times A_T/A_S \times V/V_0}{C} 	imes 100
\]

\(M_S\): Amount (mg) of cinoxacin for assay
\(C\): Labeled amount (mg) of cinoxacin (C\(_{12}\)H\(_{10}\)N\(_2\)O\(_5\)) in 1 capsule

**Assay** Weigh accurately the mass of not less than 20 Cinoxacin Capsules, take out the contents, and powder. Wash the capsule shells with a small amount of diethyl ether, allow to stand at room temperature to vaporize the diethyl ether, weigh accurately the mass of the capsule shells, and calculate the mass of the contents. Weigh accurately a portion of the powder, equivalent to about 50 mg of cinoxacin (C\(_{12}\)H\(_{10}\)N\(_2\)O\(_5\)), add 10 mL of dilute sodium hydroxide TS, shake, add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 1 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of cinoxacin for assay, previously dried at 105°C for 1 hour, dissolve in 10 mL of dilute sodium hydroxide TS, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24>, and determine the absorbances, \(A_T\) and \(A_S\), at 351 nm.
Cisplatin

シスプラチン

\[ \text{C}_{2}\text{H}_{6}\text{N}_{2}\text{Pt}: 300.05 \]

\[(\text{SP}-4-2)-\text{Diamminedichloroplatinum} \]

[15663-27-1]

Cisplatin, when dried, contains not less than 98.0% and not more than 102.0% of C\textsubscript{2}H\textsubscript{6}N\textsubscript{2}Pt.

**Description**

Cisplatin occurs as a yellow crystalline powder.

It is sparingly soluble in \( N, N \)-dimethylformamide, slightly soluble in water, and practically insoluble in ethanol (99.5).

**Identification**

1. To 5 mL of a solution of Cisplatin (1 in 2000) add 2 to 3 drops of a solution of tin (II) chloride dihydrate (1 in 100); a brown precipitate is formed.

2. Determine the absorption spectrum of a solution of Cisplatin in a solution of sodium chloride in 0.01 mol/L hydromorphic acid TS (9 in 1000) (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cisplatin RS prepared in the same manner as the sample solution; both spectra exhibit similar intensities of absorption at the same wavelengths.

3. Determine the infrared absorption spectrum of Cisplatin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cisplatin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

4. A solution of Cisplatin (1 in 2000) responds to the Qualitative Tests <1.09> (1) for chloride.

**Purity**

Ammonium amminetrichloroplatinate—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Cisplatin in a solution of sodium chloride (9 in 1000) to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of ammonium amminetrichloroplatinate for liquid chromatography, previously dried at 80°C for 3 hours, in the solution of sodium chloride (9 in 1000) to make exactly 200 mL. Pipet 2 mL of this solution, add the solution of sodium chloride (9 in 1000) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 40 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak area of ammonium amminetrichloroplatinate and the peak area of Cisplatin by the automatic integration method: the peak area from the sample solution is not larger than that from the standard solution.

### Operating conditions—

**Detector**

An ultraviolet absorption photometer (wave-length: 209 nm).

**Column**

A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with silica gel for liquid chromatography having quaternary ammonium groups (10 \( \mu \)m in particle diameter).

**Column temperature**

A constant temperature of about 25°C.

**Mobile phase**

A solution of ammonium sulfate (1 in 1000).

**Flow rate**

Adjust the flow rate so that the retention time of ammonium amminetrichloroplatinate is about 8 minutes.

### System suitability—

**System performance**

When the procedure is run with 40 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ammonium amminetrichloroplatinate are not less than 1500 and not more than 2.0, respectively.

**System repeatability**

When the test is repeated 6 times with 40 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ammonium amminetrichloroplatinate is not more than 3.0%.

**Loss on drying**

Not more than 0.1% (1 g, 105°C, 4 hours).

**Assay**

Conduct this procedure using light-resistant vessels. Weigh accurately about 25 mg each of Cisplatin and Cisplatin RS, previously dried, dissolve in \( N, N \)-dimethylformamide to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 40 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of cisplatin by the automatic integration method.

\[
M_S = \text{Amount (mg) of Cisplatin RS} \\
M_C = \text{Amount (mg) of Cisplatin} \\
M = M_S \times A_T/A_S
\]

**Operating conditions—**

**Detector**

An ultraviolet absorption photometer (wave-length: 310 nm).

**Column**

A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

**Column temperature**

A constant temperature of about 25°C.

**Mobile phase**

A mixture of ethyl acetate, methanol, water and \( N, N \)-dimethylformamide (25:16:5:5).

**Flow rate**

Adjust the flow rate so that the retention time of cisplatin is about 4 minutes.

**System suitability—**

**System performance**

When the procedure is run with 40 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cisplatin are not less than 3000 and not more than 2.0, respectively.

**System repeatability**

When the test is repeated 6 times with 40 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cisplatin is not more than 1.0%.
Containers and storage  Containers—Tight containers.

Anhydrous Citric Acid

無水クエン酸

C₆H₈O₇: 192.12
2-Hydroxypropane-1,2,3-tricarboxylic acid
[77-92-9]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbol (*) •).

Anhydrous Citric Acid contains not less than 99.5% and not more than 100.5% of C₆H₈O₇, calculated on the anhydrous basis.

*Description*  Anhydrous Citric Acid occurs as colorless crystals, white granules or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95%).

*Identification*  Determine the infrared absorption spectrum of Anhydrous Citric Acid, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity  (1) Clarity and color of solution—Dissolve 2.0 g of Anhydrous Citric Acid in water to make 10 mL: the solution is clear and has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To 1.5 mL of Cobalt (II) Chloride CS and 6.0 mL of Iron (III) Chloride CS add water to make 1000 mL.

Control solution (2): To 0.15 mL of Cobalt (II) Chloride CS, 7.2 mL of Iron (III) Chloride CS and 0.15 mL of Copper (II) Sulfate CS add water to make 1000 mL.

Control solution (3): To 2.5 mL of Cobalt (II) Chloride CS, 6.0 mL of Iron (III) Chloride CS and 1.0 mL of Copper (II) Sulfate CS add water to make 1000 mL.

(2) Sulfates—Dissolve 2.0 g of Anhydrous Citric Acid in water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in 1000 mL. Pipet 5 mL of this solution, and add 5 mL of water to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of potassium hexacyanoferrate (III) (1 in 20), mix, and add lead solution (not more than 10 ppm). •

(5) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Anhydrous Citric Acid, provided that the solution is heated at 90°C for 1 hour and then cool quickly: the solution has no more color than Matching Fluid K.

Water <2.48>  Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition <2.48>  Not more than 0.1% (1 g).

Assay  Weigh accurately about 0.55 g of Anhydrous Citric Acid, dissolve in 50 mL of water, and titrate with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS = 64.04 mg of C₆H₈O₇.

*Containers and storage*  Containers—Tight containers.

C₆H₈O₇·H₂O: 210.14
2-Hydroxypropane-1,2,3-tricarboxylic acid monohydrate
[59469-29-7]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbol (•). Citric Acid Hydrate contains not less than 99.5% and not more than 100.5% of anhydrous citric acid (C₆H₈O₇: 192.12), calculated on the anhydrous basis.

*Description*  Citric Acid Hydrate occurs as colorless crystals, white granules or crystalline powder.

It is soluble in water, and freely soluble in ethanol (95).

It is efflorescent in dry air.

*Identification*  Determine the infrared absorption spectrum of Citric Acid Hydrate, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit...
similar intensities of absorption at the same wave numbers.

**Purity** (1) Clarity and color of solution—Dissolve 2.0 g of Citric Acid Hydrate in water to make 10 mL; the solution is clear and has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To 1.5 mL of Cobalt (II) Chloride CS and 6.0 mL of Iron (III) Chloride CS add water to make 1000 mL.

Control solution (2): To 0.15 mL of Cobalt (II) Chloride CS, 7.2 mL of Iron (III) Chloride CS and 0.15 mL of Copper (II) Sulfate CS add water to make 1000 mL.

Control solution (3): To 2.5 mL of Cobalt (II) Chloride CS, 6.0 mL of Iron (III) Chloride CS and 1.0 mL of Copper (II) Sulfate CS add water to make 1000 mL.

(2) Sulfates—Dissolve 2.0 g of Citric Acid Hydrate in water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as above using this solution instead of the sample solution.

(3) Oxalic acid—Dissolve 0.80 g of Citric Acid Hydrate in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of zinc, and boil for 1 minute. After allowing to stand for 2 minutes, take the supernatant liquid, add 0.25 mL of a solution of phenylhydrazinium hydrochloride (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution prepared at the same time.

Control solution: To 4 mL of a solution of oxalic acid dihydrate (1 in 10,000) add 3 mL of hydrochloric acid and 1 g of zinc, and proceed in the same manner as the test solution.

(4) Heavy metals <1.07—Proceed with 2.0 g of Citric Acid Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Readily carbonizable substances <1.15—Perform the test with 0.5 g of Citric Acid Hydrate, provided that the solution is heated at 90°C for 1 hour and then cool quickly: the solution has no more color than Matching Fluid K.

**Water** <2.48 Not less than 7.5% and not more than 9.0% (0.5 g, volumetric titration, direct titration).

**Residue on ignition** <2.44 Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.55 g of Citric Acid Hydrate, dissolve in 50 mL of water, and titrate <2.50 with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS = 64.04 mg of C6H8O7

*Containers and storage* Containers—Tight containers.

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**Clarithromycin**

クラリスロマイシン

C38H69NO13: 747.95 (2R,3S,4S,5R,6R,8R,10R,11S,12S,13R)-5-(3,4,6-Trideoxy-3-dimethylamino-β-D-xylo-hexopyanosyl)-3-(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)-11,12-dihydroxy-6-methoxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide [81103-11-9]

Clarithromycin is a derivative of erythromycin. It contains not less than 950 μg (potency) and not more than 1050 μg (potency) per mg, calculated on the anhydrous basis. The potency of Clarithromycin is expressed as mass (potency) of clarithromycin (C38H69NO13).

**Description** Clarithromycin occurs as a white crystalline powder and has a bitter taste. It is soluble in acetone and in chloroform, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

**Identification** (1) To 5 mg of Clarithromycin add 2 mL of sulfuric acid, and shake gently: a red-brown color develops.

(2) Dissolve 3 mg of Clarithromycin in 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color develops and changes immediately to red to deep purple.

(3) Determine the infrared absorption spectra of Clarithromycin and Clarithromycin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Dissolve 10 mg each of Clarithromycin and Clarithromycin RS in 4 mL of chloroform, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of chloroform, methanol and ammonia water (28) (100:5:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat at 105°C for 10 minutes: the principal spot from the sample solution and the spot from the standard solution show a dark purple color and have the same Rf value.
Optical rotation $< 2.4^\circ$  $[\alpha]_D^{25} < -87^\circ$ to $-97^\circ$ (0.25 g calculated on the anhydrous basis, chloroform, 25 mL, 100 mm).

Melting point 2.60°C  220 - 227°C

Purity (1)  Heavy metals $< 1.07$—Proceed with 2.0 g of Clarithromycin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2)  Arsenic—Prepare the test solution with 1.0 g of Clarithromycin according to Method 3, and perform the test (not more than 2 ppm).

(3)  Related substances—Weigh accurately about 0.1 g of Clarithromycin, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Clarithromycin RS, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography $< 2.01 >$ according to the following conditions, and determine the peak area by the automatic integration method: the amount of each related substance calculated on the anhydrous basis is not more than 2.0%, and the total of them is not more than 5.0%. Exclude any peak with an area of less than 0.05%.

Amount (%) of each related substance calculated on the anhydrous basis

\[ M_S / M_T \times A_T / A_S \times 100 \]

Total amount (%) of the related substances calculated on the anhydrous basis

\[ M_S / M_T \times \Sigma A_T / A_S \times 100 \]

$M_S$: Amount (mg) of Clarithromycin RS

$M_T$: Amount (mg) of the sample, calculated on the anhydrous basis

$A_T$: Peak area of clarithromycin obtained with the standard solution

$A_S$: Peak area of each related substance obtained with the sample solution

\[ \Sigma A_T \]: Total area of the peaks other than clarithromycin obtained with the sample solution

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of the main peak after 2 minutes of sample injection.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL, and use this solution as the solution for system suitability test. Confirm that when the procedure is run with 10 µL of the solution for system suitability test, the peak area of clarithromycin is equivalent to 14 to 26% of that obtained with 10 µL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 µL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of clarithromycin is not more than 3.0%.

Water $< 2.4^\circ$  Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition $< 2.4^\circ$  Not more than 0.1% (2 g).

Assay  Weigh accurately a sufficient amount of Clarithromycin and Clarithromycin RS, equivalent to about 0.1 g (potency), and dissolve in the mobile phase to make exactly 20 mL. Pipet 2 mL each of these solutions, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography $< 2.01 >$ according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of clarithromycin to that of the internal standard.

Amount [µg (potency)] of $C_{38}H_{60}NO_{13}$

\[ M_S = M_s \times Q_T / Q_S \times 1000 \]

$M_S$: Amount [mg (potency)] of Clarithromycin RS

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilsilica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogenphosphate TS (1 in 3) and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of clarithromycin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, clarithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clarithromycin to that of the internal standard is not more than 2.0%.

Containers and storage  Containers—Well-closed containers.

Clarithromycin Tablets

クラリスロマイシン錠

Clarithromycin Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of clarithromycin ($C_{38}H_{60}NO_{13}$: 747.95).

Method of preparation  Prepare as directed under Tablets, with Clarithromycin.

Identification  Shake a quantity of pulverized Clarithromycin Tablets, equivalent to 60 mg (potency) of Clarithromycin
according to the labeled amount, with 40 mL of acetone for 10 minutes, and centrifuge at 4000 rpm for 5 minutes. Evaporate 30 mL of the supernatant liquid, and determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25Y; it exhibits absorption at the wave numbers of about 2980 cm⁻¹, 2940 cm⁻¹, 1734 cm⁻¹, 1693 cm⁻¹, 1459 cm⁻¹, 1379 cm⁻¹ and 1171 cm⁻¹.

**Uniformity of dosage units** 6.02  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Clarithromycin Tablets add exactly V/20 mL of the internal standard solution (1), then add the mobile phase so that each mL contains about 5 mg (potency) of clarithromycin (C₃₈H₆₉NO₁₃) to make V mL, and disperse to fine particles with the aid of ultrasonic waves for 20 minutes while occasional vigorous shaking. Centrifuge this solution at 4000 rpm for 15 minutes, and filter the supernatant liquid through a membrane filter with a pore size of not more than 0.45 μm. Then, proceed as directed in the Assay.

Amount [mg (potency)] of clarithromycin (C₃₈H₆₉NO₁₃) = Mₜ × Q₁/Qₕ × V/10

Mₜ: Amount [mg (potency)] of Clarithromycin RS

**Internal standard solution (1)—** A solution of butyl parahydroxybenzoate in the mobile phase (1 in 1000).

**Internal standard solution (2)—** To exactly 1 mL of the internal standard solution (1) add the mobile phase to make exactly 20 mL.

**Dissolution 6.10** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 as the dissolution medium, the dissolution rates in 30 minutes of a 50-mg tablet and a 200-mg tablet are not less than 80% and not less than 75%, respectively.

Start the test with 1 tablet of Clarithromycin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V mL so that each mL contains about 28 μg (potency) of Clarithromycin according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Clarithromycin RS, equivalent to about 28 mg (potency), and disperse in acetonitrile for liquid chromatography to make exactly 100 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q₁ and Qₕ, of the peak areas of clarithromycin to that of the internal standard.

Amount [mg (potency)] of clarithromycin (C₃₈H₆₉NO₁₃) = Mₜ × Q₁/Qₕ × V/10

Mₜ: Amount [mg (potency)] of Clarithromycin RS

**Operating conditions—**

Produce as directed in the operating conditions in the Assay.

**System suitability—**

**System performance:** When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clarithromycin are not less than 3000 and not more than 2.0, respectively.

**System repeatability:** When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clarithromycin is not more than 2.0%.

**Assay** To not less than 5 Clarithromycin Tablets add diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 3) so that each mL contains about 8 mg (potency) of clarithromycin (C₃₈H₆₉NO₁₃), disperse to fine particles with the aid of ultrasonic waves, add exactly 1 mL of the internal standard solution (1) per 100 mg (potency) of clarithromycin according to the labeled amount, then add acetonitrile for liquid chromatography so that each mL contains about 5 mg (potency) of clarithromycin (C₃₈H₆₉NO₁₃), and disperse to fine particles with the aid of ultrasonic waves for 10 minutes while occasional vigorous shaking. Centrifuge of this solution at 4000 rpm for 15 minutes, and filter the supernatant liquid through a membrane filter with a pore size of not more than 0.45 μm. Discard the first 3 mL of the filtrate, to 2 mL of the subsequent filtrate add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Clarithromycin RS, equivalent to about 50 mg (potency), and dissolve in the mobile phase to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution (2) and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q₁ and Qₕ, of the peak area of clarithromycin to that of the internal standard.

Amount [mg (potency)] of clarithromycin (C₃₈H₆₉NO₁₃) = Mₜ × Q₁/Qₕ × V/10

Mₜ: Amount [mg (potency)] of Clarithromycin RS

**Internal standard solution (1)—** A solution of butyl parahydroxybenzoate in the mobile phase (1 in 1000).

**Internal standard solution (2)—** To exactly 1 mL of the internal standard solution (1) add the mobile phase to make exactly 20 mL.

**Operating conditions—**

**Dissolution 6.10** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 as the dissolution medium, the dissolution rate in 30 minutes of a 50-mg tablet and a 200-mg tablet are not less than 80% and not less than 75%, respectively.

Start the test with 1 tablet of Clarithromycin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V mL so that each mL contains about 28 μg (potency) of Clarithromycin according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Clarithromycin RS, equivalent to about 28 mg (potency), and disperse in acetonitrile for liquid chromatography to make exactly 100 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the peak areas, A₁ and Aₕ, of clarithromycin.

Dissolution rate (%) with respect to the labeled amount of clarithromycin (C₃₈H₆₉NO₁₃) = Mₜ × A₁/Aₕ × V/V × 1/C × 90

Mₜ: Amount [mg (potency)] of Clarithromycin RS

C: Labeled amount [mg (potency)] of clarithromycin (C₃₈H₆₉NO₁₃) in 1 tablet

**Operating conditions—**

Produce as directed in the operating conditions in the Assay.
Clebopride Malate

**Chemical Name:** 4-Amino-N-(1-benzylpiperidin-4-yl)-5-chloro-2-methoxybenzamido mono-(2RS)-malate

**CAS Number:** 57645-91-7

**Molar Mass:** 507.96

**Description:** Clebopride Malate occurs as a white crystalline powder. It is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (99.5).

A solution of Clebopride Malate in methanol (1 in 25) shows no optical rotation.

**Identification (1):** Determine the absorption spectrum of a solution of Clebopride Malate in methanol (1 in 80000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Identification (2):** Determine the infrared absorption spectrum of Clebopride Malate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Assay:** Weigh accurately about 0.5 g of Clebopride Malate, previously dried, dissolve in 20 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS contains 50.80 mg of C_{20}H_{24}ClN_{3}O_{2}.C_{4}H_{6}O_{5}.

**Containers and Storage:** Containers—Well-closed containers.

**System Suitability:**

- **System performance:** When the procedure is run with 10 μL of the standard solution under the above operating conditions, clarithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.
- **System repeatability:** When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of clarithromycin to that of the internal standard is not more than 2.0%.

**Loss on Drying (<2.4>)**

Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on Ignition (<2.4>)**

Not more than 0.1% (1 g).

**Heavy Metals (<1.07>)**

Proceed with 2.0 g of Clebopride Malate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Related Substances**

Dissolve 0.10 g of Clebopride Malate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 0.2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of clebopride obtained from the sample solution is not larger than the peak area of clebopride from the standard solution.

**Operating Conditions:**

- **Detector:** An ultraviolet absorption photometer (wavelength: 240 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecysilane silanized silica gel for liquid chromatography (7 μm in particle diameter).
- **Column temperature:** A constant temperature of about 25°C.
- **Mobile phase:** Dissolve 3.85 g of ammonium acetate in water to make 500 mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. To 400 mL of the filtrate add 600 mL of methanol.
- **Flow rate:** Adjust the flow rate so that the retention time of clebopride is about 15 minutes.
- **Time span of measurement:** About 2 times as long as the retention time of clebopride.

**System Suitability:**

Test for required detectability: Pipet 10 mL of the standard solution, and add water to make exactly 100 mL. Confirm that the peak area of clebopride obtained from 10 μL of this solution is equivalent to 7 to 13% of that of clebopride from 10 μL of the standard solution.

System performance: Dissolve 30 mg Clebopride Malate and 5 mg of propyl parahydroxybenzoate in the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, propyl parahydroxybenzoate and clebopride are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: The test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clebopride is not more than 2.5%.

**Residual Solvent**

- Being specified separately.
Clemastine Fumarate

克伦斯汀布马酸盐

C₂₁H₂₆ClNO.C₄H₄O₄: 459.96

(2R)-2-[2-[(1R)-1-(4-Chlorophenyl)-1-phenylethoxy]ethyl]-1-methylpyrrolidine monofumarate

[Clemastine Fumarate occurs as a white, crystalline powder. It is odorless. It is sparingly soluble in methanol and in acetic acid (100), slightly soluble in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

**Identification**  (1) To 5 mg of Clemastine Fumarate add 5 mL of sulfuric acid, and shake to dissolve: a yellow color develops. Slowly drop this solution into 10 mL of water: the yellow color immediately disappears.

(2) To 0.01 g of Clemastine Fumarate add 1 mL of fuming nitric acid, and evaporate on a water bath to dryness. Then add 2 mL of diluted hydrochloric acid (1 in 2) and 0.2 g of zinc powder, heat for 10 minutes on a water bath, cool, and filter. Add 20 mL of water to the filtrate. The solution responds to the Qualitative Tests <1.00> for primary aromatic amines.

(3) To 5 mL of a solution of Clemastine Fumarate (1 in 50,000), add 5 mL of 4-dimethylaminobenzaldehyde TS, and warm for 10 minutes: a red-purple color develops.

(4) Perform the test with Clemastine Fumarate as directed under Flame Coloration Test <1.04> (2): a green color appears.

(5) Dissolve 0.04 g of Clemastine Fumarate and 0.01 g of fumaric acid for thin-layer chromatography in 2 mL each of a mixture of ethanol (95) and water (4:1) by gentle warming, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol to make exactly 250 mL, and use this solution as the standard solution (1). Pipet 5 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (90:10:1) to a distance of about 10 cm, and air-dry the plate. After spraying evenly Dragendorff’s TS on the plate, immediately spray evenly hydrogen peroxide TS: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and not more than 2 spots from the sample solution are more intense than the spot from the standard solution (2).

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.4 g of Clemastine Fumarate, previously dried, dissolved in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 46.00 mg of C₂₁H₂₆ClNO.C₄H₄O₄

**Containers and storage** Containers—Tight containers.

Clindamycin Hydrochloride

クリンダマイシン塩酸塩

C₁₈H₁₇ClN₅O₆S.HCl: 461.44

Methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-propylypyrrolidine-2-carboxamido]-1-thio-L-threo-α-D-galacto-octopyranoside monohydrochloride

[21662-39-5]

Clindamycin Hydrochloride is the hydrochloride of a derivative of lincomycin.
It contains not less than 838 μg (potency) and not more than 940 μg (potency) per mg, calculated on the anhydrous basis. The potency of Clindamycin Hydrochloride is expressed as mass (potency) of clindamycin (C18H33ClN2O5S: 424.98).

**Description** Clindamycin Hydrochloride occurs as white to grayish white, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (99.5).

**Identification** (1) Determine the infrared absorption spectrum of Clindamycin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Clindamycin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Clindamycin Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

**Optical rotation** <2.49> [α]D: 135° to 150° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

**Purity** Heavy metals <1.07>—Proceed with 2.0 g of Clindamycin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Water** <2.48> Not more than 6.0% (0.3 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Clindamycin Hydrochloride and an amount of Clindamycin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve each in the mobile phase to make exactly 20 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A1 and A5, of clindamycin in each solution.

\[ \text{Amount} [\mu g \text{ (potency)}] \text{ of clindamycin (} C_{18}H_{33}ClN_{2}O_{5}S) = M_s \times A_1/A_5 \times 1000 \]

\[ M_s: \text{Amount [mg (potency)] of Clindamycin Hydrochloride RS} \]

**Operating conditions**

- **Detector:** An ultraviolet absorption photometer (wavelength: 210 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature:** A constant temperature of about 25°C.
- **Mobile phase:** To 0.05 mol/L potassium dihydrogen phosphate TS add 8 mol/L potassium hydroxide TS to adjust the pH to 7.5. To 550 mL of this solution add 450 mL of acetonitrile for liquid chromatography.
- **Flow rate:** Adjust the flow rate so that the retention time of clindamycin is about 10 minutes.

**System suitability**—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clindamycin are not less than 6000 and not more than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clindamycin is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

### Clindamycin Hydrochloride Capsules

Clindamycin Hydrochloride Capsules contain not less than 93.0% and not more than 107.0% of the labeled potency of clindamycin (C18H33ClN2O5S: 424.98).

**Method of preparation** Prepare as directed under Capsules, with Clindamycin Hydrochloride.

**Identification** To an amount of the contents of Clindamycin Hydrochloride Capsules, equivalent to 10 mg (potency) of Clindamycin Hydrochloride, add 2 mL of methanol, shake well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Clindamycin Hydrochloride RS in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 10 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, toluene and ammonia solution (28) (140:60:3) to a distance of about 12 cm, and air-dry the plate. Spray evenly a mixture of 500 mL of a solution of L-tartaric acid (1 in 5) and 50 mL of bismuth subnitrate TS on the plate: the Rf values of the principal spot with the sample solution and the spot with the standard solution are not different each other.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Clindamycin Hydrochloride Capsules add a suitable amount of the mobile phase, shake for 30 minutes, and add the mobile phase to make exactly V mL so that each mL contains 0.75 mg (potency) of Clindamycin Hydrochloride. Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

\[ \text{Amount [mg (potency)] of clindamycin (} C_{18}H_{33}ClN_{2}O_{5}S) = M_s \times A_1/A_5 \times V/100 \]

\[ M_s: \text{Amount [mg (potency)] of Clindamycin Hydrochloride RS} \]

**Dissolution** <6.1D> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate of a 75-mg capsule in 15 minutes and that of a 150-mg capsule in 30 minutes are not less than 80%.

Start the test with 1 capsule of Clindamycin Hydrochlo-
ride Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V’ so that each mL contains about 83 µg (potency) of Clindamycin Hydrochloride according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Clindamycin Hydrochloride RS, equivalent to about 17 mg (potency), dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.0\% > \), and determine the peak areas, \( A_f \) and \( A_s \), of clindamycin.

Dissolution rate (%) with respect to the labeled amount of clindamycin hydrochloride

\[
M_s = M_f \times \frac{A_f}{A_s} \times \frac{V}{V'} \times \frac{C}{C'} \times 450
\]

\( M_f: \) Amount [mg (potency)] of Clindamycin Hydrochloride RS

\( C: \) Labeled amount [mg (potency)] of clindamycin \((C_{18}H_{33}ClN_2O_5S)\) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclysilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.5 with 8 mol/L potassium hydroxide TS to adjust the pH to 7.5. To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of clindamycin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clindamycin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clindamycin is not more than 1.0%.

Containers and storage Containers—Tight containers.

**Clindamycin Phosphate**

クリンダマイシンリン酸エステル

\( C_{18}H_{35}ClIN_2O_5SP: \) 504.96

Methyl 7-chloro-6,7,8-trideoxy-6-\( \{2S,4R\}\)-1-methyl-4-propylpyrrolidine-2-carboxamido-1-thio-L-threo-a-D-galacto-octopyranoside 2-dihydrogenphosphate [24729-96-2]

Clindamycin Phosphate is a derivative of clindamycin.

It contains not less than 800 µg (potency) and not more than 846 µg (potency) per mg, calculated on the anhydrous basis. The potency of Clindamycin Phosphate is expressed as mass (potency) of clindamycin \((C_{18}H_{35}ClIN_2O_5S): \) 424.98.

**Description** Clindamycin Phosphate occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95).

**Identification** Determine the infrared absorption spectrum...
of Clindamycin Phosphate, previously dried at 100°C for 2 hours, as directed in the paste method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum or the spectrum of Clindamycin Phosphate RS previously dried at 100°C for 2 hours: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> $\alpha_D^{25} + 115 - +130^\circ$ (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

**pH** <2.54> Dissolve 0.1 g of Clindamycin Phosphate in 10 mL of water. The pH of the solution is between 3.5 and 4.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Clindamycin Phosphate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Prepare with 2.0 g of Clindamycin Phosphate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 5 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Clindamycin Phosphate according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.1 g of Clindamycin Phosphate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of clindamycin phosphate to that of the internal standard.

Amount [µg (potency)] of clindamycin (C$_{18}$H$_{33}$ClN$_2$O$_5$S) 

\[ M_S \times Q_T/Q_S \times 1000 \]

$M_S$: Amount [mg (potency)] of Clindamycin Phosphate RS

**Internal standard solution**—A solution of methyl parahydroxybenzoate in the mobile phase (3 in 50,000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 10.54 g of potassium dihydrogen phosphate in 775 mL of water, adjust the pH to 2.5 with phosphoric acid, and add 225 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of clindamycin phosphate is about 8 minutes.

**System suitability**—

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, clindamycin phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clindamycin phosphate to that of the internal standard is not more than 2.5%.

**Containers and storage** Containers—Tight containers.

### Clindamycin Phosphate Injection

クリンダマイシンリン酸エステル注射液

Clindamycin Phosphate Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of clindamycin phosphate (C$_{18}$H$_{33}$ClN$_2$O$_5$S: 504.96).

**Method of preparation** Prepare as directed under Injections, with Clindamycin Phosphate.

**Description** Clindamycin Phosphate Injection is a clear, colorless or light yellow liquid.

**Identification** To a volume of Clindamycin Phosphate Injection, equivalent to 0.15 g (potency) of Clindamycin Phosphate according to the labeled amount, add 4 mL of water, 2 mL of 8 mol/L sodium hydroxide TS and 0.1 mL of sodium pentacyanonitrosylferrate (III) TS, mix, heat in a water bath for 10 minutes, and add 2 mL of hydrochloric acid: a blue-green color develops.

**Osmotic pressure ratio** Being specified separately.
Clinofibrate / Official Monographs

**pH** \(<2.5\rightarrow 6.0 \sim 7.0\)

**Bacterial endotoxins** \(<4.01\>\) Less than 0.1 EU/mg (potency).

**Extractable volume** \(<6.05\>\) It meets the requirement.

**Foreign insoluble matter** \(<6.06\>\) Perform the test according to Method 1; it meets the requirement.

**Insoluble particulate matter** \(<6.07\>\) It meets the requirement.

**Sterility** \(<4.06\>\) Perform the test according to the Membrane filtration method; it meets the requirement.

**Assay** Measure exactly a volume of Clindamycin Phosphate Injection, equivalent to about 0.3 g (potency) of Clindamycin Phosphate, and add the mobile phase to make exactly 100 mL. Pipet 7 mL of this solution, add exactly 25 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Clindamycin Phosphate RS, equivalent to about 20 mg (potency), dissolve in exactly 25 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Clindamycin Phosphate.

\[
\text{Amount [mg (potency)] of clindamycin phosphate (C_{16}H_{39}CIN_{2}O_{5}PS)} = M_S \times \frac{Q_1}{Q_3} \times 100/7
\]

\[
M_S: \text{Amount [mg (potency)] of Clindamycin Phosphate RS}
\]

**Internal standard solution**—A solution of methyl parahydroxybenzoate in the mobile phase (3 in 50,000).

**Containers and storage** Containers—Hermetic containers.

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**Clinofibrate**

**クリノフィブラート**

\[
\text{C}_{28}\text{H}_{35}\text{O}_{4}: 468.58
\]

2,2’-(4,4’-Cyclohexyldenediphenoxy)-2,2’-dimethyl dibutanoic acid

[30299-08-2]

Clinofibrate, when dried, contains not less than 98.5% of \(\text{C}_{28}\text{H}_{35}\text{O}_{4}\).

**Description** Clinofibrate occurs as a white to yellowish white powder.

It is odorless and has no taste.

It is freely soluble in methanol, in ethanol (99.5), in acetone and in diethyl ether, and practically insoluble in water.

A solution of Clinofibrate in methanol (1 in 20) shows no optical rotation.

Melting point: about 146°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Clinofibrate in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clinofibrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** (1) Heavy metals \(<1.07\>\)—Proceed with 1.0 g of Clinofibrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \(<1.11\>\)—Prepare the test solution with 1.0 g of Clinofibrate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Clinofibrate in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 50 mL. Pipet 5 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\>\). Spot 50 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, cyclohexane and acetic acid (100) (12:5:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.41\>\) Not more than 1.0% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** \(<2.44\>\) Not more than 0.2% (1 g).

**Isomer ratio** To 50 mg of Clinofibrate add 0.4 mL of thionyl chloride, stopper tightly, heat on a water bath of 60°C for 5 minutes with occasional shaking, and evaporate the excess thionyl chloride at a temperature not exceeding 60°C under reduced pressure. Dissolve the residue in 2 mL of toluene previously dried with synthetic zeolite for drying, add 2 mL of a solution of \(\text{d}-(+)-\alpha\)-methylbenzylamine in toluene previously dried with synthetic zeolite for drying (3 in 100), mix gently, allow to stand for 10 minutes, and evaporate the toluene at a temperature not exceeding 60°C under reduced pressure. Dissolve the residue in 5 mL of chloroform, and use this solution as the sample solution. Perform the test with 5 \(\mu\)L of the sample solution as directed under Liquid Chromatography \(<2.07\>\) according to the following conditions. Determine each peak area, \(A_a, A_b, A_c\) and \(A_d\), of three peaks appear in order near the retention time of 40 minutes: a value, \(A_d/(A_a + A_b + A_c) \times 100\), is between 40 and 70.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
Column temperature: A constant temperature of about 20°C.
Mobile phase: A mixture of hexane and 2-propanol (500:3).
Flow rate: Adjust the flow rate so that the retention time of the peak appearing first is about 35 minutes.
Selection of column: Proceed with 5 μL of the sample solution under the above operating conditions. Use a column giving a complete separation of the three peaks.

**Assay**
Weigh accurately about 0.45 g of Clobetasol Propionate RS, previously dried, dissolve in 40 mL of ethanol (99.5), add 30 mL of water, and titrate \( <2.3 Dream > \) with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 23.43 mg of C\(_25\)H\(_{32}\)ClFO\(_5\).

**Containers and storage**
Containers—Tight containers.

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**Clobetasol Propionate**

\( \text{C}_{25}\text{H}_{32}\text{ClFO}_{5} \): 466.97
21-Chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propanoate

[C2122-46-7]

Clobetasol Propionate, when dried, contains not less than 97.0\%\( \text{w} \)\( \text{w} \) and not more than 102.0\%\( \text{w} \)\( \text{w} \) of C\(_25\)H\(_{32}\)ClFO\(_5\).

**Description**
Clobetasol Propionate occurs as a white to pale yellowish white crystalline powder.
It is soluble in methanol and in ethanol (99.5), and practically insoluble in water.
It gradually turns yellow by light.
Melting point: about 196°C (with decomposition).

**Identification**
Determine the infrared absorption spectra of Clobetasol Propionate as directed in the paste method under Infrared Spectrophotometry \( <2.2 Dream > \), and compare the spectrum with the Reference Spectrum or the spectrum of Clobetasol Propionate RS: both spectra exhibit similar intensities of absorbance at the same wave numbers.

**Optical rotation** \( <2.4 Dream > \) \( [\alpha]_D^{10} +109 - +115^\circ \) (after drying, 0.1 g, methanol, 10 mL, 100 mm).

**Purity**

1. Heavy metals \( <1.07 \) — Proceed with 1.0 g of Clobetasol Propionate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
2. Related substances — Dissolve 10 mg of Clobetasol Propionate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 \) according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than clobetasol propionate obtained from the sample solution is not larger than 2/5 times the peak area of clobetasol propionate from the standard solution. Furthermore, the total area of the peaks other than clobetasol propionate is not larger than the peak area of clobetasol propionate from the standard solution.

**Operating conditions**
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 2.5 times as long as the retention time of clobetasol propionate, beginning after the solvent peak.

**System suitability**
Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of clobetasol propionate obtained from 10 μL of this solution is equivalent to 2.8 to 5.2% of that from 10 μL of the standard solution.

**System performance**
Dissolve 20 mg of Clobetasol Propionate in 20 mL of methanol. To 5 mL of this solution add 10 mL of a solution of beclometasone dipropionate in methanol (1 in 1000), and then add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above conditions, clobetasol propionate and beclometasone dipropionate are eluted in this order with the resolution between these peaks being not less than 8.

**System repeatability**
When the test is repeated 6 times with 10 μL of the standard solution under the above conditions, the relative standard deviation of the peak area of clobetasol propionate is not more than 2.0%.

**Loss on drying** \( <2.4 Dream > \) Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** \( <2.4 Dream > \) Not more than 0.1% (1 g, platinum crucible).

**Assay**
Weigh accurately about 10 mg each of Clobetasol Propionate and Clobetasol Propionate RS, both previously dried, dissolve each in the mobile phase, add exactly 100 mL of the internal standard solution, add the mobile phase to make 250 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 \) according to the following conditions, and calculate the ratios, \( Q_S \) and \( Q_S \), of the peak area of clobetasol propionate to that of the internal standard.

\[
\text{Amount (mg) of C}_{25}\text{H}_{32}\text{ClFO}_{5} = M_S \times \frac{Q_S}{Q_S}
\]

**Internal standard solution**
A solution of beclometasone dipropionate in the mobile phase (1 in 5000).

**Operating conditions**
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 2.5 with phosphoric acid, and then add water to make 1000 mL. To 425 mL of this solution add 475 mL of acetonitrile and 100 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of clobetasol propionate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above conditions, clobetasol propionate and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of clobetasol propionate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clocapramine Hydrochloride Hydrate

クロカプラミン塩酸塩水和物

C_{28}H_{37}ClN_{4}O.2HCl.H_{2}O: 572.01

1’-[3-(3-Chloro-10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)propyl]-1,4-bipiperidine-4’-carboxamide dihydrochloride monohydrate [60789-62-0]

Clocapramine Hydrochloride Hydrate, when dried, contains not less than 98.0% of clocapramine hydrochloride (C_{28}H_{37}ClN_{4}O.2HCl: 553.99).

Description Clocapramine Hydrochloride Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, slightly soluble in ethanol (95), in chloroform and in isopropylamine, and practically insoluble in acetic anhydride and in diethyl ether.

It is gradually colored by light.

Melting point: about 260°C (with decomposition, after drying).

Identification (1) To 5 mL of a solution of Clocapramine Hydrochloride Hydrate (1 in 2500) add 1 mL of nitric acid: a blue color develops at first, and rapidly changes to deep blue, and then changes to green to yellow-green.

(2) Determine the absorption spectrum of a solution of Clocapramine Hydrochloride Hydrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clocapramine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Dissolve 0.1 g of Clocapramine Hydrochloride Hydrate in 10 mL of water by warming, and after cooling, add 2 mL of ammonia TS, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Sulfate <1.14>—Dissolve 0.5 g of Clocapramine Hydrochloride Hydrate in 40 mL of water by warming, after cooling, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clocapramine Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Clocapramine Hydrochloride Hydrate in 10 mL of a mixture of chloroform and isopropylamine (99:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and isopropylamine (99:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, ethyl acetate, methanol and ammonia solution (28:100:70:40:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 2.0–3.5% (0.5 g, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clocapramine Hydrochloride Hydrate, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.
Each mL of 0.1 mol/L perchloric acid VS = 27.70 mg of C_{17}H_{20}ClNO.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Clofedan Hydrochloride

クロフェダノール塩酸塩

C_{17}H_{20}ClNO.HCl: 326.26
(1R5)-1-(2-Chlorophenyl)-3-dimethylamino-1-phenylpropan-1-ol monohydrochloride
[511-13-7]

Clofedan Hydrochloride, when dried, contains not less than 98.5% of C_{17}H_{20}ClNO.HCl.

Description Clofedan Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetic acid (100), sparingly soluble in water, and practically insoluble in diethyl ether.

A solution of Clofedan Hydrochloride in methanol (1 in 20) does not show optical rotation.

Melting point: about 190°C (after drying, with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Clofedan Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.2>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clofedan Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Clofedan Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Clofedan Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.05 g of Clofedan Hydrochloride in 25 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than clofedanol from the sample solution is not larger than the peak area of clofedanol from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1.34 g of potassium methanesulfonate in diluted phosphoric acid (1 in 1000) to make 1000 mL, and to 650 mL of this solution add 350 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of clofedanol is about 9 minutes.

Selection of column: Dissolve 0.01 g each of Clofedan Hydrochloride and ethyl parahydroxybenzoate in methanol to make 100 mL. Proceed with 3 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of clofedanol and ethyl parahydroxybenzoate in this order with the resolution of these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of clofedanol obtained from 3 μL of the standard solution composes between 20% and 50% of the full scale.
Time span of measurement: About three times as long as the retention time of clofedanol beginning after the solvent peak.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, silica gel, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clofedan Hydrochloride, previously dried, dissolve in 15 mL of acetic acid (100), add 35 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.63 mg of C_{17}H_{20}ClNO.HCl

Containers and storage Containers—Tight containers.

Clofibrate

クロフィブラート

C_{12}H_{15}ClO_{3}: 242.70
Ethyl 2-(4-chlorophenoxy)-2-methylpropanoate
[637-07-0]

Clofibrate contains not less than 98.0% of C_{12}H_{15}ClO_{3}, calculated on the unhydrous basis.
Separately, determine the absorption spectrum of a solution similar to the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Identification

(1) Determine the absorption spectrum of a solution of Clofibrate in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Clofibrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Clofibrate in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Clofibrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clofibrate as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Clofibrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> \( n^\prime \): 1.500 - 1.505

Specific gravity <2.50> \( d^\prime \): 1.137 - 1.144

Purity (1) Acidity—Dissolve 2.0 g of Clofibrate in 100 mL of neutralized ethanol, and add 1 drop of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clofibrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.13>—To 5.0 g of Clofibrate add 20 mL of nitric acid and 5 mL of sulfuric acid, and heat until white fumes are evolved. After cooling, if necessary, add further 5 mL of nitric acid, heat until white fumes are evolved, and repeat this procedure until the solution is colorless to light yellow. After cooling, add 15 mL of saturated ammonium oxalate solution, and heat again until white fumes are evolved. Cool, add water to make 25 mL, use 5 mL of this solution as the test solution, and perform the test.

Color standard: Prepare a solution according to the above procedure without using Clofibrate as the blank. Transfer 5 mL of the solution to a generator bottle, add 2.0 mL of Standard Arsenic Solution, and then proceed as directed in the test solution (not more than 20 ppm).

(4) p-Chlorophenol—To 1.0 g of Clofibrate add exactly 1 mL of the internal standard solution, then add the mobile phase to make 5 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 4-chlorophenol in a mixture of hexane and 2-propanol (9:1) to make exactly 100 mL. Pipet 10 mL of this solution, and add a mixture of hexane and 2-propanol (9:1) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of 4-chlorophenol to that of the internal standard: \( Q_1 \) is not greater than \( Q_2 \).

Internal standard solution—A solution of 4-ethoxyphenol in the mobile phase (1 in 30,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with cyanopropyl-silanized silica gel for liquid chromatography (5 to 10 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of hexane, 2-propanol and acetic acid (100) (1970:30:1).

Flow rate: Adjust the flow rate so that the retention time of clofibrate is about 2 minutes.

Selection of column: Dissolve 10.0 g of clofibrate, 6 mg of 4-chlorophenol and 6 mg of 4-ethoxyphenol in 1000 mL of hexane. Proceed with 20 \( \mu \)L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of clofibrate, 4-chlorophenol and 4-ethoxyphenol in this order, with the resolution between the peaks of clofibrate and 4-chlorophenol is not less than 5, and with the resolution between the peaks of 4-chlorophenol and 4-ethoxyphenol is not less than 2.

Water <2.48> Not more than 0.2% (5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clofibrate, add exactly 50 mL of 0.1 mol/L potassium hydroxide-ethanol VS, and heat in a water bath under a reflux condenser with a carbon dioxide absorbing tube (soda-lime) for 2 hours with frequent shaking. Cool, and titrate <2.50> immediately the excess potassium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 24.27 mg of C\(_{12}\)H\(_{15}\)ClO\(_3\)

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clofibrate Capsules

クロフィブラートカプセル

Clofibrate Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of clofibrate (C\(_{12}\)H\(_{15}\)ClO\(_3\)): 242.70.

Method of preparation Prepare as directed under Capsules, with Clofibrate.

Identification Cut and open Clofibrate Capsules, and use the contents as the sample. Determine the absorption spectrum of a solution of the sample in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectropho-
JP XVI

Official Monographs / Clomifene Citrate 659

Clomifene Citrate

クロミフェンクエン酸塩

\[
\begin{align*}
C_{26}H_{28}ClNO.C_6H_8O_7: \text{Molecular weight} = 598.08
\end{align*}
\]

2-[4-(2-Chloro-1,2-diphenylvinlylphenoxy)-N,N-dieethylamino mononitrate

Clomifene Citrate, when dried, contains not less than 98.0% of \( C_{26}H_{28}ClNO.C_6H_8O_7 \).

Description

Clomifene Citrate occurs as a white to pale yellowish white powder. It is odorless.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

It gradually changes in color by light.

Melting point: about 115°C

Identification

(1) To 2 mL of a solution of Clomifene Citrate in methanol (1 in 200) add 2 mL of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Clomifene Citrate in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Clomifene Citrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Clomifene Citrate in methanol (1 in 200) responds to the Qualitative Tests \( <1.09> \) (1) and (2) for citrate salt.

Purity

(1) Clarity and color of solution—A solution of 1.0 g of Clomifene Citrate in 30 mL of methanol is clear and colorless.

(2) Heavy metals \( <1.07> \)—Proceed with 2.0 g of Clomifene Citrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying \( <2.47> \) Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition \( <2.47> \) Not more than 0.1% (1 g).

Isomer ratio

To 0.10 g of Clomifene Citrate add 10 mL of water and 1 mL of sodium hydroxide TS, and extract with three 15-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 20 mL of water, add 10 g of anhydrous sodium sulfate to the combined diethyl ether extracts, shake for 1 minute, filter, and evaporate the diethyl ether of the filtrate. Dissolve the residue in 10 mL of chloroform, and use this solution as the sample solution. Perform the test with 2 \( \mu \)L of the sample solution as directed under Gas Chromatography \( <2.02> \) according to the following conditions. Determine the areas of two adjacent peaks, \( A_a \) and \( A_b \), hav-
ing retention times of about 20 minutes, where \( A_s \) is the peak area of shorter retention time and \( A_l \) is the peak area of longer retention time: \( A_l/(A_s + A_l) \) is between 0.3 and 0.5. 

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, having methylsilicone polymer coated at the ratio of 1% on siliceous earth for gas chromatography (125 to 150 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 195°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of the first peak of clomifene citrate is about 20 minutes.

System suitability—

System performance: When the procedure is run with 2 \( \mu \)L of the sample solution under the above operating conditions, the resolution between the two peaks is not less than 1.3.

System repeatability: When the test is repeated 5 times with 2 \( \mu \)L of the sample solution under the above operating conditions, the relative standard deviation of \( A_l/(A_s + A_l) \) is not more than 5.0%.

Assay

Weigh accurately about 1 g of Clomifene Citrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 59.81 mg of \( \text{C}_2\text{H}_2\text{ClNO.C}_6\text{H}_8\text{O}_7 \).

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.

Clomifene Citrate Tablets

クロミフェンクエン酸塩錠

Clomifene Citrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of clomifene citrate (\( \text{C}_{26}\text{H}_{28}\text{ClNO.C}_6\text{H}_8\text{O}_7 \): 598.08).

Method of preparation

Prepare as directed under Tablets, with Clomifene Citrate.

Identification (1)

Weigh a portion of powdered Clomifene Citrate Tablets, equivalent to 1 g of Clomifene Citrate according to the labeled amount, shake vigorously with 100 mL of chloroform, and filter. Concentrate the filtrate on a water bath, allow to stand at room temperature, collect the crystals formed by filtration, wash with a small quantity of chloroform. Proceed with the crystals as directed in the Identification (1) and (3) under Clomifene Citrate.

(2) Determine the absorption spectrum of a solution of the crystals obtained in (1) in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 233 nm and 237 nm, and between 290 nm and 294 nm.

Uniformity of dosage units

Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Clomifene Citrate Tablets add 10 mL of water, and shake until the tablets are disintegrated. To this solution add 50 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet 4 mL of the supernatant liquid, add methanol to make exactly \( V \) mL so that each mL contains about 20 \( \mu \)g of clomifene citrate (\( \text{C}_{26}\text{H}_2\text{ClNO.C}_6\text{H}_8\text{O}_7 \)), and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of clomifene citrate (\( \text{C}_{26}\text{H}_2\text{ClNO.C}_6\text{H}_8\text{O}_7 \))

\[ M_5 = M_s \times A_l/\left(A_s + A_l\right) \times V/100 \]

\( M_5 \): Amount (mg) of Clomifene Citrate RS

Dissolution

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Clomifene Citrate Tablets is not less than 80%.

Start the test with 1 tablet of Clomifene Citrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \)m. Discard the first 10 mL of the filtrate, pipet \( V \) mL of the subsequent filtrate, add the dissolution medium to make exactly \( V \) mL so that each mL contains about 28 \( \mu \)g of clomifene citrate (\( \text{C}_{26}\text{H}_2\text{ClNO.C}_6\text{H}_8\text{O}_7 \)) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Clomifene Citrate RS, previously dried in vacuum using phosphorus (V) oxide as a desiccant for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), at 291 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of clomifene citrate (\( \text{C}_{26}\text{H}_2\text{ClNO.C}_6\text{H}_8\text{O}_7 \))

\[ M_5 = M_s \times A_T/\left(A_S + A_T\right) \times 100 \]

\( M_5 \): Amount (mg) of Clomifene Citrate RS

C: Labeled amount (mg) of clomifene citrate (\( \text{C}_{26}\text{H}_2\text{ClNO.C}_6\text{H}_8\text{O}_7 \)) in 1 tablet

Assay

Weigh accurately, and powder not less than 20 Clomifene Citrate Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of clomifene citrate (\( \text{C}_{26}\text{H}_2\text{ClNO.C}_6\text{H}_8\text{O}_7 \)), add 50 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Centrifuge a portion of this solution, pipet 4 mL of the supernatant liquid, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Clomifene Citrate RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and dilute with methanol to make exactly 100 mL. Centrifuge this solution, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and the standard solution, respectively, at 295 nm as directed under Ultraviolet-visible Spectrophotometry.

Amount (mg) of clomifene citrate (\( \text{C}_{26}\text{H}_2\text{ClNO.C}_6\text{H}_8\text{O}_7 \))

\[ M_5 = M_s \times A_T/\left(A_S + A_T\right) \]
Clomipramine Hydrochloride

Clomipramine Hydrochloride, when dried, contains not less than 98.5% of C_{19}H_{23}ClN_{2}HCl.

Description
Clomipramine Hydrochloride occurs as a white to pale yellow, crystalline powder. It is odorless.

It is very soluble in acetic acid (100), freely soluble in water, in methanol and in chloroform, soluble in ethanol (95), sparingly soluble in acetic anhydride, slightly soluble in acetone, and practically insoluble in ethyl acetate and in diethyl ether.

Identification
(1) Dissolve 3 mg of Clomipramine Hydrochloride in 1 mL of nitric acid: a deep blue color develops.

(2) Determine the absorption spectrum of a solution of Clomipramine Hydrochloride in 0.1 mol/L hydrochloric acid in 1 mL of nitric acid: a deep blue color develops.

Loss on drying
<2.4% Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition
<2.4% Not more than 0.1% (1 g).

Assay
Weigh accurately about 0.5 g of Clomipramine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 35.13 mg of C_{19}H_{23}ClN_{2}HCl.

Containers and storage
Containers—Well-closed containers.

Storage—Light-resistant.

Clonazepam

Clonazepam, when dried, contains not less than 99.0% of C_{15}H_{10}ClN_{3}O_{3}.

C_{15}H_{10}ClN_{3}O_{3}: 315.71

5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-2H-1,4-benzodiazepin-2-one
[1622-61-3]
Clonidine Hydrochloride / Official Monographs

**Description**
Clonazepam occurs as white to light yellow, crystals or crystalline powder.

It is sparingly soluble in acetic anhydride and in acetone, slightly soluble in methanol and in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored by light.

Melting point: about 240°C (with decomposition).

**Identification (1)**
Determine the absorption spectrum of a solution of Clonazepam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clonazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Clonazepam as directed under Flame Coloration Test \(<1.04>\) (2): a green color appears.

**Purity (1)**
Chloride \(<1.07>\)—To 1.0 g of Clonazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. Discard the first 20 mL portion of the filtrate, and take the subsequent 20 mL portion of the filtrate, and add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.022%).

(2) Heavy metals \(<1.07>\)—Proceed with 1.0 g of Clonazepam according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.25 g of Clonazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, then pipet 1 mL of this solution, add acetone to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.07>\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of nitromethane and acetone (10:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.41>\) Not more than 0.30% (1 g, 105°C, 4 hours).

**Residue on ignition** \(<2.44>\) Not more than 0.1% (1 g).

**Assay**
Weigh accurately about 0.5 g of Clonazepam, previously dried, dissolve in 70 mL of acetic anhydride, and titrate \(<2.50>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 31.57 mg of C\(_{15}\)H\(_{10}\)ClN\(_3\)O\(_3\)

**Containers and storage**
Containers—Well-closed containers.

Storage—Light-resistant.

**Clonidine Hydrochloride**
クロニジン塩酸塩

\[
\text{C}_9\text{H}_9\text{Cl}_2\text{N}_3\cdot\text{HCl}: 266.55
\]

2-(2,6-Dichlorophenylimino)imidazolidine monohydrochloride

【4205-91-8】

Clonidine Hydrochloride, when dried, contains not less than 99.0% of C\(_9\)H\(_9\)Cl\(_2\)N\(_3\)H\(_2\)Cl.

**Description**
Clonidine Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in methanol, soluble in water and in ethanol (95), and practically insoluble in acetic anhydride and in diethyl ether.

**Identification (1)**
To 5 mL of a solution of Clonidine Hydrochloride (1 in 1000) add 6 drops of Dragendorff’s TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of a solution of Clonidine Hydrochloride in 0.01 mol/L hydrochloric acid TS (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clonidine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Clonidine Hydrochloride (1 in 50) responds to the Qualitative Tests \(<1.09>\) for chloride.

**pH** \(<2.54>\) Dissolve 1.0 g of Clonidine Hydrochloride in 20 mL of water: the pH of this solution is between 4.0 and 5.5.

**Purity (1)**
Clarity and color of solution—Dissolve 1.0 g of Clonidine Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals \(<1.07>\)—Proceed with 2.0 g of Clonidine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic \(<1.17>\)—Prepare the test solution with 0.5 g of Clonidine Hydrochloride according to Method 3, and perform the test (not more than 4 ppm).

(4) Related substances—Dissolve 0.20 g of Clonidine Hydrochloride in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, to each add methanol to make exactly 20 mL, and use these solutions as the standard solution (1) and
the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.05\). Spot 2 \(\mu\)L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene, 1,4-dioxane, ethanol (99.5) and ammonia solution (28) (10:8:2:1) to a distance of about 12 cm, air-dry the plate, and then dry at 100°C for 1 hour. Spray evenly sodium hypochlorite TS on the plate, air-dry the plate for 15 minutes, and then spray evenly potassium iodide starch TS on the plate: the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution (2), and the numbers of spots other than the principal spot and the spot of the starting point, which are more intense than the spot from the standard solution (1), are not more than 3.

**Loss on drying** \(<2.4\>) Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** \(<2.44\>) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Clonidine Hydrochloride, previously dried, and dissolve in 30 mL of acetic acid (100) by warming. After cooling, add 70 mL of acetic chloride, previously dried, and dissolve in 30 mL of acetic acid. Weigh accurately about 0.3 g of Clonidine Hydrochloride (2) and dissolve in 25 mL of acetic acid (100). Pipet 2 mL of the solutions as directed under Thin-layer Chromatography (1 in 100) to a distance of about 12 cm, air-dry the plate, and then dry at 100°C for 1 hour. Spray evenly sodium hypochlorite TS on the plate, air-dry the plate for 15 minutes, and then spray evenly potassium iodide starch TS on the plate: the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution (2), and the numbers of spots other than the principal spot and the spot of the starting point, which are more intense than the spot from the standard solution (1), are not more than 3.

**Containers and storage** Containers—Tight containers.

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**Cloperastine Hydrochloride**

クロペラスチン塩酸塩

![](image)

C₂₀H₂₄ClNO.HCl: 366.32

1-[2-[(RS)-(4-Chlorophenyl) (phenyl)methoxy]ethyl]piperidine monohydrochloride

[14984-68-0]

Cloperastine Hydrochloride, when dried, contains not less than 98.5% of C₂₀H₂₃ClNO.HCl.

**Description** Cloperastine Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in water, in methanol, in ethanol (95) and in acetic acid (100), and soluble in acetic anhydride.

A solution of Cloperastine Hydrochloride (1 in 10) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Cloperastine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Cloperastine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cloperastine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry \(<2.25\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Shake 10 mL of a solution of Cloperastine Hydrochloride (1 in 100) with 2 mL of ammonia TS and 20 mL of diethyl ether, separate the water layer, wash the water layer with 20 mL of diethyl ether, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests \(<1.09\) for chloride.

**Melting point** \(<2.60\> 148 – 152°C

**Purity (1)** Heavy metals \(<1.07≥—Proceed with 1.0 g of Cloperastine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 40 mg of Cloperastine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and determine each peak area of both solutions by the automatic integration method: The areas of two peaks corresponding to the relative retention times about 0.8 and about 3.0 with respect to cloperastine obtained from the sample solution are not larger than the peak area of cloperastine from the standard solution, respectively, and the area of the peak corresponding to the relative retention time about 2.0 is not larger than 5/3 times the peak area of cloperastine from the standard solution, and the areas of the peaks other than cloperastine and other than the peaks mentioned above are all not larger than 5/3 times the peak area of cloperastine from the standard solution. The total area of these peaks is not larger than 2 times the peak area of cloperastine from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol, 0.1 mol/L mono-basic potassium phosphate TS and perchloric acid (500:30:1).

Flow rate: Adjust the flow rate so that the retention time of cloperastine is about 7 minutes.

Selection of column: Dissolve 0.03 g of Cloperastine Hydrochloride and 0.04 g of benzophenone in 100 mL of the
mobile phase. To 2.0 mL of this solution add the mobile phase to make 50 mL. Perform the test with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of cloperastine and benzophenone in this order with the resolution between these peaks being not less than 6.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cloperastine obtained from 20 μL of the standard solution is about 30% of the full scale.

Time span of measurement: About 4 times as long as the retention time of cloperastine, beginning after the solvent peak.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Cloperastine Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate chloric acid VS add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: To 0.40 mL of 0.01 mol/L perchloric acid VS add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.014%).

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Clorazepate Dipotassium**

**モノ（塩化カリウム）**

![Chemical structure](image)

C$_{16}$H$_{10}$ClKN$_2$O$_3$.KOH: 408.92

Monopotassium 7-chloro-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepine-3-carboxylate mono (potassium hydroxide)

[57109-90-7]

Clorazepate Dipotassium, when dried, contains not less than 98.5% and not more than 101.0% of C$_{16}$H$_{10}$ClKN$_2$O$_3$.KOH.

**Description** Clorazepate Dipotassium occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in water, and very slightly soluble in ethanol (99.5). It dissolves in acetic acid (100).

The pH of a solution obtained by dissolving 1 g of Clorazepate Dipotassium in 100 mL of water is between 11.5 and 12.5.

It gradually turns yellow on exposure to light.

**Identification** (1) Carefully and gradually ignite to redness 30 mg of Clorazepate Dipotassium with 50 mg of sodium. After cooling, add 3 drops of ethanol (99.5) and 5 mL of water, mix well, and filter: the filtrate responds to the Qualitative Tests <1.09> for chloride.

(2) Determine the absorption spectrum of a solution of Clorazepate Dipotassium (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clorazepate Dipotassium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Clorazepate Dipotassium responds to the Qualitative Tests <1.09> (1) for potassium salt.

**Purity** (1) Chloride <1.03>>—Dissolve 1.0 g of Clorazepate Dipotassium in 20 mL of water, add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this sample as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Clorazepate Dipotassium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 2 ppm).

(3) Arsenic <1.11>>—Prepare the test solution with 1.0 g of Clorazepate Dipotassium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 15 mg of Clorazepate Dipotassium in 25 mL of a mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make exactly 200 mL, and use this solution as the standard solution. Prepare these solutions quickly and perform the test within 3 minutes. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of nordiazepam, having the relative retention time of about 3.0 with respect to clorazepic acid, obtained from the sample solution is not larger than the peak area of clorazepic acid from the standard solution, the area of the peak other than clorazepic acid and nordiazepam is not larger than 1/5 times the peak area of clorazepic acid from the standard solution, and the total area of the peaks other than clorazepic acid is not larger than 2 times the peak area of clorazepic acid from the standard solution. For this comparison, use the peak area of nordiazepam, having the relative retention time of about 3.0 with respect to clorazepic acid, after multiplying by the relative response factor, 0.64.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 13.8 g of sodium dihydrogen phos-
Perform a blank determination in the same manner, and until the color of solution changes from violet to blue—Dipotassium, previously dried, dissolve in 100 mL of acetic acid (100), and titrate.

Containers—Tight containers.

Loss on drying Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 5 hours).

Assay Weigh accurately about 0.15 g of Clorazepate Dipotassium, previously dried, dissolve in 100 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS until the color of solution changes from violet to blue-green through blue (indicator: 3 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

\[ = 13.63 \, \text{mg of C}_{16}\text{H}_{10}\text{ClKNO}_{3} \times \text{KO}_{2} \]

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clorazepate Dipotassium Capsules

クロラゼプ酸ニカリウムカプセル

Clorazepate Dipotassium Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of clorazepate dipotassium (C_{16}H_{10}ClKNO_{3} \times \text{KO}_{2}; 408.92).

Method of preparation Prepare as directed under Capsules, with Clorazepate Dipotassium.

Identification To 10 mL of the sample solution obtained in the Assay add water to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits a maximum between 228 nm and 232 nm.

Purity Related substances—Take out the contents of Clorazepate Dipotassium Capsules, and powder. To a portion of the powder, equivalent to 15 mg of Clorazepate Dipotassium according to the labeled amount, add a mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make 25 mL, and mix for 10 minutes. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, and use subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Purity (4) under Clorazepate Dipotassium: the peak area of nor Diazepam, having the relative retention time of about 3.0 with respect to clorazepic acid, obtained from the sample solution is not larger than 3 times the peak area of clorazepic acid from the standard solution, and the total area of the peaks other than clorazepic acid and nor Diazepam is not larger than the peak area of clorazepic acid from the standard solution. For this comparison, use the peak area of nor Diazepam after multiplying by the relative response factor, 0.64.

Uniformity of dosage units (6.02) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Clorazepate Dipotassium Capsules add 70 mL of water, shake for 15 minutes, and add water to make exactly 100 mL. Centrifuge the solution, pipet V mL of the supernatant liquid, add water to make exactly V mL so that each mL contains about 12 μg of clorazepate dipotassium (C_{16}H_{10}ClKNO_{3} \times \text{KO}_{2}), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of clorazepate dipotassium

\[ (\text{C}_{16}\text{H}_{10}\text{ClKNO}_{3} \times \text{KO}_{2}) = \frac{M_{s} \times A_{T} \times A_{S} \times V/V 	imes 2/25}{M_{A}} \]

M_{s}: Amount (mg) of clorazepate dipotassium for assay

Dissolution (6.10) When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Clorazepate Dipotassium Capsules is not less than 80%.

Start the test with 1 capsule of Clorazepate Dipotassium Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 8.3 μg of clorazepate dipotassium (C_{16}H_{10}ClKNO_{3} \times \text{KO}_{2}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of clorazepate dipotassium for assay, previously dried in vacuum over phosphorus (V) oxide at 60°C for 5 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_{T} and A_{S}, at 252 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry (2.24).

Dissolution rate (% with respect to the labeled amount of clorazepate dipotassium (C_{16}H_{10}ClKNO_{3} \times \text{KO}_{2})

\[ = \frac{M_{s} \times A_{T}/A_{S} \times V/V \times 1/C \times 36}{M_{A}} \]

M_{s}: Amount (mg) of clorazepate dipotassium for assay

C: Labeled amount (mg) of clorazepate dipotassium (C_{16}H_{10}ClKNO_{3} \times \text{KO}_{2}) in 1 capsule
Assay Carefully take out the contents of not less than 20 Clorazepate Dipotassium Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 15 mg of clorazepate dipotassium (C₁₆H₁₀ClKN₂O₃.KOH), add 70 mL of water, shake for 15 minutes, and add water to make exactly 100 mL. Centrifuge the solution, pipet 4 mL of the supernatant liquid, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of clorazepate dipotassium for assay, previously dried in vacuum over phosphorus (V) oxide at 60°C for 5 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Make a blank determination in and make any necessary correction.

\[
M_S = M_T \times A_T / A_S
\]

where:
- \(M_S\) = Amount (mg) of clorazepate dipotassium for assay
- \(M_T\) = Amount (mg) of clorazepate dipotassium
- \(A_T\) = Absorbance of the sample solution
- \(A_S\) = Absorbance of the standard solution

Containers and storage Containers—Tight containers.

Clotiazepam

クロチアゼパム

\[
\text{Clotiazepam, when dried, contains not less than 98.5\% of } \text{C}_{16}\text{H}_{15}\text{ClN}_{2}\text{O}_{5}\text{.}
\]

Description Clotiazepam occurs as white to light yellowish white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in chloroform, freely soluble in methanol, in ethanol (95), in acetone, in acetic acid (100) and in ethyl acetate, soluble in diethyl ether, and practically insoluble in water.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Clotiazepam in 3 mL of sulfuric acid: the solution shows a light yellow fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Clotiazepam in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Prepare the test solution with 0.01 g of Clotiazepam as directed under Oxygen Flask Combustion Method <1.007>, using 10 mL of diluted hydrogen peroxide (30) (1 in 5) as the absorbing liquid. Apply a small amount of water to the upper part of the Apparatus A, pull out C carefully, wash C, B and the inner side of A with 15 mL of methanol, and use the obtained solution as the test solution. Add 0.5 mL of dilute nitric acid to 15 mL of the test solution: this solution responds to the Qualitative Tests <1.099> (2) for chloride. The remaining test solution responds to the Qualitative Tests <1.099> (1) for sulfate.

Melting point 20°C to 106°C to 109°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Clotiazepam in 10 mL of ethanol (95): the solution is clear and is not more colored than the following control solution.

Control solution: To 5 mL of Matching Fluid C add 0.01 mol/L hydrochloric acid TS to make 10 mL.

(2) Chloride <1.03>—To 1.0 g of Clotiazepam add 50 mL of water, shake for 30 minutes, and filter. To 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Clotiazepam according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Clotiazepam, according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.25 g of Clotiazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 20 mL, pipet 2 mL of this solution, add acetone to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 80°C, 3 hours).

Residue on ignition <2.44> not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clotiazepam, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid (potentiometric titration). Perform a blank determination in, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 31.88 mg of \(\text{C}_{16}\text{H}_{15}\text{ClN}_{2}\text{O}_{5}\)

Containers and storage Containers—Tight containers. Storage—Light-resistant.
Clotrimazole

クロトリマゾール

\[ \text{C}_{22}\text{H}_{17}\text{ClN}_{2} : 344.84 \]
1-[(2-Chlorophenyl)(diphenyl)methyl]-1H-imidazole

[23593-75-1]

Clotrimazole, when dried, contains not less than 98.0% of C\textsubscript{22}H\textsubscript{17}ClN\textsubscript{2}.

**Description** Clotrimazole occurs as a white, crystalline powder. It is odorless and tasteless.

It is freely soluble in dichloromethane and in acetic acid (100), soluble in N,N-dimethylformamide, in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

**Identification** (1) To 0.1 g of Clotrimazole add 10 mL of 5 mol/L hydrochloric acid TS, dissolve by heating, and cool. To this solution add 3 drops of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Clotrimazole in methanol (1 in 5000) as directed under Ultra-violet-visible Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clotrimazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Clotrimazole as directed under Flame Coloration Test (2) \(<1.42>\): a green color appears.

**Melting point** \(<2.60>\) 142 – 145°C

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Clotrimazole in 10 mL of dichloromethane: the solution is clear and colorless.

(2) Chloride \(<1.03>\)—Dissolve 1.0 g of Clotrimazole in 40 mL of N,N-dimethylformamide, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.60 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of N,N-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(3) Sulfate \(<1.14>\)—Dissolve 0.5 g of Clotrimazole in 10 mL of methanol, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.05 mL of 0.005 mol/L sulfuric acid VS, 10 mL of methanol, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(4) Heavy metals \(<1.07>\)—Proceed with 2.0 g of Clotrimazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic \(<1.17>\)—Prepare the test solution with 1.0 g of Clotrimazole according to Method 3, and perform the test (not more than 2 ppm).

(6) Imidazole—Dissolve 0.10 g of Clotrimazole in exactly 10 mL of dichloromethane, and use this solution as the sample solution. Separately, dissolve 25 mg of imidazole for thin-layer chromatography in dichloromethane to make exactly 50 mL. Pipet 5 mL of this solution, add dichloromethane to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03>\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and chloroform (3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly sodium hydrochloride TS on the plate, and air-dry the plate for 15 minutes, then spray evenly potassium iodide-starch TS on the plate: the spot from the sample solution, corresponding to that from the standard solution, is not more intense than that from the standard solution.

(7) (2-Chlorophenyl)-diphenylmethanol—Dissolve 0.20 g of Clotrimazole in exactly 10 mL of dichloromethane, and use this solution as the sample solution. Separately, dissolve 0.010 g of (2-chlorophenyl)-diphenylmethanol for thin-layer chromatography in dichloromethane to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03>\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluororescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ammonia solution (28:50:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution, corresponding to that from the standard solution, is not more intense than that from the standard solution.

**Loss on drying** \(<2.47>\) Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** \(<2.44>\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.35 g of Clotrimazole, previously dried, and dissolve in 80 mL of acetic acid (100), and titrate \(<2.50>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 34.48 mg of C\textsubscript{22}H\textsubscript{17}ClN\textsubscript{2}.

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.
Cloxacin Sodium Hydrate

クロキサシリンナトリウム水和物

C_{19}H_{17}ClN_3NaO_5S.H_2O: 475.88
Monosodium (2S,5R,6R)-6-[(3-(2-chlorophenyl)-5-methylisoxazole-4-carbonyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [7081-44-9]

Cloxacillin Sodium Hydrate contains not less than 900 μg (potency) and not more than 960 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cloxacillin Sodium Hydrate is expressed as mass (potency) of cloxacillin (C_{19}H_{18}ClN_3O_5S: 435.88).

Description Cloxacillin Sodium Hydrate occurs as white to light yellowish white, crystals or crystalline powder.

It is freely soluble in water, in N,N-dimethylformamide and in methanol, and sparingly soluble in ethanol (95%).

Identification (1) Determine the absorption spectrum of a solution of Cloxacillin Sodium Hydrate in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cloxacillin Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cloxacillin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum or the spectrum of Cloxacillin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Cloxacillin Sodium Hydrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> [α]_D^20^0: +163 - +171° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH <2.5> Dissolve 1.0 g of Cloxacillin Sodium Hydrate in 10 mL of water: the pH of the solution is between 5.0 and 7.5.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cloxacillin Sodium Hydrate in 10 mL of water is clear and colorless to light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cloxacillin Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.12>—Prepare the test solution with 1.0 g of Cloxacillin Sodium Hydrate according to Method 5, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Cloxacillin Sodium Hydrate in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cloxacillin obtained from the sample solution is not larger than the peak area of cloxacillin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.953 g of diammonium hydrogen phosphate in 700 mL of water, and add 250 mL of acetonitrile. Adjust the pH to 4.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cloxacillin is about 24 minutes.

Time span of measurement: About 3 times as long as the retention time of cloxacillin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cloxacillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Weigh accurately about 50 mg of Cloxacillin Sodium RS, dissolve in a suitable amount of the mobile phase, add 5 mL of a solution of guaifenesin in the mobile phase (1 in 200), then add the mobile phase to make exactly 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, guaifenesin and cloxacillin are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of cloxacillin to that of guaifenesin is not more than 1.0%.

Water <2.48> 3.0 - 4.5% (0.2 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Cloxacillin Sodium RS equivalent to about 20 mg (potency), and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the
high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Cloxacillin Sodium Hydrate equivalent to about 20 mg (potency), and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Cloxazolam

クロキサゾラム

\[
\text{C}_{17}\text{H}_{14}\text{Cl}_{2}\text{N}_{2}\text{O}_{2} : 349.21
\]

\(11b\text{RS})\)-10-Chloro-11b-(2-chlorophenyl)-2,3,7,11b-tetrahydro[1,3]oxazolo[3,2-\(\text{d}\)][1,4]benzodiazepin-6(5\(\text{H}\))one \[24166-13-0\]

Cloxazolam, when dried, contains not less than 99.0% of \(\text{C}_{17}\text{H}_{14}\text{Cl}_{2}\text{N}_{2}\text{O}_{2}\).

Description Cloxazolam occurs as white crystals or crystalline powder.

It is odorless and tasteless.

It is freely soluble in acetic acid (100), sparingly soluble in dichloromethane, slightly soluble in ethanol (99.5) and in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Melting point: about 200°C (with decomposition).

Identification (1) Dissolve 0.01 g of Cloxazolam in 10 mL of ethanol (99.5) by heating, and add 1 drop of hydrochloric acid: the solution shows a light yellow color and a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). Add 1 mL of sodium hydroxide TS to this solution: the color and fluorescence of this solution disappear immediately.

(2) Dissolve 0.01 g of Cloxazolam in 5 mL of dilute hydrochloric acid by heating in a water bath for 10 minutes. After cooling, 1 mL of this solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) Place 2 g of Cloxazolam in a 200-mL flask, add 50 mL of ethanol (95) and 25 mL of sodium hydroxide TS, and boil under a reflux condenser for 4 hours. After cooling, neutralize with dilute hydrochloric acid, and extract with 30 mL of dichloromethane. Dehydrate with 3 g of anhydrous sodium sulfate, filter, and evaporate the dichloromethane of the filtrate. Dissolve the residue in 5 mL of methanol by heating on a water bath, and cool immediately in an ice bath. Collect the crystals, and dry the crystals is vacuum at 60°C for 1 hour: it melts \(\approx 2.60\) between 87°C and 91°C.

(4) Determine the absorption spectrum of a solution of Cloxazolam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Proceed with Cloxazolam as directed under Flame Coloration Test <1.04> (2), and perform the test: a green color appears.

Absorbance \(\leq 2.24\) \(E_{1\%}^{1\text{cm}}\) (244 nm): 390 – 410 (after drying, 1 mg, ethanol (99.5), 100 mL).

Purity (1) Chloride \(\leq 1.03\)—To 1.0 g of Cloxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of this filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals \(\leq 1.07\)—Proceed with 1.0 g of Cloxazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic \(\leq 1.17\)—Place 1.0 g of Cloxazolam in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat gently. Repeat the addition of 2 to 3 mL of nitric acid at times, and continue heating until a colorless to light yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate solution, and heat the solution until dense white fumes are evolved, and evaporate to a volume of 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(4) Related substances—Dissolve 0.05 g of Cloxazolam in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of toluene and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than that from the standard solution.

Loss on drying \(\leq 2.4\%\) Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition \(\leq 2.4\%\) Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Cloxazolam, previously dried, and dissolve in 50 mL of acetic acid (100). Titrate \(\leq 2.50\) with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 34.92 mg of \(\text{C}_{17}\text{H}_{14}\text{Cl}_{2}\text{N}_{2}\text{O}_{2}\).
Containers and storage  Containers—Tight containers.  Storage—Light-resistant.

Cocaine Hydrochloride

コカイン塩酸塩

\[
\begin{align*}
\text{C}_{17}\text{H}_{21}\text{NO}_4\cdot\text{HCl} &: 339.81 \\
(1R,2R,3S,5S)-2-\text{Methoxycarbonyl}-8-\text{methyl}-8-\text{azabicyclo}[3.2.1]\text{oct}-3-\text{yl} \text{ benzoate monohydrochloride} \[53-21-4]\n\end{align*}
\]

Cocaine Hydrochloride, when dried, contains not less than 98.0% of \(\text{C}_{17}\text{H}_{21}\text{NO}_4\cdot\text{HCl}\).

Description  Cocaine Hydrochloride occurs as colorless crystals or a white crystalline powder.

It is very soluble in water, freely soluble in ethanol (95%) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification  (1) Determine the absorption spectrum of a solution of Cocaine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Cocaine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cocaine Hydrochloride, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Cocaine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation  <2.49> \([\alpha]_D^0\) = \(-70 \text{ to } -73^\circ\) (after drying, 0.5 g, water, 20 mL, 100 mm).

Purity  (1) Acidity—Dissolve 0.5 g of Cocaine Hydrochloride in 10 mL of water, add 1 drop of methyl red TS, and neutralize with 0.01 mol/L sodium hydroxide VS: the consumed volume is not more than 1.0 mL.

(2) Cinnamyl cocaine—Dissolve 0.10 g of Cocaine Hydrochloride in 5 mL of water, and add 0.3 mL of diluted sulfuric acid (1 in 20) and 0.10 mL of 0.02 mol/L potassium permanganate VS: the red color does not disappear within 30 minutes.

(3) Isoatropyl cocaine—Dissolve 0.10 g of Cocaine Hydrochloride in 30 mL of water in a beaker. Transfer 5 mL of this solution to a test tube, add 1 drop of ammonia TS, and mix. After the precipitate is coagulated, add 10 mL of water, and transfer the mixture to the former beaker, to which 30 mL of water has been added previously. Wash the test tube with 10 mL of water, combine the washings with the mixture in the beaker, add 3 drops of ammonia TS to the combined mixture, and mix gently: a crystalline precipitate is produced. Allow to stand for 1 hour: the supernatant liquid is clear.

Loss on drying  <2.47> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition  <2.44> Not more than 0.1% (0.5 g).

Assay  Weigh accurately about 0.5 g of Cocaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 33.98 mg of \(\text{C}_{17}\text{H}_{21}\text{NO}_4\cdot\text{HCl}\).

Containers and storage  Containers—Tight containers. Storage—Light-resistant.

Coconut Oil

Oleum Cocos

ヤシ油

Coconut oil is the fixed oil obtained from the seeds of \(\text{Cocos nucifera}\) Linné (\text{Palmae}).

Description  Coconut Oil is a white to light yellow mass or a colorless or light yellow, clear oil. It has a slight, characteristic odor and a mild taste. It is freely soluble in diethyl ether and in petroleum ether. It is practically insoluble in water. At a temperature below 15°C, it congeals to a hard and brittle solid.

Melting point: 20 – 28°C

Acid value  <1.13> Not more than 0.2.

Saponification value  <1.13> 246 – 264

Unsaponifiable matter  <1.13> Not more than 1.0%.

Iodine value  <1.13> 7 – 11

Containers and storage  Containers—Tight containers.
Codeine Phosphate Hydrate

Codeine Phosphate Hydrate occurs as white to yellowish white crystals or crystalline powder. It is freely soluble in water and in acetic acid (100), slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether. The pH of a solution of Codeine Phosphate Hydrate (1 in 10) is between 3.0 and 5.0. It is affected by light.

Identification (1) Determine the absorption spectrum of a solution of Codeine Phosphate Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Codeine Phosphate Hydrate, previously dried at 105°C same wavelengths. Both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Codeine Phosphate Hydrate (1 in 20) responds to the Qualitative Tests <2.09> (1) for phosphate. Optical rotation <2.49> [α]D20 = −98 to −102° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

Purity (1) Chloride <1.03>—Perform the test with 0.5 g of Codeine Phosphate Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(2) Sulfate <1.14>—Perform the test with 0.20 g of Codeine Phosphate Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

(3) Related substances—Dissolve 0.20 g of Codeine Phosphate Hydrate in 10 mL of a mixture of 0.01 mol/L hydrochloric acid TS and ethanol (99.5) (4:1). Use this solution as the standard solution. Prepare the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 1.5–3.0% (0.5 g, volumetric titration, direct titration).

Assay Dissolve about 0.5 g of Codeine Phosphate Hydrate, accurately weighed, in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction. Each mL of 0.1 mol/L perchloric acid VS = 39.74 mg of C18H21NO3.H3PO4.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

1% Codeine Phosphate Powder

10% Codeine Phosphate Powder contains not less than 10.0% and not more than 11.0% of codeine phosphate hydrate C18H21NO3.H3PO4·1/2H2O: 406.37.

Method of preparation

<table>
<thead>
<tr>
<th>Codeine Phosphate Hydrate</th>
<th>10 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose Hydrate</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g Prepare as directed under Granules or Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 1% Codeine Phosphate Powder (1 in 100) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm.

Assay Weigh accurately about 5 g of 1% Codeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay (previously determine the water <2.48> in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Qf and Qs, of the peak area of codeine to that of the internal standard.
Weigh accurately about 2.5 g of 10% Assay exhibits a maximum between 283 nm and 287 nm.

Separately, weigh accurately about 50 mg of codeine phosphate for assay (previous-solution as the sample solution). Separately, weigh accurately about 50 mg of codeine phosphate powder, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution.

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.
Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of codeine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

10% Codeine Phosphate Powder

コデインリン酸塩散 10%

10% Codeine Phosphate Powder contains not less than 9.3% and not more than 10.7% of codeine phosphate hydrate (C18H21NO3.H3PO4.1/2H2O: 406.37).

Method of preparation

<table>
<thead>
<tr>
<th>Codeine Phosphate Hydrate</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose Hydrate</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td></td>
<td>To make 1000 g</td>
</tr>
</tbody>
</table>

Prepare as directed under Powders, with the above ingredients.

Identification—Determine the absorption spectrum of a solution of 10% Codeine Phosphate Powder (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.242>: it exhibits a maximum between 283 nm and 287 nm.

Assay—Weigh accurately about 2.5 g of 10% Codeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay (previous-

\[ M_S = M_5 \times \frac{Q_T}{Q_S} \times 1.023 \]

where \( M_5 \) is the amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis.

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.
Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Codeine Phosphate Tablets

コデインリン酸塩錠

Codeine Phosphate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of codeine phosphate hydrate (C18H21NO3.H3PO4.1/2H2O: 406.37).

Method of preparation—Prepare as directed under Tablets, with Codeine Phosphate Hydrate.

Identification—To a quantity of powdered Codeine Phosphate Tablets, equivalent to 0.1 g of Codeine Phosphate Hydrate according to the labeled amount, add 20 mL of water, shake, and filter. To 2 mL of the filtrate add water to make 100 mL, and determine the absorption spectrum as directed
under Ultraviolet-visible Spectrophotometry ($\leq 2.40$): it exhibits a maximum between 283 nm and 287 nm.

**Uniformity of dosage units** ($\leq 6.02$) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Codeine Phosphate Tablets add 3 V/25 mL of water to disintegrate, add 2 V/25 mL of diluted dilute sulfuric acid (1 in 20), and treat with ultrasonic waves for 10 minutes. To this solution add exactly 2 V/25 mL of the internal standard solution, add water to make $V$ mL so that each mL contains about 0.2 mg of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.1/2H_2O$), filter, and use the filtrate as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay (separately, determine the water $<2.4\sigma$ in the same manner as Codeine Phosphate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution, add water to make exactly 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

**Operating conditions**
- Proceed as directed in the operating conditions in the Assay.

**System suitability**

- System performance: When the procedure is run with 100 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of codeine are not less than 5000 and not more than 1.5, respectively.
- System repeatability: When the test is repeated 6 times with 100 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of codeine is not more than 2.0%.

**Assay** Weigh accurately and powder not less than 20 Codeine Phosphate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.1/2H_2O$), add 30 mL of water, shake, add 20 mL of diluted dilute sulfuric acid (1 in 20), treat the mixture with ultrasonic waves for 10 minutes, and add water to make exactly 100 mL. Filter the solution, then pipet 5 mL of the filtrate, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay (previously determine the water $<2.4\sigma$ in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography ($\leq 2.0\sigma$) according to the following conditions, and calculate the ratios, $Q_1$ and $Q_2$, of the peak area of codeine to that of the internal standard.

\[
\text{Amount (mg) of codeine phosphate hydrate} = M_5 \times \frac{Q_1}{Q_2} \times \frac{V}{250} \times 1.023
\]

\[
M_5: \text{Amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis}
\]

**Internal standard solution**—A solution of ethylefurin hydrochloride (3 in 2000).

**Dissolution** ($\leq 6.10$) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Codeine Phosphate Tablets is not less than 80%.

Start the test with 1 tablet of Codeine Phosphate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 $\mu$m. Discard the first 10 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add water to make exactly $V$ mL so that each mL contains about 5.6 $\mu$g of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.1/2H_2O$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of codeine phosphate for assay (separately, determine the water $\leq 2.4\sigma$ in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography ($\leq 2.0\sigma$) according to the following conditions, and calculate the ratios, $Q_1$ and $Q_2$, of the peak area of codeine to that of the internal standard.

\[
\text{Amount (mg) of codeine phosphate hydrate} = M_5 \times \frac{Q_1}{Q_2} \times 2 \times 1.023
\]

\[
M_5: \text{Amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis}
\]

**Internal standard solution**—A solution of etilefrine hydrochloride (3 in 10,000).

**Operating conditions**
- Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of distilled phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.
- Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.

**System suitability**

- System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
- System repeatability: When the test is repeated 5 times...
with 20 \mu L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

### Cod Liver Oil

肝油

Cod Liver Oil is the fatty oils obtained from fresh livers and pyloric appendages of *Gadus macrocephalus* Tilesius or *Theragra chalcogramma* Pallas (Gadidae).

Cod Liver Oil contains not less than 2000 Vitamin A Units and not more than 5000 Vitamin A Units per g.

**Description** Cod Liver Oil is a yellow to orange oily liquid. It has a characteristic, slightly fishy odor and a mild taste. It is miscible with chloroform. It is slightly soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air or by light.

**Identification** Dissolve 0.1 g of Cod Liver Oil in 10 mL of chloroform, and to 1 mL of this solution add 3 mL of antimony (III) chloride TS: a blue color develops immediately, but the color fades rapidly.

**Specific gravity** \(<1.13>\ d^2_2: 0.918 – 0.928\)

**Acid value** \(<1.13>\ Not more than 1.7.\)

**Saponification value** \(<1.13>\ 180 – 192\)

**Unsaponifiable matter** \(<1.13>\ Not more than 3.0%.\)

**Iodine value** \(<1.13>\ 130 – 170\)

**Purity** Rancidity—No unpleasant odor of rancid oil is perceptible on warming Cod Liver Oil.

**Assay** Proceed with about 0.5 g of Cod Liver Oil, accurately weighed, as directed in Method 2 under the Vitamin A Assay. Proceed with about 0.5 g of Cod Liver Oil, accurately weighed, as directed in Method 2 under the Vitamin A Assay, and perform the test.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under nitrogen atmosphere.

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### Colchicine

コルヒチン

![Colchicine structure](image)

C_{22}H_{25}NO_6: 399.44

\[\text{N-[}(7S)-(1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl)]acetamide [64-86-8]\]

Colchicine contains not less than 97.0% and not more than 102.0% of C_{22}H_{26}NO_6, calculated on the anhydrous basis and corrected by the amount of ethyl acetate.

**Description** Colchicine occurs as a yellowish white powder. It is very soluble in methanol, freely soluble in \(N,N\)-dimethylformamide, in ethanol (95) and in acetic anhydride, and sparingly soluble in water.

It is colored by light.

**Identification (1)** Determine the absorption spectrum of a solution of Colchicine in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \(2.24\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) To 1 g of potassium bromide for infrared absorption spectrum add 0.5 mL of a solution of Colchicine in methanol (1 in 50), grind thoroughly, and dry in vacuum at 80°C for 1 hour. Determine the infrared absorption spectrum of this powder as directed in the potassium bromide disk method under Infrared Spectrophotometry \(2.25\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \(2.49\) \([\alpha]_D^2\): \(-235\) to \(250^\circ\) (0.1 g calculated on the anhydrous basis and corrected by the amount of ethyl acetate, ethanol (95), 10 mL, 100 mm).

**Purity (1)** Colchicine—Dissolve 0.10 g of Colchicine in 10 mL of water, and to 5 mL of this solution add 2 drops of iron (III) chloride TS: no definite green color develops.

(2) Chloroform and ethyl acetate—Weigh accurately about 0.6 g of Colchicine, dissolve in exactly 2 mL of the internal standard solution, add \(N,N\)-dimethylformamide to make 10 mL, and use this solution as the sample solution. Separately, weigh 0.30 g of chloroform using a 100-mL volumetric flask containing about 20 mL of \(N,N\)-dimethylformamide, and add \(N,N\)-dimethylformamide to make exactly 100 mL. Pipet 2 mL of this solution, add \(N,N\)-dimethylformamide to make exactly 200 mL, and use this solution as the standard solution (1). Separately, weigh accurately about 1.8 g of ethyl acetate using a 100-mL volumetric flask containing about 20 mL of \(N,N\)-dimethylformamide, and add \(N,N\)-dimethylformamide to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution and \(N,N\)-dimethylformamide to make 10 mL,
and use this solution as the standard solution (2). Perform the test with exactly 2 μL each of the sample solution and standard solutions (1) and (2) as directed under Gas Chromatography <2.02> according to the following conditions: the peak area of chloroform from sample solution is not larger than that from the standard solution (1). Calculate the ratios of the peak area of ethyl acetate to that of the internal standard, $Q_{1}$ and $Q_{2}$, of the sample solution and standard solution (2), and calculate the amount of ethyl acetate by the following formula: the amount of ethyl acetate is not more than 6.0%.

\[
\text{Amount (g) of ethyl acetate (C}_4\text{H}_8\text{O}_2) = \frac{M_5}{M_1 \times Q_1/2} \\
\text{M}_5: \text{Amount (g) of ethyl acetate} \\
\text{M}_1: \text{Amount (g) of the sample}
\]

**Internal standard solution**—A solution of 1-propanol in N,N-dimethylformamide (3 in 200).

**Operating conditions**
- Detector: A hydrogen flame-ionization detector.
- Column: A fused silica column 0.53 mm in inside diameter and 30 m in length, coated inside surface with polyethylene glycol 20 M for gas chromatography 1.0 μm in thickness.
- Column temperature: 60°C for 7 minutes, then up to 100°C at a rate of 40°C per minute if necessary, and hold at 100°C for 10 minutes.
- Injection port temperature: A constant temperature of about 130°C.
- Detector temperature: A constant temperature of about 200°C.
- Carrier gas: Helium.
- Flow rate: Adjust the flow rate so that the retention time of ethyl acetate is about 3 minutes.

**System suitability**—
Test for required detectability: Pipet 2 mL of the standard solution (2), and add N,N-dimethylformamide to make exactly 25 mL. Pipet 1 mL of this solution, and add N,N-dimethylformamide to make exactly 50 mL. Confirm that the peak area of ethyl acetate obtained from 2 μL of this solution is equivalent to 0.11 to 0.21% of that from 2 μL of the standard solution (2).

System performance: To 1 mL of chloroform add N,N-dimethylformamide to make 10 mL. To 1 mL of this solution add 2 mL of ethyl acetate and N,N-dimethylformamide to make 50 mL. Confirm that the peak area of ethyl acetate obtained from 2 μL of this solution is equivalent to 0.11 to 0.21% of that from 2 μL of the standard solution (2).

System performance: To 1 mL of chloroform add N,N-dimethylformamide to make 10 mL. To 1 mL of this solution add 2 mL of ethyl acetate and N,N-dimethylformamide to make 50 mL. When the procedure is run with 2 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of ethyl acetate is not more than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of chloroform is not more than 2.0%.

**Water**<2.48> Not more than 2.0% (0.5 g, volumetric titration, back titration).

**Assay**
Weigh accurately about 0.4 g of Colchicine, dissolve in 25 mL of acetic anhydride, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

\[
\text{Each mL of 0.05 mol/L perchloric acid VS} = 19.97 \text{ mg of C}_2\text{H}_2\text{NO}_6
\]

**Containers and storage**
Containers—Tight containers.
Storage—Light-resistant.
Colistin Sodium Methanesulfonate
コリスチンメタンスルホン酸ナトリウム

Colistin Sodium Methanesulfonate is the sodium salt of colistin derivatives, and is a mixture of colistin A sodium methanesulfonate and colistin B sodium methanesulfonate. It, when dried, contains not less than 11,500 Units per mg. The unit of Colistin Sodium Methanesulfonate is expressed as mass of colistin A (R = 6-methyloctanic acid, R' = H; C_{53}H_{100}N_{16}O_{13}: 1169.46).

**Description**
Colistin Sodium Methanesulfonate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

**Identification (1)**
Dissolve 20 mg of Colistin Sodium Methanesulfonate in 2 mL of water, add 0.5 mL of sodium hydroxide TS, and add 5 drops of copper (II) sulfate TS while shaking: a blue-purple color develops.

(2) Dissolve 40 mg of Colistin Sodium Methanesulfonate in 1 mL of 1 mol/L hydrochloric acid TS, and add 0.5 mL of dilute iodine TS: the color of iodine disappears.

(3) Determine the infrared absorption spectrum of Colistin Sodium Methanesulfonate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Colistin Sodium Methanesulfonate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Colistin Sodium Methanesulfonate responds to the Qualitative Tests <1.09> (1) for sodium salt.

**pH**
Dissolve 0.1 g of Colistin Sodium Methanesulfonate in 10 mL of water, and allow to stand for 30 minutes: the pH of the solution is between 6.5 and 8.5.

**Purity**
Clarity and color of solution—Dissolve 0.16 g of Colistin Sodium Methanesulfonate in 10 mL of water: the solution is clear and colorless.

Heavy metals <1.07>—Proceed with 1.0 g of Colistin Sodium Methanesulfonate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

Arsenic <1.11>—Prepare the test solution with 1.0 g of Colistin Sodium Methanesulfonate according to Method 4, and perform the test (not more than 2 ppm).

Free colistin—Dissolve 80 mg of Colistin Sodium Methanesulfonate in 3 mL of water, add 0.05 mL of a solu-
tion of silicotungstic acid 26-water (1 in 10), and immediately compare the solution with the reference suspension described under Test Methods for Plastic Containers <7.02>: the turbidity is not greater than that of the reference suspension (not more than 0.25%).

**Loss on drying**
Not more than 3.0% (0.1 g, reduced pressure, 60°C, 3 hours).

**Assay**
Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—Escherichia coli NIHJ

(ii) Culture medium—To 10.0 g of peptone, 30.0 g of sodium chloride, 3.0 g of meat extract and 20.0 g of agar add 1000 mL of water, then add a suitable amount of sodium hydroxide TS so that the pH of the medium is being 6.5 to 6.6 after sterilization, sterile, and use this as the seeded agar medium and the agar medium for base layer.

(iii) Standard solutions—Weigh accurately an amount of Colistin Sodium Methanesulfonate RS, previously dried, dissolve in phosphate buffer solution, pH 6.0 to make a solution containing 100,000 Units per mL, and use this solution as the standard stock solution. Keep the standard stock solution at 10°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, and add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 Units and 2500 Units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Colistin Sodium Methanesulfonate, previously dried, dissolve in phosphate buffer solution, pH 6.0 to make a solution containing about 100,000 Units per mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 Units and 2500 Units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage**
Containers—Tight containers.
Colistin Sulfate

コリスチン硫酸塩

Colistin Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of Bacillus polymyxa var. colistinus.

It, when dried, contains not less than 16,000 units per mg. The potency of Colistin Sulfate is expressed as unit calculated from the amount of colistin A (C₅₃H₁₀₀N₁₆O₁₃·2H₂SO₄: 1169.46). One unit of Colistin Sulfate is equivalent to 0.04 µg of colistin A (C₅₃H₁₀₀N₁₆O₁₃).

**Description** Colistin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

**Identification** (1) Dissolve 20 mg of Colistin Sulfate in 2 mL of water, add 0.5 mL of sodium hydroxide, then add 5 drops of copper (II) sulfate. A purple color develops.

(2) Dissolve 50 mg of Colistin Sulfate in 10 mL of diluted hydrochloric acid (1 in 2). Transfer 1 mL of this solution in a tube for hydrolysis, seal, and heat at 135°C for 5 hours. After cooling, open the tube, and evaporate the content to dryness until the odor of hydrochloric acid is no more perceptible. Dissolve the residue in 0.5 mL of water, and use this solution as the sample solution. Separately, dissolve 20 mg each of L-leucine, L-threonine, phenylalanine and L-serine in 10 mL of water, and use these solutions as the standard solutions.

**Purity** (1) Sulfuric acid—Weigh accurately about 0.25 g of previously dried Colistin Sulfate, dissolve in a suitable amount of water, adjust the pH to 11 with ammonia solution (28), and add water to make 100 mL. To this solution add exactly 10 mL of 0.1 mol/L barium chloride VS and 50 mL of ethanol (99.5), and titrate with 2.50 M 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the blue-purple color of the solution disappears (indicator: 0.5 mg of phthalein purple): the amount of sulfuric acid (SO₄) is 16.0 to 18.0%.

Each mL of 0.1 mol/L barium chloride VS = 9.606 mg of SO₄

(2) Related substances—Dissolve 50 mg of Colistin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of pyridine, 1-butanol, water, 25 mL, 100 mm).

**Residue on drying** <2.41> Not more than 6.0% (1 g, in vacuum, 60°C, 3 hours).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—Escherichia coli NIHJ

(ii) Culture medium—Dissolve 10.0 g of peptone, 30.0 g of sodium chloride, 3.0 g of meat extract and 15.0 g of agar in 1000 mL of water, adjust the pH with sodium hydroxide TS so that the solution will be 6.5 to 6.6 after sterilization, and use as the agar media for seed layer and for base layer.

(iii) Standard solutions—Weigh accurately an amount of Colistin Sulfate RS, previously dried, equivalent to about 1,000,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 10 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 10°C, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 units and 2500 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Colistin Sulfate, previously dried, equivalent to about 1,000,000 units, and dissolve in phosphate buffer solution, pH 6.0 to make exactly 10 mL. Take exactly a suitable
amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 units and 2500 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

**Corn Oil**

*Oleum Maydis*

トウモロコシ油

Corn Oil is the fixed oil obtained from the embryo of *Zea mays* Linné (*Gramineae*).

**Description** Corn Oil is a clear, light yellow oil. It is odorless or has a slight odor, and a mild taste.

It is miscible with diethyl ether and with petroleum ether. It is slightly soluble in ethanol (95), and practically insoluble in water.

At –7°C, it congeals to an unguentary mass.

**Specific gravity** $d_{25}^2$: 0.915 – 0.921

**Acid value** <1.13 Not more than 0.2.

**Saponification value** <1.13 187 – 195

**Unsaponifiable matter** <1.13 Not more than 1.5%.

**Iodine value** <1.13 103 – 130

**Containers and storage** Containers—Tight containers.

**Corn Starch**

*Amylum Maydis*

トウモロコシデンプン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (*,* *).

Corn Starch consists of starch granules derived from the ripe seeds of *Zea mays* Linné (*Gramineae*).

**Description** Corn Starch occurs as white to pale yellowish white masses or powder.

It is practically insoluble in water and in ethanol (99.5). •

**Identification** (1) Under a microscope <5.0D, using mixture of water and glycerin (1:1), Corn Starch appears as either angular polyhedral granules of irregular sizes with diameters ranging from about 2 μm to about 23 μm or as rounded or spheroidal granules of irregular sizes with diameters ranging from about 25 μm to about 35 μm. The central hilum consists of a distinct cavity or two- to five-rayed cleft, and there are no concentric striations. Between orthogonally oriented polarizing plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Corn Starch add 50 mL of water, boil for 1 minute, and allow to cool: a subtle white-turbid, pasty liquid is formed.

(3) To 1 mL of the pasty liquid obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): an orange-red to deep blue color is formed and the color disappears by heating.

**pH** <2.54 Put 5.0 g of Corn Starch in a non-metal vessel, add 25.0 mL of freshly boiled and cooled water, mix gently for 1 minute, and allow to stand for 15 minutes: the pH of the solution is between 4.0 and 7.0.

**Purity** (1) Iron—To 1.5 g of Corn Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (1 in 5) and 0.1 mL of mercapo acetic acid, and mix. Alkalize with ammonia solution (28) to litmus paper, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Corn Starch add 50.0 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate <2.50 with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm, calculated as hydrogen peroxide).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the following figure.

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through

---

The figures are in mm.
Reisdue on ignition 90 minutes.

Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromphenol blue TS, and titrate with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

\[ \text{Amount (ppm) of sulfur dioxide} = \frac{V \times M}{1000 \times 3.203} \]

\[ M: \text{Amount (g) of the sample} \]

\[ V: \text{Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed} \]

* Foreign matter—Under a microscope <5.0>, Corn Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant.

**Loss on drying** <2.4> Not more than 15.0% (1 g, 130°C, 90 minutes).

**Residue on ignition** <2.4> Not more than 0.6% (1 g).

**Containers and storage** Containers—Well-closed containers.

Cortisone Acetate

コルチゾン酢酸エステル

\[
\text{C}_{23}\text{H}_{30}\text{O}_6: 402.48
\]

17,21-Dihydroxypregn-4-ene-3,11,20-trione 21-acetate [50-04-4]

Cortisone Acetate, when dried, contains not less than 97.0% and not more than 102.0% of C_{23}H_{30}O_{6}.

**Description** Cortisone Acetate occurs as white crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Melting point: about 240°C (with decomposition).

**Identification** (1) To 2 mg of Cortisone Acetate add 2 mL of sulfuric acid, and allow to stand for a while: a yellowish green color is produced, and it gradually changes to yellow-orange. Examine the solution under ultraviolet light: the solution shows a light green fluorescence. Add carefully 10 mL of water to this solution: the color of the solution is discharged, and the solution remains clear.

(2) Determine the absorption spectrum of a solution of Cortisone Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cortisone Acetate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Cortisone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Cortisone Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Cortisone Acetate and Cortisone Acetate RS in acetonitrile, respectively, then evaporate the acetonitrile to dryness, and repeat the test on the residues.

**Optical rotation** <2.4> [α]_{D}^{20}: +207 – +216° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

**Purity** Related substances—Dissolve 25 mg of Cortisone Acetate in 10 mL of a mixture of acetonitrile, water and acetic acid (100) (70:30:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution and add the mixture of acetonitrile, water and acetic acid (100) (70:30:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than cortisone acetate obtained with the sample solution is not larger than 1/2 times the peak area of cortisone acetate with the standard solution, and the total area of the peaks other than cortisone acetate is not larger than 1.5 times the peak area of cortisone acetate with the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water and acetonitrile (7:3). Mobile phase B: A mixture of acetonitrile and water (7:3). Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 5</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>5 – 25</td>
<td>90 → 10</td>
<td>10 → 90</td>
</tr>
<tr>
<td>25 – 30</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

Flow rate: About 1 mL per minute.

Time span of measurement: About 3 times as long as the retention time of cortisone acetate beginning after the sol-
Cresol

クレゾール

C₆H₅O: 108.14

Cresol is a mixture of isomeric cresols.

Description Cresol is a clear, colorless or yellow to yellow-brown liquid. It has a phenol-like odor. It is miscible with ethanol (95) and with diethyl ether. It is sparingly soluble in water. It dissolves in sodium hydroxide TS. A saturated solution of Cresol is neutral to bromocresol purple TS. It is a highly refractive liquid. It becomes dark brown by light or on aging.

Identification To 5 mL of a saturated solution of Cresol add 1 to 2 drops of dilute iron (III) chloride TS: a blue-purple color develops.

Specific gravity <2.56> dₑ°: 1.032 – 1.041

Purity (1) Hydrocarbons—Dissolve 1.0 mL of Cresol in 60 mL of water: the solution shows no more turbidity than that produced in the following control solution.

Control solution: To 54 mL of water add 6.0 mL of 0.005 mol/L sulfuric acid and 1.0 mL of barium chloride TS, and after thorough shaking, allow to stand for 5 minutes.

(2) Sulfur compounds—Transfer 20 mL of Cresol in a 100-mL conical flask, place a piece of moistened lead (II) acetate paper on the mouth of the flask, and warm for 5 minutes on a water bath: the lead (II) acetate paper may develop a yellow color, but neither a brown nor a dark tint.

Distilling range <2.57> 196 – 206°C, not less than 90 vol%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cresol Solution

クレゾール水

Cresol Solution contains not less than 1.25 vol% and not more than 1.60 vol% of cresol.

Method of preparation

| Saponated Cresol Solution | 30 mL |
| Water, Purified Water or Purified Water in Containers | a sufficient quantity |

To make 1000 mL

Prepare by mixing the above ingredients.

Description Cresol Solution is a clear or slightly turbid, yellow solution. It has the odor of cresol.

Identification Shake 0.5 mL of the oily layer obtained in the Assay with 30 mL of water, filter, and perform the following tests using this filtrate as the sample solution:

(1) To 5 mL of the sample solution add 1 to 2 drops of iron (III) chloride TS: a blue-purple color develops.
Containers and storage
Containers—Tight containers.

Saponated Cresol Solution

クロゾール石ケン液

Saponated Cresol Solution contains not less than 42 vol% and not more than 52 vol% of cresol.

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cresol</td>
<td>500 mL</td>
<td></td>
</tr>
<tr>
<td>Fixed Oil</td>
<td>300 mL</td>
<td></td>
</tr>
</tbody>
</table>
| Potassium Hydroxide| a suitable quantity | Added to
| Water, Purified Water or Purified Water in Containers | a sufficient quantity |

To make 1000 mL

Dissolve Potassium Hydroxide, in required quantity for saponification, in a sufficient quantity of Water, Purified Water or Purified Water in Containers. Add this solution to fixed oil, previously warmed, add a sufficient quantity of Ethanol, if necessary, heat in a water bath by thorough stirring, and continue the saponification. After complete saponification, add Cresol, stir thoroughly until the mixture becomes clear, and add sufficient Water, Purified Water or Purified Water in Containers to make 1000 mL. A corresponding amount of Sodium Hydroxide may be used in place of Potassium Hydroxide.

Description
Saponated Cresol Solution is a yellow-brown to red-brown, viscous liquid. It has the odor of cresol.

It is miscible with water, with ethanol (95) and with glycerin.

It is alkaline.

Identification
Proceed as directed in the Identification under Cresol, using the distillate in the Purity (3).

Purity
(1) Alkalinity—Mix well 0.50 mL of Saponated Cresol Solution with 10 mL of neutralized ethanol, add 2 to 3 drops of phenolphthalein TS and 0.10 mL of 1 mol/L hydrochloric acid VS: no red color develops.

(2) Unsaponified matter—To 1.0 mL of Saponated Cresol Solution add 5 mL of water, and shake: the solution is clear.

(3) Cresol fraction—Transfer 180 mL of Saponated Cresol Solution to a 2000-mL distilling flask, add 300 mL of water and 100 mL of dilute sulfuric acid, and distil with steam until the distillate becomes clear. Draw off the water from the condenser, and continue the distillation until water vapor begins to come out of the tip of the condenser. Cool the condenser again, and continue distillation for 5 minutes. Dissolve 20 g of sodium chloride per 100 mL of the distillate, allow to stand, and collect the separated clear oil layer. After adding about 15 g of powdered calcium chloride for drying in small portions with frequent shaking, allow to stand for 4 hours. Filter, and distil exactly 50 mL of the filtrate: the distillate is not less than 43 mL between 196°C and 206°C.

Assay
Transfer 5 mL of Saponated Cresol Solution, exactly measured, to a 500-mL distilling flask, holding the pipet vertically for 15 minutes to draw off the solution into the flask. Add 200 mL of water, 40 g of sodium chloride and 3 mL of dilute sulfuric acid, connect the distilling apparatus with the distilling flask, and distil into a cassia flask which contains 30 g of powdered sodium chloride and exactly 3 mL of kerosene, until the distillate reaches 90 mL. Draw off the water from the condenser, and continue the distillation until water vapor begins to come out of the tip of the condenser. Allow the cassia flask to stand in warm water for 15 minutes to dissolve the sodium chloride, and allow to stand for 4 hours. Filter, and distil exactly 50 mL of the filtrate: the distillate is not less than 43 mL between 196°C and 206°C.

Storage—Light-resistant.

Croconazole Hydrochloride

クロコナゾール塩酸塩

Croconazole Hydrochloride, when dried, contains not less than 98.5% of C_{16}H_{15}ClN_{2}O.HCl.

Description
Croconazole Hydrochloride occurs as white to pale yellowish white crystals or crystalline powder.

It is very soluble in water, freely soluble in methanol, in ethanol (95) and in acetic acid (100), and practically insolu-
ble in diethyl ether.

**Identification** (1) Determine the absorption spectrum of a solution of Croconazole Hydrochloride in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.2d>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Croconazole Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.2s>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.05 g of Croconazole Hydrochloride in 10 mL of water, add 2 mL of sodium hydroxide TS and 20 mL of diethyl ether, and shake. Wash the separated aqueous layer with two 10-mL portions of diethyl ether, and acidify the solution with 2 mL of dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

**Melting point** <2.6d> 148 ~ 153°C

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Croconazole Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Croconazole Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol and ammonia solution (28) (30:15:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and other than the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 60°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.6 g of Croconazole Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS [indicator: 1 to 2 drops of a solution of malachite green oxalate in acetic acid (100) (1 in 100)] until the color of the solution changes from blue-green through green to yellow-green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 34.72 mg of C₁₈H₁₅ClN₂O.HCl

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Croscarmellose Sodium**

クロスカルメロースナトリウム

[74811-65-7]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the test that are not harmonized are marked with symbol (••).

**Croscarmellose Sodium** is the sodium salt of a cross-linked poly carboxymethylcellulose.

**Description** Croscarmellose Sodium occurs as a white to yellowish white powder.

It is practically insoluble in ethanol (99.5) and in diethyl ether.

It swells with water and becomes a suspension.

It is hygroscopic. •

**Identification** (1) To 1 g of Croscarmellose Sodium add 100 mL of a solution of methylene blue (1 in 250,000), stir well, and allow to stand: blue cotton-like precipitates appear.

(2) To 1 g of Croscarmellose Sodium add 50 mL of water, and stir well to make a suspension. To 1 mL of this suspension add 1 mL of water and 5 drops of freshly prepared solution of 1-naphthol in methanol (1 in 25), and gently add 2 mL of sulfuric acid along a wall of the vessel: a red-purple color appears at the zone of contact.

(3) The suspension obtained in (2) responds to the Qualitative Tests <1.09> (1) for sodium salt.

**pH** <2.54> To 1.0 g of Croscarmellose Sodium add 100 mL of water, and stir for 5 minutes: the pH of the supernatant liquid is between 5.0 and 7.0.

**Purity** •(1) Heavy metals <1.07>—Proceed with 2.0 g of Croscarmellose Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm). •

•(2) Sodium chloride and sodium glycolate—The total amount of sodium chloride and sodium glycolate is not more than 0.5%, calculated on the dried basis.

(i) Sodium chloride: Weigh accurately about 5 g of Croscarmellose Sodium, add 50 mL of water and 5 mL of hydrogen peroxide (30), and heat on a water bath for 20 minutes with occasional stirring. After cooling, add 100 mL of water and 10 mL of nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

(ii) Sodium glycolate: Weigh accurately about 0.5 g of Croscarmellose Sodium, add 2 mL of acetic acid (100) and 5 mL of water, and stir for 15 minutes. Add gradually 50 mL of acetone with stirring, then add 1 g of sodium chloride, stir for 3 minutes, and filter through a filter paper moistened with acetone. Wash the residue thoroughly with 30 mL of acetone, combine the filtrate and washings, add acetone to make exactly 100 mL, and use this solution as the sample stock solution. Separately, dissolve 0.100 g of glycolic acid
in water to make 200 mL. Pipet 0.5 mL, 1 mL, 2 mL, 3 mL, and 4 mL of this solution, add water to make them exactly 5 mL, then add 5 mL of acetic acid (100) and acetone to make exactly 100 mL, and designate them standard stock solution (1), standard stock solution (2), standard stock solution (3), standard stock solution (4) and standard stock solution (5), respectively. Pipet 2 mL each of the sample stock solution and the standard stock solutions (1), (2), (3), (4) and (5), and heat them in a water bath for 20 minutes to evaporate acetone. After cooling, add exactly 5 mL of 2,7-dihydroxy- naphthalene TS, mix, then add 15 mL of 2,7-dihydroxy- naphthalene TS, mix, cover the mouth of the vessels with aluminum foil, and heat in a water bath for 20 minutes. After cooling, add sulfuric acid to make exactly 25 mL, mix, and designate them sample solution, standard solution (1), standard solution (2), standard solution (3), standard solution (4) and standard solution (5), respectively. Separately, to 10 mL of a mixture of water and acetic acid (100) (1:1) add acetone to make exactly 100 mL, and proceed with exactly 2 mL of this solution in the same manner for preparation of the sample solution, and use the solution so obtained as the blank solution. Determine the absorbances, A1, A21, A32, A43, A54 and A55, of the sample solution and the standard solutions (1), (2), (3), (4) and (5), respectively, at 540 nm as directed under Ultraviolet-visible Spectrophotometry 2.2.4, using the blank solution as the control. Determine the amount (g) of glycolic acid, X, in 100 mL of the sample solution from the calibration curve obtained with the standard solutions, and calculate the amount of sodium glycolate by the following formula.

\[
A = \frac{1150M/(7102 - 412M - 80C)}{(162 + 584C)/(7102 - 80C)}
\]

\[
S = \frac{A}{(162 + 584C)/(7102 - 80C)}
\]

M: Amount (mmol) of sodium hydroxide needed to neutralize 1 g of sample, calculated on the dried basis

C: The value (%) obtained in Residue on ignition

Loss on drying <2.41 Not more than 10.0% (1 g, 105°C, 6 hours).

Residue on ignition <2.45 14.0 - 28.0% (after drying, 1 g).

Containers and storage Containers—Tight containers.

Cyanamide

シアナミド

\[\text{H}_2\text{N—CN}\]

CH₃N₂: 42.04
Aminonitrile [420-04-2]

Cyanamide contains not less than 97.0% and not more than 101.0% of CH₃N₂, calculated on the anhydrous basis.

Description Cyanamide occurs as white crystals or crystalline powder.

It is very soluble in water, in methanol, in ethanol (99.5) and in acetone.

The pH of a solution of Cyanamide (1 in 100) is between 5.0 and 6.5.

It is hygroscopic.

Melting point: about 46°C

Identification (1) To 1 mL of a solution of Cyanamide (1 in 100) add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(2) Drop one or two drops of a solution of Cyanamide in acetone (1 in 100) onto a potassium bromide disk prepared as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.2.5, and air-dry the disk. Determine the infrared absorption spectrum of the disk as directed in the film method under Infrared Spectrophotometry 2.2.5, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
Cyanocobalamin

Vitamin B12

Cyanocobalamin contains not less than 96.0% and not more than 102.0% of C₆₃H₈₈CoN₁₄O₁₄P, calculated on the dried basis.

Description
Cyanocobalamin occurs as dark red crystals or powder.

It is sparingly soluble in water, and slightly soluble in ethanol (99.5).

It is hygroscopic.

Identification
(1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ≤2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cyanocobalamin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Cyanocobalamin with 50 mg of potassium hydrogen sulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

(3) Transfer 5 mg of Cyanocobalamin to a 50-mL distilling flask, dissolve in 5 mL of water, and add 2.5 mL of hypophosphorous acid. Connect the flask with a short condenser, and dips its tip into a test tube containing 1 mL of a solution of sodium hydroxide (1 in 50). Heat gently for 10 minutes, then distil 1 mL into a test tube. To the test tube add 4 drops of a saturated solution of ammonium iron (II) sulfate hexahydrate, shake gently, then add about 30 mg of sodium fluoride, and heat the contents to boil. Immediately add dropwise diluted sulfuric acid (1 in 7) until a clear solution results, then add 3 to 5 drops more of diluted sulfuric acid (1 in 7): a blue to blue-green color develops.

pH ≤2.54> Dissolve 0.10 g of Cyanocobalamin in 20 mL of water: the pH of this solution is between 4.2 and 7.0.

Purity
(1) Clarity and color of solution—Dissolve 20 mg of Cyanocobalamin in 10 mL of water: the solution is clear and red in color.

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 10 mg of Cyanocobalamin in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ≤2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peak other than cyanocobalamin obtained from the sample solution is not larger than the peak area of cyanocobalamin from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 361 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: Dissolve 10 g of anhydrous disodium hydrogen phosphate in 1000 mL of water, and adjust to pH 3.5 with phosphoric acid. To 147 mL of this solution add 53 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cyanocobalamin is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of cyanocobalamin, beginning after the solvent peak.

**System suitability—**

Test for required detectability: Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cyanocobalamin obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the solution for system suitability test.

System performance: Perform this procedure quickly after the solution is prepared. To 25 μg of cyanocobalamin add 10 mL of water, and warm, if necessary, to dissolve. After cooling, add 0.5 mL of sodium toluenesulfonchloramide TS, 0.5 mL of 0.05 mol/L hydrochloric acid TS and water to make 25 mL, mix, and allow the solution to stand for 5 minutes. To 1 mL of the solution add the mobile phase to make 10 mL. When the procedure is run with 20 μL of the solution under the above operating conditions, two principal peaks appear with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cyanocobalamin is not more than 3.0%.

**Loss on drying** <2.41> Not more than 12% (50 mg, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 100°C, 4 hours).

**Assay** Weigh accurately about 20 mg each of Cyanocobalamin and Cyanocobalamin RS (previously determine the loss on drying <2.41> in the same conditions as Cyanocobalamin), dissolve in water to make exactly 1000 mL, respectively, and use these solutions as the sample solution and the standard solution. Determine the absorbances, $A_1$ and $A_2$, of the sample solution and the standard solution, respectively, at 361 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Amount (mg) of } C_{63}H_{88}CoN_{14}O_{14}P = M_5 \times \frac{A_1}{A_2}
\]

$M_5$: Amount (mg) of Cyanocobalamin RS, calculated on the dried basis

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Cyanocobalamin Injection**

**Vitamin B₁₂ Injection**

シアノコバラミン注射液

Cyanocobalamin Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 115.0% of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$: 1355.37)

**Method of preparation** Prepare as directed under Injections, with Cyanocobalamin.

**Description** Cyanocobalamin Injection is a clear, light red to red liquid.

**Identification** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 277 nm and 279 nm, between 360 nm, and 362 nm and between 548 nm and 552 nm. Determine the absorbances, $A_1$ and $A_2$, of this solution at the wavelengths of maximum absorption between 360 nm and 362 nm, and between 548 nm and 552 nm, respectively: the ratio $A_2 / A_1$ is not less than 0.29 and not more than 0.32.

**Bacterial endotoxins** <4.01> Less than 0.30 EU/μg.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Measure exactly a volume of Cyanocobalamin Injection, equivalent to about 2 mg of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Cyanocobalamin RS (previously determine the loss on drying <2.41> in the same conditions as Cyanocobalamin), add water to make exactly 1000 mL, and use this solution as the standard solution. With these solutions, proceed as directed in the Assay under Cyanocobalamin.

\[
\text{Amount (mg) of cyanocobalamin } C_{63}H_{88}CoN_{14}O_{14}P = M_5 \times \frac{A_1}{A_2} \times \frac{1}{10}
\]

$M_5$: Amount (mg) of Cyanocobalamin RS, calculated on the dried basis

**Containers and storage** Containers—Hermetic containers, and colored containers may be used. Storage—Light-resistant.
Cyclopentolate Hydrochloride

シクロペントラート塩酸塩

\[ \text{C}_{17}\text{H}_{25}\text{NO}_{3}\cdot\text{HCl} : 327.85 \]

2-(Dimethylamino)ethyl (2-hydroxycyclopentyl)phenylacetate monohydrochloride [5370-29-1]

Cyclopentolate Hydrochloride, when dried, contains not less than 98.5% of \( \text{C}_{17}\text{H}_{25}\text{NO}_{3}\cdot\text{HCl} \).

**Description**
Cyclopentolate Hydrochloride occurs as a white, crystalline powder. It is odorless, or has a characteristic odor.

It is very soluble in water, freely soluble in ethanol (95), in acetic acid (100) and in chloroform, sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

**Identification (1)**
To 1 mL of a solution of Cyclopentolate Hydrochloride (1 in 100) add 1 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Dissolve 0.2 g of Cyclopentolate Hydrochloride in 2 mL of water, add 2 mL of sodium hydroxide TS, and boil for 1 minute. After cooling, add 2 drops of nitric acid: a phenylacetic acid-like odor is perceptible.

(3) Determine the infrared absorption spectrum of Cyclopentolate Hydrochloride, previously dried, as directed in Method 1, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Cyclopentolate Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

**pH** &lt;2.54\&gt; Dissolve 0.20 g of Cyclopentolate Hydrochloride in 20 mL of water: the pH of this solution is between 4.5 and 5.5.

**Melting point** &lt;2.60\&gt; 135 – 138°C

**Purity (1)**
Clarity and color of solution—Dissolve 1.0 g of Cyclopentolate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals &lt;1.07\&gt;—Proceed with 1.0 g of Cyclopentolate Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Cyclopentolate Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography &lt;2.03\&gt;. Spot 10 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, \( n \)-butyl acetate, water and ammonia solution (28:100:60:23:17) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sulfuric acid in ethanol (99.5) (1 in 10) on the plate, and heat at 120°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** &lt;2.44\&gt; Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** &lt;2.44\&gt; Not more than 0.05% (1 g).

**Assay**
Weigh accurately about 0.5 g of Cyclopentolate Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), and titrate &lt;2.50\&gt; with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to yellow-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.79 mg of \( \text{C}_{17}\text{H}_{25}\text{NO}_{3}\cdot\text{HCl} \).

**Containers and storage**
Containers—Tight containers.

Cyclophosphamide Hydrate

シクロホスファミド水和物

\[ \text{C}_{17}\text{H}_{32}\text{Cl}_2\text{N}_2\text{O}_2\text{P} \cdot \text{H}_2\text{O} : 279.10 \]

\( \text{N},\text{N-Bis}(2\text{-chloroethyl})-3,4,5,6\text{-tetrahydro-2H-1,3,2-} \]

oxazaphosphorin-2-amine 2-oxide monohydrate [6055-19-2]

Cyclophosphamide Hydrate contains not less than 97.0% of \( \text{C}_{17}\text{H}_{32}\text{Cl}_2\text{N}_2\text{O}_2\text{P} \cdot \text{H}_2\text{O} \).

**Description**
Cyclophosphamide Hydrate occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in acetic acid (100), freely soluble in ethanol (95), in acetic anhydride and in chloroform, and soluble in water and in diethyl ether.

Melting point: 45 – 53°C

**Identification (1)**
Dissolve 0.1 g of Cyclophosphamide Hydrate in 10 mL of water, and add 5 mL of silver nitrate TS: no precipitate is produced. Then boil this solution: a white precipitate is produced. Collect the precipitate, and wash with 0.1 mol/L perchloric acid VS until the color of the precipitate changes from violet through blue-green to yellow-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.79 mg of \( \text{C}_{17}\text{H}_{32}\text{Cl}_2\text{N}_2\text{O}_2\text{P} \cdot \text{H}_2\text{O} \).

**Residue on ignition** &lt;2.44\&gt; Not more than 0.05% (1 g).

**Assay**
Weigh accurately about 0.5 g of Cyclophosphamide Hydrate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), and titrate &lt;2.50\&gt; with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to yellow-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.79 mg of \( \text{C}_{17}\text{H}_{32}\text{Cl}_2\text{N}_2\text{O}_2\text{P} \cdot \text{H}_2\text{O} \).

**Containers and storage**
Containers—Tight containers.
Cycloserine

Cycloserine contains not less than 950 \( \mu g \) (potency) and not more than 1020 \( \mu g \) (potency) per mg, calculated on the dried basis. The potency of Cycloserine is expressed as mass (potency) of cycloserine \( \text{C}_3\text{H}_6\text{N}_2\text{O}_2 \).

**Description** Cycloserine occurs as white to light yellowish white, crystals or crystalline powder.

It is soluble in water, and sparingly soluble in ethanol (95).

**Identification** Determine the infrared absorption spectrum of Cycloserine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \( \langle 2.25 \rangle \), and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Cycloserine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \( \langle 2.49 \rangle \) \([\alpha]_D^2 = +108\) – +114° (2.5 g calculated on the dried basis, 2 mol/L sodium hydroxide TS, 50 mL, 100 mm).

**pH** \( \langle 2.54 \rangle \) Dissolve 1.0 g of Cycloserine in 20 mL of water: the pH of the solution is between 5.0 and 7.4.

**Purity** (1) Heavy metals \( \langle 1.07 \rangle \) — Proceed with 1.0 g of Cycloserine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Condensation products—Dissolve 20 mg of Cycloserine in sodium hydroxide TS to make exactly 50 mL, and determine the absorbance of this solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry \( \langle 2.24 \rangle \): not more than 0.8.

**Loss on drying** \( \langle 2.41 \rangle \) Not more than 1.5% (0.5 g, reduced pressure, 60°C, 3 hours).

**Residue on ignition** \( \langle 2.44 \rangle \) Not more than 0.5% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics \( \langle 4.02 \rangle \) according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium

(iii) Standard solutions—Weigh accurately an amount of Cycloserine RS, previously dried at 60°C for 3 hours under reduced pressure of not exceeding 0.67 kPa, equivalent to about 40 mg (potency), dissolve in water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, and add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 100 \( \mu g \) (potency) and 50 \( \mu g \) (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Cycloserine equivalent to about 40 mg (potency), dissolve in water to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 100 \( \mu g \) (potency) and 50 \( \mu g \) (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Well-closed containers.

**Storage**—Not exceeding 30°C.

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Cycloserine

C\(_3\)H\(_6\)N\(_2\)O\(_2\): 102.09

(4R)-4-Aminoisoxazolidin-3-one

[68-41-7]

Cycloserine occurs as white to light yellowish white, crystals or crystalline powder.

It is soluble in water, and sparingly soluble in ethanol (95).
Cyproheptadine Hydrochloride Hydrate

シプロヘプタジン塩酸塩水和物

Cyproheptadine Hydrochloride Hydrate, when dried, contains not less than 98.5% of cyproheptadine hydrochloride (C₂₁H₂₁N.HCl: 323.86).

Description Cyproheptadine Hydrochloride Hydrate occurs as a white to pale yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in methanol and in acetic acid (100), soluble in chloroform, sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Dissolve 0.1 g of Cyproheptadine Hydrochloride Hydrate in 10 mL of methanol, apply 1 drop of this solution on filter paper, air-dry, and examine under ultraviolet light (main wavelength: 254 nm): the solution shows a pale blue fluorescence.

(2) Weigh 0.1 g of Cyproheptadine Hydrochloride Hydrate, transfer to a separator, dissolve in 5 mL of chloroform, add 4 mL of water and 1 mL of sodium carbonate TS, and shake. Transfer the chloroform layer to another separator, and wash with 4 mL of water by shaking well. It dissolves in 1 mol/L hydrochloric acid TS.

(3) Determine the absorption spectrum of a solution of Cyproheptadine Hydrochloride Hydrate in ethanol (95) (1 in 100,000) as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A saturated solution of Cyproheptadine Hydrochloride Hydrate responds to the Qualitative Tests (1.09) (2) for chloridate.
solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.041%).

(3) Sulfate $<1.14>$—Dissolve 0.6 g of L-Cysteine in 30 mL of water and 3 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 3 mL of dilute hydrochloric acid and water to make 50 mL. Prepare the test solution and the control solution with 4 mL of barium chloride TS, respectively (not more than 0.028%).

(4) Ammonium $<1.02>$—Prepare the test with 0.25 g of L-Cysteine, using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals $<1.07>$—Proceed with 1.0 g of L-Cysteine according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron $<1.10>$—Prepare the test solution with 1.0 g of L-Cysteine according to Method 1, and perform the test using Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Cysteine in N-ethylmaleimide solution (1 in 50) to make exactly 10 mL, leave for 30 minutes, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL, pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution (1). Separately, dissolve 0.10 g of L-cysteine in 0.5 mol/L hydrochloric acid TS to make exactly 20 mL. Pipet 1 mL of this solution, add water to make 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate for 30 minutes at 80°C. Spray the plate evenly with a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100), and then heat at 80°C for 10 minutes: the spot obtained from the sample solution corresponding to the spot obtained from the standard solution (2) is not more intense than the spot from the standard solution (2). Also, the spots other than the principal spot and the spots mentioned above from the sample solution are not more intense than the spot from the standard solution (1).

Loss on drying $<2.41>$ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition $<2.44>$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of L-Cysteine, place it in a stoppered flask, and dissolve in 20 mL of water. Dissolve 4 g of potassium iodide in this solution, immediately place in ice cold water, add 5 mL of dilute hydrochloric acid and exactly 25 mL of 0.05 mol/L iodine VS, leave in a dark place for 20 minutes, and then titrate $<2.50>$ an excess amount of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination using the same method.

Each mL of 0.05 mol/L iodine VS = 12.12 mg of C₅H₇NO₃S

Containers and storage Containers—Tight containers.

### L-Cysteine Hydrochloride Hydrate

L-システイン塩酸塩水和物

C₅H₇NO₃S.HCl·H₂O: 175.63
(2R)-2-Amino-3-sulfanylpropanoic acid monohydrochloride monohydrate

$[7048-04-6]$

L-Cysteine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of L-cysteine hydrochloride (C₅H₇NO₃S.HCl: 157.62), calculated on the dried basis.

**Description** L-Cysteine Hydrochloride Hydrate occurs as white crystals or crystalline powder. It has a characteristic odor and a strong acid taste.

It is very soluble in water, and soluble in ethanol (99.5). It dissolves in 6 mol/L hydrochloric acid TS.

**Identification** (1) Determine the infrared absorption spectrum of L-Cysteine Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 10 mL of a solution of L-Cysteine Hydrochloride Hydrate (1 in 50) add 1 mL of hydrogen peroxide (30), heat on a water bath for 20 minutes, and cool: the solution responds to the Qualitative Tests $<1.09>$ (2) for chloride.

**Optical rotation** $<2.49>$ $\alpha_1^D$ +6.0° to +7.5° (2 g, calculated on the dried basis, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** $<2.54>$ The pH of a solution prepared by dissolving 1.0 g of L-Cysteine Hydrochloride Hydrate in 100 mL of water is between 1.3 and 2.3.

**Purity** (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of L-Cysteine Hydrochloride Hydrate in 10 mL of water is clear and colorless.

(2) Sulfate $<1.14>$—Dissolve 0.8 g of L-Cysteine Hydrochloride Hydrate in 30 mL of water and 3 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 3 mL of dilute hydrochloric acid and water to make 50 mL. To both of the test solution and the control solution add 4 mL of barium chloride TS (not more than 0.021%).

(3) Ammonium $<1.02>$—Prepare the test with 0.25 g of L-Cysteine Hydrochloride Hydrate using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals $<1.07>$—Proceed with 1.0 g of L-Cysteine Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 1.0
mL of Standard Lead Solution (not more than 10 ppm).

(5) Iron <1.10>—Prepare the test solution with 1.0 g of L-Cysteine Hydrochloride Hydrate according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(6) Related substances—Dissolve 0.10 g of L-Cysteine Hydrochloride Hydrate in N-ethylmaleimide solution (1 in 50) to make 10 mL, allow to stand for 30 minutes, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-Layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and then heat at 80°C for 10 minutes: the spots other than the principal spot obtained with the sample solution is not more intense than the spot obtained with the standard solution.

Loss on drying <2.41> 8.5 – 12.0% (1 g, in vacuum, phosphorus (V) oxide, 20 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of L-Cysteine Hydrochloride Hydrate, place in a glass-stoppered flask, and dissolve in 20 mL of water. Dissolve 4 g of potassium iodide in this solution, soak immediately in ice cold water, add 5 mL of dilute hydrochloric acid and exactly 25 mL of 0.05 mol/L iodine VS, allow to stand for 20 minutes in a dark place, titrate <2.50> the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 15.76 mg of C₆H₇NO₃S.HCl

Containers and storage Containers—Tight containers.

Cytarabine

シタラビン

C₉H₁₅N₃O₅: 243.22
1-β-D-Arabinofuranosylcytosine
[147-94-4]

Cytarabine, when dried, contains not less than 98.5% and not more than 101.0% of C₉H₁₅N₃O₅.

Description Cytarabine occurs as white crystals or crystalline powder.

It is freely soluble in water, soluble in acetic acid (100), and very slightly soluble in ethanol (99.5). It dissolves in 0.1 mol/L hydrochloric acid TS.

Melting point: about 214°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Cytarabine in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cytarabine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D: +154 – +160° (after drying, 0.1 g, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.20 g of Cytarabine in 20 mL of water: the pH of this solution is between 6.5 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cytarabine in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.07>—Perform the test with 1.0 g of Cytarabine. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Cytarabine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.10 g of Cytarabine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add water to make exactly 25 mL and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with 1-butanol saturated with water to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the number of them which are more intense than the spot from the standard solution (2) is not more than two. Spray evenly acidic potassium permanganate TS on the plate: any spot other than the principal spot does not appear.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Weigh accurately about 0.2 g of Cytarabine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any
necessary correction.
Each mL of 0.05 mol/L perchloric acid VS = 12.16 mg of C₈H₁₇N₃O₅

Containers and storage  Containers—Tight containers.

Danazol

Danazol, when dried, contains not less than 98.5% and not more than 101.0% of C₂₂H₂₇NO₂.

Description  Danazol occurs as a white to pale yellow crystalline powder.
It is soluble in acetone, sparingly soluble in ethanol (99.5), and practically insoluble in water.
Melting point: about 225°C (with decomposition).

Identification (1)  Determine the absorption spectrum of a solution of Danazol in ethanol (95%) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Danazol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)  Determine the infrared absorption spectrum of Danazol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Danazol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49>  [α]D: +8° to +11° (after drying, 0.25 g, ethanol (99.5), 50 mL, 100 mm).

Purity (1)  Chloride <1.07>—To 2.0 g of Danazol add 80 mL of water, shake well, and boil for 5 minutes. After cooling, add water to make 100 mL, and filter through a glass filter (G4). Discard the first 30 mL of the filtrate, take 40 mL of the subsequent filtrate, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(2)  Heavy metals <1.07>—Proceed with 2.0 g of Danazol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3)  Related substances—Dissolve 0.20 g of Danazol in 4 mL of acetonitrile, and use this solution as the standard solution. Pipet 2 mL of the sample solution, add acetone to make exactly 200 mL. Pipet 4 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (3:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point obtained from the sample solution are not more intense than the spot from the standard solution.

(4)  Residual solvent—Being specified separately.

Loss on drying <2.41>  Not more than 0.2% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition <2.44>  Not more than 0.1% (1 g).

Assay  Weigh accurately about 25 mg each of Danazol and Danazol RS, previously dried, dissolve separately in ethanol (95) to make exactly 50 mL. Pipet 2 mL each of these solutions, add ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_S and A_T, at 285 nm.

Amount (mg) of danazol (C₂₂H₂₇NO₂) = M_S × A_T/A_S

M_S: Amount (mg) of Danazol RS

Containers and storage  Containers—Well-closed containers.

Storage—Light-resistant.

Dantrolene Sodium Hydrate

Dantrolene Sodium Hydrate contains not less than 98.0% of dantrolene sodium (C₁₄H₉N₄NaO₅: 336.23), calculated on the anhydrous basis.

Description  Dantrolene Sodium Hydrate occurs as a yellowish orange to deep orange, crystalline powder.
It is soluble in propylene glycol, sparingly soluble in methanol, slightly soluble in ethanol (95), very slightly soluble in water and in acetic acid (100), and practically insoluble in acetone, in tetrahydrofuran and in diethyl ether.

Identification (1)  Determine the absorption spectrum of a solution of Dantrolene Sodium Hydrate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Refer-
ence Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dantrolene Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Dantrolene Sodium Hydrate add 20 mL of water and 2 drops of acetic acid (100), shake well, and filter: the filtrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Purity (1) Alkalinity—To 0.7 g of Dantrolene Sodium Hydrate add 10 mL of water, shake well, and centrifuge or filter through a membrane filter. To 5 mL of the supernatant liquid or the filtrate add 45 mL of water, 3 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L hydrochloric acid liquid or the filtrate add 45 mL of water, 3 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L hydrochloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 33.62 mg of C_{14}H_{9}N_{3}NaO_{7}

Containers and storage Containers—Tight containers.

Daunorubicin Hydrochloride

ダウノルビシン塩酸塩

\[
\text{C}_{27}\text{H}_{29}\text{NO}_{10}\cdot\text{HCl} : 563.98
\]

(25.45)-2-Acetyl-4-(3-amino-2,3,6-trideoxy-a-L-lyxo-hexopyranosyloxy)-2,5,12-trihydroxy-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione monohydrochloride [23541-50-6]

Daunorubicin Hydrochloride is the hydrochloride of an anthracycline substance having antitumor activity produced by the growth of Streptomyces peucetius.

It contains not less than 940 μg (potency) and not more than 1050 μg (potency) per mg, calculated on the dried basis. The potency of Daunorubicin Hydrochloride is expressed as mass (potency) of daunorubicin hydrochloride (C_{27}H_{25}NO_{10}·HCl).

Description Daunorubicin Hydrochloride occurs as a red powder.

It is soluble in water and in methanol, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Daunorubicin Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Daunorubicin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) A solution of Daunorubicin Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> \[ [\alpha]_{D}^{27} : +250 - +275 \] (15 mg calculated on the dried basis, methanol, 10 mL, 100 mm).

pH <2.54> Dissolve 0.15 g of Daunorubicin Hydrochloride in 30 mL of water: the pH of the solution is between 4.5 and 6.0.
Purity (1) Clarity and color of solution—Dissolve 20 mg of Daunorubicin Hydrochloride in 10 mL of water: the solution is clear and red.

(2) Heavy metals $<1.07$—Proceed with 1.0 g of Daunorubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Daunorubicin Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.07>$. Spot 10 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, water and acetic acid (100:15:5:1:1) to a distance of about 10 cm, and air-dry the plate. Examine the spots with the naked eye: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying $<2.41>$ Not more than 7.5% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Daunorubicin Hydrochloride and Daunorubicin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve each in a suitable amount of the mobile phase, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL of each of the sample solution and standard solution as directed under Thin-layer Chromatography $<2.07>$. Spot 2.0 μL of the sample solution and standard solution on a plate of silica gel for liquid chromatography (10 mm, 3 hours). Perform the test with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of daunorubicin to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Deferoxamine Mesilate デフェロキサミンメシル酸塩

C$_{22}$H$_{32}$N$_{6}$O$_{9}$·CH$_{4}$O$_{3}$S: 656.79
N-$\{5$-(Acetylhydroxyamino)pentyl]-N’-$\{5$-[3-[5-aminopentyl]hydroxycarbamoyl]propanoylamino]pentyl]-N’$-$hydroxyxysuccinamide monomethanesulfonate [138-14-7]

Deferoxamine Mesilate contains not less than 98.0% and not more than 102.0% of C$_{22}$H$_{32}$N$_{6}$O$_{9}$·CH$_{4}$O$_{3}$S, calculated on the anhydrous basis.

Description Deferoxamine Mesilate occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5), in 2-propanol and in diethyl ether.

Melting point: about 147°C (with decomposition).

Identification (1) To 5 mL of a solution of Deferoxamine Mesilate (1 in 500) add 1 drop of iron (III) chloride TS: a deep red color develops.

(2) A 50 mg portion of Deferoxamine Mesilate responds to the Qualitative Tests $<1.092$ for mesilate.

(3) Determine the infrared absorption spectrum of Deferoxamine Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum or the Spectrum of Deferoxamine Mesilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH $<2.54>$ Dissolve 1.0 g of Deferoxamine Mesilate in 10 mL of water: the pH of this solution is between 3.5 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Deferoxamine Mesilate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride $<1.03>$—Perform the test with 1.0 g of Deferoxamine Mesilate. Prepare the control solution with 0.90 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.032%).

(3) Sulfate $<1.14>$—Perform the test with 0.6 g of Deferoxamine Mesilate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.040%).

(4) Heavy metals $<1.07>$—Proceed with 2.0 g of Deferoxamine Mesilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
(5) Arsenic <1,11>—Prepare the test solution with 1.0 g of Deferoxamine Mesilate according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(6) Related substances—Dissolve 50 mg of Deferoxamine Mesilate in 50 mL of the mobile phase, and use this solution as the standard solution. Perform the test with exactly 20 mL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of peaks other than deferoxamine obtained from the sample solution is not larger than the peak area of deferoxamine from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecysilanol silica gel for liquid chromatography (10 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1.52 g of diammonium hydrogen phosphate, 0.37 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 1.08 g of sodium 1-heptanesulfonate in 950 mL of water, and adjust the pH of this solution to 2.8 with phosphoric acid. To 800 mL of this solution add 100 mL of 2-propanol.
Flow rate: Adjust the flow rate so that the retention time of deferoxamine is about 15 minutes.
Time span of measurement: About two times as long as the retention time of deferoxamine, beginning after the solvent peak.
System suitability—
Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of deferoxamine obtained from 20 µL of this solution is equivalent to 1.5 to 2.5% of that of deferoxamine obtained from 20 µL of the standard solution.

System performance: Dissolve 16 mg of Deferoxamine Mesilate and 4 mg of methyl parahydroxybenzoate in 50 mL of the mobile phase. When the procedure is run with 20 µL of this solution under the above operating conditions, deferoxamine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of deferoxamine is not more than 3.0%.

Water <2.48>—Not more than 2.0% (0.2 g, volumetric titration, direct titration).
Residue on ignition <2.44>—Not more than 0.1% (1 g).

Assay Weigh accurately about 60 mg of Deferoxamine Mesilate and Deferoxamine Mesilate RS (previously determine the water <2.48>) in the same manner as Deferoxamine Mesilate), dissolve each in 20 mL of water, add exactly 10 mL of 0.05 mol/L sulfuric acid TS, and add water to make exactly 50 mL. Pipet 5 mL of each of these solutions, add exactly 5 mL of 0.05 mol/L ferric chloride TS and exactly 0.2 mL of iron (III) chloride TS, then add water to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by adding 0.05 mol/L sulfuric acid TS to 0.2 mL of iron (III) chloride TS to make exactly 50 mL as the blank, and determine the absorbances, $A_T$ and $A_s$, of each solution from the sample solution and the standard solution at 340 nm.

Amount (mg) of C$_2_{11}$H$_{28}$N$_6$O$_8$.CH$_4$O$_3$S

$$M_s = M_s \times A_T/A_s$$

$M_s$: Amount (mg) of Deferoxamine Mesilate RS, calculated on the anhydrous basis
Containers and storage Containers—Tight containers.

**Dehydrocholic Acid**

デヒドロコール酸

C$_{24}$H$_{32}$O$_2$: 402.52
3,7,12-Trioxo-5β-cholan-24-oic acid [81-23-2]

Dehydrocholic Acid, when dried, contains not less than 98.5% of C$_{24}$H$_{32}$O$_5$.

Description Dehydrocholic Acid occurs as a white, crystalline powder. It is odorless, and has a bitter taste.
It is sparingly soluble in 1,4-dioxane, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.
It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Dehydrocholic Acid in 1 mL of sulfuric acid and 1 drop of formaldehyde solution, and allow to stand for 5 minutes. Add 5 mL of water to the solution: the solution shows a yellow color and a blue-green fluorescence.
(2) To 0.02 g of Dehydrocholic Acid add 1 mL of ethanol (95), shake, add 5 drops of 1,3-dinitrobenzene TS and 0.5 mL of a solution of sodium hydroxide (1 in 8), and allow to stand: a purple to red-purple color develops, and gradually changes to brown.

Optical rotation <2.49> [α]$D$: +29$^\circ$ to +32$^\circ$ (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 233 – 242°C
Purity (1) Odor—To 2.0 g of Dehydrocholic Acid add 100 mL of water, and boil for 2 minutes: the solution is odorless.
(2) Clarity and color of solution—To 0.10 g of Dehydrocholic Acid, previously powdered in a mortar, add 30 mL of
ethanol (95), and dissolve by shaking for 10 minutes: the solution is clear and colorless.

(3) Chloride <1.03>—To 2.0 g of Dehydrocholic Acid add 100 mL of water, shake for 5 minutes and filter, and use this filtrate as the sample solution. To 25 mL of the sample solution add 6 mL of dilute nitric acid, heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(4) Sulfate <1.14>—Add 1 mL of dilute hydrochloric acid to 25 mL of the sample solution obtained in (3), heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of Dehydrocholic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Barium—To the solution obtained in (1) add 2 mL of hydrochloric acid, and boil for 2 minutes. Cool, filter, and wash with water until 100 mL of the filtrate is obtained. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is produced.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Dehydrocholic Acid, previously dried, add 40 mL of neutralized ethanol and 20 mL of water, and dissolve by warming. Add 2 drops of phenolphthalein TS, titrate <2.5D> with 0.1 mol/L sodium hydroxide VS, adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS = 40.25 mg of C₂₄H₃₄O₅.

Containers and storage Containers—Well-closed containers.

Purified Dehydrocholic Acid

精製デヒドロコール酸

C₂₄H₃₄O₅: 402.52
3,7,12-Trioxo-5β-cholan-24-oic acid
[81-23-2]

Purified Dehydrocholic Acid, when dried, contains not less than 99.0% of C₂₄H₃₄O₅.

Description Purified Dehydrocholic Acid occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is sparingly soluble in 1,4-dioxane, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Purified Dehydrocholic Acid in 1 mL of sulfuric acid and 1 drop of formaldehyde solution, and allow to stand for 5 minutes. Add 5 mL of water to the solution: the solution shows a yellow color and blue-green fluorescence.

(2) To 0.02 g of Purified Dehydrocholic Acid add 1 mL of ethanol (95), shake, add 5 drops of 1,3-dinitrobenzene TS and 0.5 mL of a solution of sodium hydroxide (1 in 8), and allow to stand: a purple to red-purple color develops, and gradually changes to brown.

Optical rotation <2.49> [α]₀^2θ: +29° to +32° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 237 – 242°C

Purity (1) Odor—To 2.0 g of Purified Dehydrocholic Acid add 100 mL of water, and boil for 2 minutes: the solution is odorless.

(2) Clarity and color of solution—Dissolve 0.10 g of Purified Dehydrocholic Acid, previously powdered in a mortar, in 30 mL of ethanol (95) by shaking for 10 minutes: the solution is clear and colorless.

(3) Chloride <1.03>—To 2.0 g of Purified Dehydrocholic Acid add 100 mL of water, shake for 5 minutes and filter, and use this filtrate as the sample solution. To 25 mL of the sample solution add 6 mL of dilute nitric acid, heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(4) Sulfate <1.14>—Add 1 mL of dilute hydrochloric acid to 25 mL of the sample solution obtained in (3), heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).
mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals \(<1.07\)—Proceed with 1.0 g of Purified Dehydrocholic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Barium—To the solution obtained in (1) add 2 mL of hydrochloric acid, and boil for 2 minutes, cool, filter, and wash the filter with water until 100 mL of the filtrate is obtained. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is produced.

Loss on drying \(<2.4\) Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition \(<2.44\) Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Purified Dehydrocholic Acid, previously dried, add 40 mL of neutralized ethanol and 20 mL of water, and dissolve by warming. Add 2 drops of phenolphthalein TS, then titrate with 2.0 mL of Standard Lead Solution (not more than 20 ppm), adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS = 40.25 mg of C₂₄H₃₄O₅.

Containers and storage Containers—Well-closed containers.

Dehydrocholic Acid Injection

Dehydrocholate Sodium Injection

デヒドロコール酸注射液

Dehydrocholic Acid Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of dehydrocholic acid (C₂₄H₃₄O₅: 402.52).

Method of preparation Dissolve Purified Dehydrocholic Acid in a solution of Sodium Hydroxide, and prepare as directed under Injections.

Description Dehydrocholic Acid Injection is a clear, colorless to light yellow liquid, and has a bitter taste. pH: 9 - 11

Identification Transfer a volume of Dehydrocholic Acid Injection, equivalent to 0.1 g of Purified Dehydrocholic Acid according to the labeled amount, to a separator, and add 10 mL of water and 1 mL of dilute hydrochloric acid: a white precipitate is produced. Extract the mixture with three 15-mL portions of chloroform, combine all the chloroform extracts, evaporate the chloroform on a water bath, and dry the residue at 105°C for 1 hour: the residue so obtained melts \(>2.50\)° between 235°C and 242°C.

Purity Heavy metals \(<1.07\)—Evaporate a volume of Dehydrocholic Acid Injection, equivalent to 1.0 g of Purified Dehydrocholic Acid according to the labeled amount, on a water bath to dryness. Proceed with the residue according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Bacterial endotoxins \(<0.07\) Less than 0.30 EU/mg.

Extractable volume \(<6.05\) It meets the requirement.

Foreign insoluble matter \(<6.06\) Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter \(<6.07\) It meets the requirement.

Sterility \(<4.06\) Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Transfer an exactly measured volume of Dehydrocholic Acid Injection, equivalent to about 0.5 g of dehydrocholic acid (C₂₄H₃₄O₅), to a 100-mL separator, and add, if necessary, water to make 25 mL. Add 2 mL of hydrochloric acid, and extract with 25-mL, 20-mL and 15-mL portions of chloroform successively. Combine the chloroform extracts, wash with cold water until the washings become negative to acid, and evaporate the chloroform on a water bath. Dissolve the residue in 40 mL of neutralized ethanol and 20 mL of water by warming. Add 2 drops of phenolphthalein TS to this solution, titrate \(>2.50\)° with 0.1 mol/L sodium hydroxide VS, adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS = 40.25 mg of C₂₄H₃₄O₅.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Demethylchlortetracycline Hydrochloride

デメチルクロルテトラサイクリン塩酸塩

Demethylchlortetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of the mutant of Streptomyces aureofaciens.

It contains not less than 900 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Demethylchlortetracycline Hydrochloride is expressed as mass
(potency) of demethylchlortetracycline hydrochloride (C_{21}H_{27}ClN_{5}O_{8}HCl).

**Description** Demethylchlortetracycline Hydrochloride occurs as a yellow crystalline powder.

It is soluble in water, and slightly soluble in ethanol (99.5).

**Identification** (1) Dissolve 40 mg of Demethylchlortetra
cycline Hydrochloride in 250 mL of water. To 10 mL of this solution add 85 mL of water and 5 mL of a solution of sodium hydroxide (1 in 5). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectro-
photometry <2.25>, and compare the absorbance with the Refer-
ence Spectrum or the spectrum of a solution of Demethyl-
chlortetracycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Demethylchlortetracycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectro-
photometry <2.25>, and compare the spectrum with the Refer-
ence Spectrum or the spectrum of Demethylchlortetracycline Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Demethylchlortetracycline Hydrochloride (1 in 100) responds to the Qualitative Tests <1.07> (2) for chloride.

**Optical rotation** <2.44> \([\alpha]_D^{20} = -248 - 263^\circ\) (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** <2.54> Dissolve 1.0 g of Demethylchlortetracycline Hydrochloride in 100 mL of water: the pH of the solution is between 2.0 and 3.0.

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Demethylchlortetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Demethylchlortetracycline Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 25 mg of Demethylchlortetracycline Hydrochloride in 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area obtained from the chromatograms of these solutions by the automatic integration method: each peak area other than demethylchlortetracycline obtained from the sample solution is not larger than 1.2 times that of demethylchlortetracycline from the standard solution, and the sum of the areas of the peaks other than demethylchlortetracycline is not larger than 2 times the peak area of demethylchlortetracycline from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

**Time span of measurement:** About 2 times as long as the retention time of demethylchlortetracycline beginning after the solvent peak.

**System suitability**—

Test for required detectability: Measure exactly 10 mL of the standard solution, add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of demethylchlortetracycline obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

**System repeatability:** When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of demethylchlortetracycline is not more than 1.0%.

**Loss on drying** <2.41> Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Assay** Weigh accurately an amount of Demethylchlortetra
cycline Hydrochloride and Demethylchlortetracycline Hydro-
chloride RS, equivalent to about 25 mg (potency), dis-
solve each in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of demethylchlortetracycline.

Amount [μg (potency)] of C_{21}H_{27}ClN_{5}O_{8}HCl = M_S × A_T/A_S × 1000

M_S: Amount [mg (potency)] of Demethylchlortetracycline Hydrochloride RS

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wave-
length: 254 nm).

Column: A stainless steel column 4.1 mm in inside diam-
eter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle di-
meter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate, 1.5 g of tetrabutylammonium hydrogensulfate and 0.4 g of disodium dihydrogen ethylenediamine tetaa-
cetate dihydrate in 300 mL of water, and adjust the pH to 8.5 with sodium hydroxide TS. To this solution add 75.0 g of i-
butanol and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of demethylchlortetracycline is about 8 minutes.

**System suitability**—

System performance: Heat 10 mL of the standard solution on a water bath for 60 minutes. When the procedure is run with 20 μL of this solution so obtained under the above operating conditions, 4-epidemethylchlortetracycline and demethylchlortetracycline are eluted in this order with the resolution between these peaks being not less than 3. The
relative retention time of 4-epidemethylchlortetracycline with respect to demethylchlortetracycline is about 0.7.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of demethylchlortetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Deslanoside
デスラノシド

Deslanoside, when dried, contains not less than 90.0% and not more than 102.0% of C_{47}H_{74}O_{19}.

Description Deslanoside occurs as colorless or white crystals or a white, crystalline powder. It is odorless.

It is freely soluble in anhydrous pyridine, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is hygroscopic.

Identification Transfer 1 mg of Deslanoside to a small test tube about 10 mm in inside diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 1000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of two liquids a brown ring is produced, and the color of the upper layer near to the contact zone changes gradually to blue through purple, and the entire acetic acid layer shows a blue-green color through a deep blue color.

Purity (1) Clarity and color of solution—Dissolve 20 mg of Deslanoside in 10 mL of ethanol (95) and 3 mL of water by warming, cool, and dilute to 100 mL with water: the solution is clear and colorless.

Related substances—Dissolve 10 mg of Deslanoside in exactly 5 mL of methanol, and use this solution as the sample solution. Dissolve 1.0 mg of Deslanoside RS in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.93). Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: the spots other than the principal spot obtained from the sample solution are not larger and not more intense than the spot from the standard solution.

Optical rotation (2.49) [α]_D^20: +6.5 to +8.5° (after drying, 0.5 g, anhydrous pyridine, 25 mL, 100 mm).

Loss on drying (2.41) Not more than 8.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition (2.44) Not more than 0.5% (0.1 g).

Assay Dissolve about 12 mg each of Deslanoside and Deslanoside RS, previously dried and accurately weighed, in 20 mL each of methanol, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of these solutions, transfer to light-resistant, 25-mL volumetric flasks, shake well with 5 mL each of 2,4,6-trinitrophenol TS and 0.5 mL each of a solution of sodium hydroxide (1 in 10), add dilute methanol (1 in 4) to make 25 mL, and allow to stand at a temperature between 18°C and 22°C for 25 minutes. Determine the absorbances, A_1 and A_2, of the subsequent solutions of the sample solution and the standard solution, respectively, at 485 nm as directed under Ultraviolet-visible Spectrophotometry (2.22), using a solution prepared with 5 mL of dilute methanol (1 in 5) in the same manner as the blank.

Amount (mg) of C_{47}H_{74}O_{19} = M_s × A_1/A_2

M_s: Amount (mg) of Deslanoside RS

Containers and storage Containers—Tight containers.

Deslanoside Injection
デスラノシド注射液

Deslanoside Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of deslanoside (C_{47}H_{74}O_{19}: 943.08).

Method of preparation Dissolve Deslanoside in 10 vol% ethanol and prepare as directed under Injections. It may contain Glycerin. It may be prepared with a suitable amount of Ethanol and Water for Injection or Sterile Water for Injection in Containers.

Description Deslanoside Injection is a clear and colorless liquid.

pH: 5.0 – 7.0
Identification (1) Place a volume of Deslanoside Injection, equivalent to 2 mg of Deslanoside according to the labeled amount, in a separator, add sodium chloride in the ratio of 0.2 g to each mL of this solution, and extract with three 10-mL portions of chloroform. Combine the chloroform extracts, mix uniformly, pipet 15 mL of this solution, and evaporate the chloroform under reduced pressure. Proceed with the residue as directed in the Identification under Deslanoside.

(2) Evaporate the remaining chloroform extract obtained in (1) under reduced pressure, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of Deslanoside RS in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{(2.03)} \). Spot 20 \( \mu \)L each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate, and heat the plate at 110 °C for 10 minutes: the spots from the sample solution and standard solution show a black color and have the same \( R_f \) value.

Bacterial endotoxins \(< 4.0 \) It meets the requirement.

Extractable volume \(< 6.0 \) It meets the requirement.

Foreign insoluble matter \(< 6.06 \) Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter \(< 6.07 \) It meets the requirement.

Sterility \(< 4.06 \) Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Deslanoside Injection, equivalent to about 3 mg of deslanoside (C47H74O19). Add 5 mL of methanol and water to make 25 mL. Use this solution as the sample solution, and proceed as directed in the Assay under Deslanoside.

\[
\text{Amount (mg) of deslanoside (C}_{47}\text{H}_{74}\text{O}_{19}) = M_s \times A_1/A_2 \times 1/4
\]

\( M_s \): Amount (mg) of Deslanoside RS

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

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### Dexamethasone

#### デキサメタゾン

**C_{22}H_{29}FO_5**: 392.46
9-Fluoro-11β,17β,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione [50-02-2]

Dexamethasone, when dried, contains not less than 97.0% and not more than 102.0% of C_{22}H_{29}FO_5.

**Description** Dexamethasone occurs as white to pale yellow crystals or crystalline powder. It is sparingly soluble in methanol, in ethanol (95) and in acetone, slightly soluble in acetonitrile, and practically insoluble in water.

Melting point: about 245°C (with decomposition).

**Identification (1)** Proceed with 10 mg of Dexamethasone as directed under Oxygen Flask Combustion Method \( \text{(1.09)} \), using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution obtained responds to the Qualitative Tests \( \text{(1.09)} \) for fluoride.

(2) Dissolve 1 mg of Dexamethasone in 10 mL of ethanol (95). Mix 2 mL of the solution with 10 mL of phenylhydrazine hydrochloride TS, heat in a water bath at 60°C for 20 minutes, and cool the solution. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \( \text{(2.24)} \), using as the blank the solution prepared with 2 mL of ethanol (95) in the same manner as the former solution, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Dexamethasone RS prepared in the same manner as the former solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Dexamethasone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \( \text{(2.25)} \), and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Dexamethasone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Dexamethasone and Dexamethasone RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

**Optical rotation** \( \text{<2.49\text{°}} \) \([\alpha]_D^2\text{°} + 86 - +94°\) (after drying, 0.1 g, methanol, 10 mL, 100 mm).

**Purity (1)** Heavy metals \(< 1.07\)—Proceed with 1.0 g of Dexamethasone according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 0.18 g of Dexamethasone in 100 mL of acetonitrile. To 33 mL of this solution add a solution, prepared by dissolving 1.32 g of ammonium for-
Dextran 40

Dextran 40 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*), and the average molecular mass is about 40,000.

When dried, it contains not less than 98.0% and not more than 102.0% of dextran 40.

**Description**

Dextran 40 occurs as a white, amorphous powder. It is odorless and tasteless. It is practically insoluble in ethanol (95%) and in diethyl ether. It dissolves gradually in water. It is hygroscopic.

**Identification**

To 1 mL of a solution of Dextran 40 (1 in 3000) add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Then to this solution add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

**pH**

Dissolve 1.0 g of Dextran 40 in 10 mL of water: the pH of this solution is between 5.0 and 7.0.

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Dextran 40 in 10 mL of water by warming: the solution is clear and colorless.

2. Chloride

**Operating conditions**

Detector: An ultraviolet absorption photometer (wave-length: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (2:1).

Flow rate: Adjust the flow rate so that the retention time of dexamethasone is about 6 minutes.

**System suitability**

System performance—When the procedure is run with 10 μL of the standard solution under the above operating conditions, dexamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dexamethasone to that of the internal standard is not more than 1.0%.

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant.

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**System suitability**

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of dexamethasone obtained with 10 μL of this solution is equivalent to 8 to 12% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dexamethasone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dexamethasone is not more than 1.0%.

**Loss on drying**

Not more than 0.5% (0.2 g, 105°C, 3 hours).

**Residue on ignition**

Not more than 0.1% (0.2 g, platinum crucible).

**Assay**

Dissolve about 10 mg each of Dexamethasone and Dexamethasone RS, previously dried and accurately weighed, in 70 mL each of diluted methanol (1 in 2), add exactly 5 mL each of the internal standard solution, then add diluted methanol (1 in 2) to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL of each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Qt and Qs, of the peak area of dexamethasone to that of the internal standard, respectively.

Amount (mg) of C22H29FO5 = M5 × Qt/Qs

**Internal standard solution**—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 1000).

**Operating conditions**

Detector: An ultraviolet absorption photometer (wave-length: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (2:1).

Flow rate: Adjust the flow rate so that the retention time of dexamethasone is about 6 minutes.

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant.

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**Description**

Dextran 40 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*), and the average molecular mass is about 40,000.

When dried, it contains not less than 98.0% and not more than 102.0% of dextran 40.

**Identification**

To 1 mL of a solution of Dextran 40 (1 in 3000) add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Then to this solution add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

**pH**

Dissolve 1.0 g of Dextran 40 in 10 mL of water: the pH of this solution is between 5.0 and 7.0.

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Dextran 40 in 10 mL of water by warming: the solution is clear and colorless.

2. Chloride
Bacterial endotoxins

Dissolve 10.0 g of Dextran 40 in isotonic sodium chloride solution to make 100 mL, sterilize, and use this solution as the sample solution. Inject 1.0 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously to each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit no signs mentioned above.

All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Assay Weigh accurately about 3 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 0.450 g of glucose, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the control solution. Pipet 5 mL each of the sample solution and the control solution, and add water to make exactly 50 mL, respectively. Pipet 5 mL each of these solutions, add 5 mL of alkaline copper TS, exactly measured, and heat for 15 minutes in a water bath. After cooling, add 1 mL of a solution of potassium iodine (1 in 40) and 1.5 mL of dilute sulfuric acid, and titrate with 0.005 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

The tinctur consumed for the sample solution is not less than that for the control solution.

**Loss on drying** Less than 5.0% (1 g, 105°C, 6 hours).

**Residue on ignition** Less than 0.1% (1 g).

**Bacterial endotoxins** Less than 2.5 EU/g.

**Viscosity** (1) Dextran 40—Weigh accurately 0.2 to 0.5 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with water as directed in Method 1 at 25°C: the intrinsic viscosity is between 0.16 and 0.19.

(2) High-molecular fraction—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 7% to 10% of the precipitate (usually 80 to 90 mL) at 25 ± 1°C with stirring. Dissolve the precipitate at 35°C in a water bath with occasional shaking, and allow to stand for more than 15 hours at 25 ± 1°C. Remove the supernatant liquid by decantation, and heat the precipitate of the lower layer to dryness on a water bath. Dry the residue, and determine the intrinsic viscosity of the dried substance as directed in (1): the value is not more than 0.27.

(3) Low-molecular fraction—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 90% to 93% of the precipitate (usually 115 to 135 mL) at 25 ± 1°C with stirring, centrifuge at 25°C, and evaporate the supernatant liquid to dryness on a water bath. Dry the residue, and determine the intrinsic viscosity of the dried substance as directed in (1): the value is not less than 0.09.

**Antigenicity** Dissolve 10.0 g of Dextran 40 in isotonic sodium chloride solution to make 100 mL, sterilize, and use this solution as the sample solution. Invert 1.0 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously to each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit no signs mentioned above.

All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

**Dextran 40 Injection**

**Dextran 40 注射液**

Dextran 40 Injection is an aqueous solution for injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of dextran 40.

**Method of preparation**

<table>
<thead>
<tr>
<th>Dextran 40</th>
<th>10 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic Sodium Chloride Solution</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 100 mL

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

**Description** Dextran 40 Injection is a clear and colorless liquid. It is slightly viscous.

**Identification** (1) Dilute 1 mL of Dextran 40 Injection with water to 200 mL, and to 1 mL of the diluted solution add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution does not change in color.

(2) Dextran 40 Injection responds to the Qualitative Tests <1.06> for sodium salt and for chloride.

**pH** 4.5 – 7.0

**Bacterial endotoxins** Less than 0.50 EU/mL.
Extractable volume <6.05> It meets the requirement.

Viscosity <2.53> Measure exactly 2 to 5 mL of Dextran 40 Injection, add isotonic sodium chloride solution to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with isotonic sodium chloride solution as directed in Method 1 at 25°C: the intrinsic viscosity is between 0.16 and 0.19. Calculate the concentration of the sample solution (g/100 mL) as directed in the Assay.

Assay To exactly 30 mL of Dextran 40 Injection add water to make exactly 50 mL, and use this solution as the sample solution. Determine the optical rotation \( \alpha_o \) with the sample solution as directed under Optical Rotation Determination <2.49> in a 100-mL cell at 20 ± 1°C.

Amount (mg) of dextran 40 in 100 mL of Dextran 40 Injection

\[ \alpha_o \times 846.0 \]

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Storage—Avoid exposure to undue fluctuations in temperature.

Dextran 70 デキストラン 70

Dextran 70 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*), and the average molecular mass is about 70,000.

When dried, it contains not less than 98.0% and not more than 102.0% of dextran 70.

Description Dextran 70 occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves gradually in water.

It is hygroscopic.

Identification To 1 mL of a solution of Dextran 70 (1 in 3000) add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Then to this solution add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

pH <2.54> Dissolve 3.0 g of Dextran 70 in 50 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dextran 70 in 10 mL of water with warming: the solution is clear and colorless.

(2) Chloride <1.07>—With 2.0 g of Dextran 70, perform the test. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Dextran 70 according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.5 g of Dextran 70 according to Method 1, and perform the test (not more than 1.3 ppm).

(5) Nitrogen—Weigh accurately about 2 g of Dextran 70, previously dried, perform the test as directed under Nitrogen Determination <1.007>, where 10 mL of sulfuric acid is used for decomposition, and 45 mL of a solution of sodium hydroxide (2 in 5) is added: the amount of nitrogen (N: 14.007) is not more than 0.101%.

(6) Reducing substances—Weigh exactly 3.00 g of Dextran 70, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 0.300 g of glucose, previously dried, dissolve in water to make exactly 500 mL, and use this solution as the control solution. Pipet 5 mL each of the sample solution and the control solution, and add water to make exactly 50 mL, respectively. Pipet 5 mL of these diluted solutions, add exactly 5 mL of alkaline copper TS, and heat for 15 minutes in a water bath. After cooling, add 1 mL of a solution of potassium iodide (1 in 40) and 1.5 mL of dilute sulfuric acid, and titrate <2.50> with 0.005 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

The titrant consumed for the sample solution is not less than that for the control solution.

Loss on drying <2.41> Not more than 5.0% (1 g, 105°C, 6 hours).

Residue on ignition <2.44> Not more than 0.10% (1 g).

Viscosity <2.53> (1) Dextran 70—Weigh accurately 0.2 to 0.5 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with water as directed in Method 1 at 25°C: the intrinsic viscosity is between 0.21 and 0.26.

(2) High-molecular fraction—Weigh accurately about 6 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 7% to 10% of the precipitate (usually, 75 to 85 mL) at 25 ± 1°C with stirring. Dissolve the precipitate in a water bath at 35°C with occasional shaking, and allow to stand for more than 15 hours at 25 ± 1°C. Remove the supernatant liquid by decantation, and heat the precipitate of the lower layer on a water bath to dryness. Dry the residue, and determine the intrinsic viscosity of the dried residue as directed in (1): the value is not more than 0.35.

(3) Low-molecular fraction—Weigh accurately about 6 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 90% to 93% of the precipitate (usually 110 to 130 mL) at 25 ± 1°C with stirring, centrifuge at 25°C, and evaporate the supernatant liquid to dryness on a water bath. Dry the residue, and determine the intrinsic viscosity of the dried residue as directed in (1): the value is not less than 0.10.

Antigenicity Dissolve 6.0 g of Dextran 70 in isotonic sodium chloride solution to make 100 mL, sterilize, and use this solution as the sample solution. Inject 1.0 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Separately, inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously to each of 2 guinea pigs of the
first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit not signs.

All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

**Pyrogen**

Dissolve 6.0 g of Dextran 70 in isotonic sodium chloride solution to make 100 mL, and perform the test: this solution meets the requirement.

**Assay**

Weigh accurately about 3 g of Dextran 70, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Determine the optical rotation $\alpha_20$ as directed under Optical Rotation Determination $<2.49>$ in a 100–mL cell at 20 $\pm$ 1°C.

Amount (mg) of dextran $70 \times \alpha_20 = 253.8$

**Containers and storage**

Containers—Tight containers.

**Dextran Sulfate Sodium Sulfur 5**

デキストラン硫酸エステルナトリウム イオウ5

Dextran Sulfate Sodium Sulfur 5 is a sodium salt of sulfate ester obtained by sulfation of partial decomposition products of dextran, which is produced by fermentation of sucrose with Lactobacillaceae Van Tieghem (Leuconostoc mesenteroides).

**Description**

Dextran Sulfate Sodium Sulfur 5 occurs as a white to light yellowish white powder. It is odorless, and has a saline taste.

It is freely soluble in water and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

**Identification**

1. To 10 mL of a solution of toluidine blue (1 in 100,000) add 0.05 mL of a solution of Dextran Sulfate Sodium Sulfur 5 (3 in 50) dropwise: a color of the solution changes from blue to red-purple.

2. To 1 mL of a solution of Dextran Sulfate Sodium Sulfur 5 (1 in 1500) add 2 mL of anthrone TS: a blue-green color develops, which turns dark blue-green gradually. Then, add 1 mL of dilute sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution remains dark blue-green.

3. A solution of Dextran Sulfate Sodium Sulfur 5 (1 in 100) responds to the Qualitative Tests $<1.09>$ (1) for sodium salt.

**Optical rotation**

$<2.49>$ $[\alpha]_20^D = [135.0 + 155.0]$° (calculated on the dried basis, 1.5 g, water, 25 mL, 100 mm).

**pH**

$<2.54>$ Dissolve 1.0 g of Dextran Sulfate Sodium Sulfur 5 in 20 mL of water: the pH of this solution is between 5.5 and 7.5.

**Purity**

Clarity of solution—Dissolve 2.5 g of Dextran Sulfate Sodium Sulfur 5 in 50 mL of water: the solution is clear. And, determine the absorbance of the solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry $<2.2>C$: not more than 0.090.

2. Chloride $<1.03>$—Perform the test with 0.10 g of Dextran Sulfate Sodium Sulfur 5. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.106%).

3. Sulfate $<1.14>$—Dissolve 0.10 g of Dextran Sulfate Sodium Sulfur 5 in 6 mL of water, add 0.6 mL of barium chloride TS, and heat in a water bath for 4 minutes. After cooling, add 1 mL of dilute hydrochloric acid and water to make 50 mL, allow to stand for 10 minutes, and observe: the turbidity of the solution is not more intense than that of the control solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 6 mL of water, and proceed in the same manner (not more than 0.240%).

4. Heavy metals $<1.07>$—Proceed with 1.0 g of Dextran Sulfate Sodium Sulfur 5 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

5. Arsenic $<1.11>$—Prepare the test solution with 1.0 g of Dextran Sulfate Sodium Sulfur 5 according to Method 3, and perform the test (not more than 2 ppm).

**Sulfur content**

Weigh accurately about 1.0 g of Dextran Sulfate Sodium Sulfur 5, dissolve in 5 mL of water, add 1.5 mL of hydrochloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. To exactly 10 mL of the sample solution add exactly 20 mL of 0.02 mol/L barium chloride VS, add 5 mL of methanol, and heat in a water bath for 30 minutes. After cooling, neutralize with sodium hydroxide TS, and add 70 mL of water, 10 mL of a solution of zinc disodium ethylenediamine tetraacetate tetrahydrate (1 in 20), 3 mL of ammonium chloride TS and 7 mL of strong ammonium water, and titrate $<2.50>$ with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to light blue (indicator: 5 drops of eriochrome black T TS). Perform a blank determination. Amount of sulfur (S: 32.07), calculated on the dried basis, is between 3.0 and 6.0%.

Each mL of 0.02 mol/L barium chloride VS $= 0.6414$ mg of S

**Loss on drying**

$<2.41>$ Not more than 10.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

**Viscosity**

Weigh accurately about 1.5 g of Dextran Sulfate Sodium Sulfur 5, calculated on the dried basis, dissolve in a solution of sodium chloride (29 in 500) to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and a solution of sodium chloride (29 in 500) at 25 $\pm$ 0.02°C as directed: the intrinsic viscosity is between 0.030 and 0.040.

**Containers and storage**

Containers—Tight containers.
Dextran Sulfate Sodium Sulfur 18
デキストラン硫酸エステルナトリウム イオウ 18

Dextran Sulfate Sodium Sulfur 18 is a sodium salt of sulfate ester obtained by sulfation of partial decomposition products of dextran, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* Van Tieghem (Lactobacillaceae).

**Description** Dextran Sulfate Sodium Sulfur 18 occurs as a white to yellowish white powder. It is odorless, and has a saline taste.

It is freely soluble in water and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

**Identification** (1) To 10 mL of a solution of toluidine blue (1 in 100,000) add 0.05 mL of a solution of Dextran Sulfate Sodium Sulfur 18 (3 in 50) dropwise: a color of the solution changes from blue to red-purple.

(2) To 1 mL of a solution of Dextran Sulfate Sodium Sulfur 18 (1 in 1500) add 2 mL of anthrone TS: a blue-green color develops, which turns dark blue-green gradually. Then, add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution remains dark blue-green.

(3) A solution of Dextran Sulfate Sodium Sulfur 18 (1 in 100) responds to the Qualitative Tests <1.09> (1) for sodium salt.

**Optical rotation** <2.49> [α]D

Optical rotation <2.49> [α]D: + 90.0 – + 110.0° (calculated on the dried basis, 1.5 g, water, 25 mL, 100 mm).

**pH** <2.54> Dissolve 1.0 g of Dextran Sulfate Sodium Sulfur 18 in 20 mL of water: the pH of this solution is between 5.5 and 7.5.

**Purity** (1) Chloride <1.03>—Perform the test with 0.10 g of Dextran Sulfate Sodium Sulfur 18. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.106%).

(2) Sulfate <1.14>—Dissolve 0.10 g of Dextran Sulfate Sodium Sulfur 18 in 6 mL of water, add 0.6 mL of barium chloride TS, and heat in a water bath for 4 minutes. After cooling, add 1 mL of dilute hydrochloric acid and water to make 50 mL, allow to stand for 10 minutes, and observe: the turbidity of the solution is not more intense than that of the control solution. Prepare the control solution as follows: to 1.0 mL of 0.005 mol/L sulfuric acid VS add 6 mL of water, and proceed in the same manner (not more than 0.480%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Dextran Sulfate Sodium Sulfur 18 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Dextran Sulfate Sodium Sulfur 18 according to Method 3, and perform the test (not more than 2 ppm).

**Sulfur content** Weigh accurately about 0.5 g of Dextran Sulfate Sodium Sulfur 18, dissolve in 5 mL of water, add 1.5 mL of hydrochloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. To exactly 10 mL of the sample solution add exactly 20 mL of 0.02 mol/L barium chloride VS, add 5 mL of methanol, and heat in a water bath for 30 minutes. After cooling, neutralize with sodium hydroxide TS, and add 70 mL of water, 10 mL of a solution of zinc disodium ethylenediamine tetraacetate tetrahydrate (1 in 20), 3 mL of ammonium chloride TS and 7 mL of strong ammonium water, and titrate <2.56> with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to light blue (indicator: 5 drops of eriochrome black T TS). Perform a blank determination. Amount of sulfur (S: 32.07), calculated on the dried basis, is between 15.0 and 20.0%.

Each mL of 0.02 mol/L barium chloride VS

\[
= 0.6414 \text{ mg of S}
\]

**Loss on drying** <2.41> Not more than 10.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

**Viscosity** <2.55> Weigh accurately about 1.5 g of Dextran Sulfate Sodium Sulfur 18, calculated on the dried basis, dissolve in a solution of sodium chloride (29 in 500) to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and a solution of sodium chloride (29 in 500) at 25 ± 0.02°C as directed: the intrinsic viscosity is between 0.020 and 0.032.

**Containers and storage** Containers—Tight containers.

Dextrin
デキストリン

**Description** Dextrin occurs as a white or light yellow, amorphous powder or granules. It has a slight, characteristic odor and a sweet taste. It does not irritate the tongue. Dextrin is freely soluble in boiling water, soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

**Identification** To 0.1 g of Dextrin add 100 mL of water, shake, and filter if necessary. To 5 mL of the filtrate add 1 drop of iodine TS: a light red-brown or light red-purple color develops.

**Purity** (1) Clarity and color of solution—Take 2.0 g of Dextrin in a Nessler tube, add 40 mL of water, dissolve by heating, cool, and add water to make 50 mL: the solution is colorless or light yellow. It is clear, and even if turbid, the turbidity is not more than that of the following control solution.

Control solution: To 1.0 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid, 46 mL of water and 2 mL of barium chloride TS, allow to stand for 10 minutes, and shake before use.

(2) Acidity—To 1.0 g of Dextrin add 5 mL of water, dissolve by heating, cool, and add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Chloride <1.03>—To 2.0 g of Dextrin add 80 mL of water, dissolve by heating, cool, add water to make 100 mL, and filter. Take 40 mL of the filtrate, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more
than 0.013%)

4. Sulfate: To 45 mL of the filtrate obtained in (3) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

5. Oxalate: To 1.0 g of Dextrin add 20 mL of water, dissolve by heating, cool, add 1 mL of acetic acid (31), and filter. To 5 mL of the filtrate add 5 drops of calcium chloride TS: no turbidity is produced immediately.

6. Calcium: To a 5-mL portion of the filtrate obtained in (5) add 5 drops of ammonium oxalate TS: no turbidity is produced immediately.

7. Heavy metals: Proceed with 0.5 g of Dextrin according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

Loss on drying: Not more than 10% (0.5 g, 105°C, 4 hours).

Residue on ignition: Not more than 0.5% (0.5 g).

Containers and storage: Containers—Well-closed containers.

Dextromethorphan Hydrobromide Hydrate

Dextromethorphan Hydrobromide Hydrate contains not less than 98.0% of dextromethorphan hydrobromide \(C_{18}H_{25}NO.HBr.H_2O\): 370.32, calculated on the anhydrous basis.

Description: Dextromethorphan Hydrobromide Hydrate occurs as white crystals or crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95) and in acetic acid (100), and sparingly soluble in water.

Melting point: about 126°C (Insert the capillary tube into the bath preheated to 116°C, and continue the heating so that the temperature rises at a rate of about 3°C per minute.)

Identification (1) Determine the absorption spectrum of a solution of Dextromethorphan Hydrobromide Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \(2.25\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dext-romethorphan Hydrobromide Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry \(2.25\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 50 mL of a solution of Dextromethorphan Hydrobromide Hydrate (1 in 100) add 2 drops of phenolphalein TS and sodium hydroxide TS until a red color develops. Add 50 mL of chloroform, shake, and add 5 mL of dilute nitric acid to 40 mL of the water layer. This solution responds to the Qualitative Tests \(1.09\) for bromide.

Optical rotation: \(2.49\) [α]D: +26 – +30° (0.34 g, calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH: Dissolve 1.0 g of Dextromethorphan Hydrobromide Hydrate in 100 mL of water: the pH of this solution is between 5.2 and 6.5.

Purity: Clarity and color of solution: Dissolve 0.20 g of Dextromethorphan Hydrobromide Hydrate in 20 mL of water: the solution is clear and colorless.

(2) N,N-dimethylaniline: To 0.50 g of Dextromethorphan Hydrobromide Hydrate add 20 mL of water, and dissolve by heating on a water bath. After cooling, add 2 mL of dilute acetic acid, 1 mL of sodium nitrite TS and water to make 25 mL: the solution has no more color than the following control solution.

Control solution: Dissolve 0.10 g of N,N-dimethylaniline in 400 mL of water by warming on a water bath, cool, and add water to make 500 mL. Pipet 5 mL of this solution, and add water to make 200 mL. To 1.0 mL of this solution add 2 mL of dilute acetic acid, 1 mL of sodium nitrite TS and water to make 25 mL.

(3) Heavy metals: Proceed with 1.0 g of Dextromethorphan Hydrobromide Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Phenolic compounds: Dissolve 5 mg of Dextromethorphan Hydrobromide Hydrate in 1 drop of dilute hydrochloric acid and 1 mL of water, add 2 drops of iron (III) chloride TS and 2 drops of potassium hexacyanoferrate (III) TS, shake, and allow to stand for 15 minutes: no blue-green color develops.

(5) Related substances: Dissolve 0.25 g of Dextromethorphan Hydrobromide Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(2.03\). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene, ethyl acetate, methanol, dichloromethane and 13.5 mol/L ammonia TS (55:20:13:10:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly bismuth potassium iodide TS on the plate, and then spray evenly hydrogen peroxide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water: \(2.48\) 4.0 – 5.5% (0.2 g, volumetric titration, back titration).
Residue on ignition  
Not more than 0.1% (1 g).

Assay  
Weigh accurately about 0.5 g of Dextromethorphan Hydrobromide Hydrate, dissolve in 10 mL of acetic acid (100) and add 40 mL of acetic anhydride. Titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.23 mg of C_{18}H_{25}NO.HBr

Containers and storage  Containers—Well-closed containers.

Diastase  
ジアスターゼ

Diastase is an enzyme drug mainly prepared from malt.

It has amylolytic activity.

It contains not less than 440 starch saccharifying activity units per g.

It is usually diluted with suitable diluents.

Description  Diastase occurs as a light yellow to light brown powder.

It is hygroscopic.

Purity  Rancidity—Diastase has no unpleasant or rancid odor, and has no unpleasant or rancid taste.

Loss on drying  Not more than 4.0% (1 g, 105°C, 5 hours).

Assay  
(i) Substrate solution—Use potato starch TS for amylolytic activity test.

(ii) Sample solution—Weigh accurately about 0.1 g of Diastase, and dissolve in water to make exactly 100 mL.


Containers and storage  Containers—Well-closed containers.

Diastase and Sodium Bicarbonate Powder  
ジアスターゼ・重曹散

Method of preparation

<table>
<thead>
<tr>
<th></th>
<th>Diastase</th>
<th>Sodium Bicarbonate</th>
<th>Precipitated Calcium Carbonate</th>
<th>Magnesium Oxide</th>
<th>Powdered Gentian</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>200 g</td>
<td>600 g</td>
<td>400 g</td>
<td>150 g</td>
<td>50 g</td>
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<tr>
<td>To make</td>
<td>1000 g</td>
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Prepare before use as directed under Powders, with the above ingredients.

Description  Diastase and Sodium Bicarbonate Powder occurs as a slightly brownish, light yellow powder. It has a characteristic odor and a bitter taste.

Containers and storage  Containers—Well-closed containers.

Diazepam  
ジアゼパム

![Diazepam structure](image)

C_{16}H_{13}ClN_{2}O: 284.74
7-Chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one
[439-14-5]

Diazepam, when dried, contains not less than 98.0% of C_{16}H_{13}ClN_{2}O.

Description  Diazepam occurs as a white to light yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetone, soluble in acetic anhydride and in ethanol (95), sparingly soluble in diethyl ether, slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification  
(1) Dissolve 10 mg of Diazepam in 3 mL of sulfuric acid, and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

(2) Dissolve 2 mg of Diazepam in 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of the solution as directed under Ultra-
Diazepam Tablets

Diazepam Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of diazepam (C16H13ClN2O: 284.74).

Method of preparation—Prepare as directed under Tablets, with Diazepam.

Identification—To a portion of the powdered Diazepam Tablets, equivalent to 50 mg of Diazepam according to the labeled amount, add 50 mL of acetone, shake, and filter. Evaporate 1 mL of the filtrate on a water bath to dryness, and dissolve the residue with 100 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 240 nm and 244 nm, between 283 nm and 287 nm, and between 360 nm and 370 nm.

Uniformity of dosage units—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Diazepam Tablets add 5 mL of water, and disintegrate the tablet by shaking. Then add 30 mL of methanol, shake for 10 minutes, add methanol to make exactly 50 mL, and centrifuge. Pipet 0.5 mL of the supernatant liquid, equivalent to 0.4 mg of diazepam (C16H13ClN2O), add exactly 5 mL of the internal standard solution, then add methanol to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of diazepam for assay, previously dried at 105°C for 2 hours, and disolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and add methanol to make 20 mL, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.07) under the following conditions, and calculate the ratios, Qs and Qo, of the peak area of diazepam to that of the internal standard.

Amount (mg) of diazepam (C16H13ClN2O) = M3 × Qo/Qs × 1/V

M3: Amount (mg) of diazepam for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 25,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the internal standard and diazepam are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of diazepam to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers. Storage—Light-resistant.

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the internal standard and diazepam are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of diazepam to that of the internal standard is not more than 1.0%.
Dissolution  Being specified separately.

Assay  Weigh accurately the mass of not less than 20 Diazepam Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of diazepam (C₁₅H₁₃ClN₂O₂), add 10 mL of water, shake, then add 60 mL of methanol, shake for 10 minutes, add methanol to make exactly 100 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of diazepam for assay, previously dried at 105°C for 2 hours, and dissolve in 10 mL of water and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.03> according to the following conditions, and calculate the ratios, QT and QS, of the peak area of diazepam to that of the internal standard.

\[
M_0: \text{Amount (mg) of diazepam for assay} \quad M_S: \text{Amount (mg) of diazepam for assay} \\
\text{Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 5000).} \\
\text{Operating conditions—} \\
\text{Detector: An ultraviolet absorption photometer (wavelength: 254 nm).} \\
\text{Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).} \\
\text{Column temperature: A constant temperature of about 40°C.} \\
\text{Mobile phase: A mixture of methanol and water (13:7).} \\
\text{Flow rate: Adjust the flow rate so that the retention time of diazepam is about 10 minutes.} \\
\text{System suitability—} \\
\text{System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and diazepam are eluted in this order with the resolution between these peaks being not more than 6.} \\
\text{System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of diazepam to that of the internal standard is not more than 1.0%.} \\
\text{Containers and storage—Containers—Tight containers.}
\]

Dibekacin Sulfate  ジベカシン硫酸塩

C₁₅H₁₃N₂O₆·xH₂SO₄
3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-[2,6-diamino-2,3,4,6-tetrahydroxy-α-D-erythro-hexopyranosyl-(1→4)]-2-deoxy-D-streptamine sulfate [58580-55-5]

Dibekacin Sulfate is the sulfate of a derivative of bekamycin.

It contains not less than 640 μg (potency) and not more than 740 μg (potency) per mg, calculated on the dried basis. The potency of Dibekacin Sulfate is expressed as mass (potency) of dibekacin (C₁₅H₁₃N₂O₄: 451.52).

Description  Dibekacin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification (1)  Dissolve 20 mg each of Dibekacin Sulfate and Dibekacin Sulfate RS in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28) and methanol (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and standard solution show a purple-brown color and the same Rf value.

(2)  To 5 mL of a solution of Dibekacin Sulfate (1 in 50) add 1 drop of barium chloride TS: a white precipitate is produced.

Optical rotation <2.49>  [α]D²⁰: +96° to +106° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <2.54>  The pH of a solution obtained by dissolving 1.0 g of Dibekacin Sulfate in 20 mL of water is between 6.0 and 8.0.

Purity (1)  Clarity and color of solution—Dissolve 1.0 g of Dibekacin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2)  Heavy metals <1.07>—Proceed with 1.0 g of Dibekacin Sulfate in 10 mL of water: the results obtained are not more than 6.0 mg per g.
Dibekacin Sulfate Ophthalmic Solution

ジベカシン硫酸塩点眼液

Dibekacin Sulfate Ophthalmic Solution is an aqueous ophthalmic preparations.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of dibekacin (C18H37N5O8: 451.52).

Method of preparation Prepare as directed under Ophthalmic Preparations, with Dibekacin Sulfate.

Description Dibekacin Sulfate Ophthalmic Solution is a clear, colorless liquid.

Identification To a volume of Dibekacin Sulfate Ophthalmic Solution add water so that each mL contains about 2.5 mg (potency) of Dibekacin Sulfate according to the labeled amount, and use this solution as the sample solution. Separately, dissolve an amount of Dibekacin Sulfate RS, equivalent to 5 mg (potency), in 2 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.02. Spot 10 μL of each solution and standard solution on a plate of silica gel for thin-layer chromatography. Proceed as directed in the Identification (1) under Dibekacin Sulfate.

Method of preparation

Prepare the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics 4.02 according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633
(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer having pH 6.5 to 6.6 after sterilization.
(iii) Standard solutions—Weigh accurately an amount of Dibekacin Sulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of this standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.
(iv) Sample solutions—Weigh accurately an amount of Dibekacin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Dibucaine Hydrochloride

ジブカイン塩酸塩

C20H29N3O2·HCl: 379.92
2-Butyloxy-N-(2-diethylaminoethyl)-4-quinolinecarboxamide monohydrochloride [61-12-1]

Dibucaine Hydrochloride, when dried, contains not less than 98.0% of C20H29N3O2·HCl.

Description Dibucaine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in water, in ethanol (95) and in acetic acid (100), freely soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Dibucaine Hydrochloride in 1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dibucaine Hydrochloride, previously dried, as directed in
Diclofenac Sodium

**Chloride**, previously dried, dissolve in 50 mL of water: the pH of this solution is between 5.0 and 6.0.

**Melting point** 2.60 95 – 100°C. Charge Dibucaine Hydrochloride into a capillary tube for melting point determination, and dry in vacuum over phosphorus (V) oxide at 80°C for 5 hours. Seal immediately the open end of the tube, and determine the melting point.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Dibucaine Hydrochloride in 20 mL of water: the solution is clear and colorless. Determine the absorbance of this solution at 430 nm as directed under Ultraviolet-visible Spectrophotometry <2.25>, using water as the blank: it is not more than 0.056%.

(2) Sulfate <1.14>—Perform the test with 0.3 g of Dibucaine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.056%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Dibucaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.20 g of Dibucaine Hydrochloride in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 20 mL, then pipet 2 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.67>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, phosphorus (V) oxide, 80°C (7:3), and air-dry the plate. Examine under ultraviolet light: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 5 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Dibucaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 19.00 mg of C₁₄H₁₀Cl₂NNaO₂.HCl

**Containers and storage** Containers—Tight containers.

Diclofenac Sodium

ジクロフェナクナトリウム

C₁₄H₁₀Cl₂NNaO₂: 318.13

Monosodium 2-(2,6-dichlorophenylamino)phenylacetate [15307-79-6]

Diclofenac Sodium, when dried, contains not less than 98.5% of C₁₄H₁₀Cl₂NNaO₂.

**Description** Diclofenac Sodium occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in water and in acetic acid (100), and practically insoluble in diethyl ether.

It is hygroscopic.

**Identification** (1) To 1 mL of a solution of Diclofenac Sodium in methanol (1 in 250) add 1 mL of nitric acid: a dark red color develops.

(2) Perform the test with 5 mg of Diclofenac Sodium as directed under Flame Coloration Test <1.04> (2): a light green color appears.

(3) Determine the infrared absorption spectrum of Diclofenac Sodium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Diclofenac Sodium (1 in 100) responds to the Qualitative Tests <1.09> for sodium salt.

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 g of Diclofenac Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Diclofenac Sodium according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Diclofenac Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of each peak other than the peak of diclofenac obtained from the sample solution is not larger than the peak area of the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diame-
and the absorbent cotton with 15 mL of chloroform, com-
moistened with chloroform. Wash the tip of the separator
with two 20-mL portions of chloroform, and filter the ex-
precipitate formed with 50 mL of chloroform. Extract again
for 3 hours. Add 2 mL of dilute hydrochloric acid, and extract the
previously dried, dissolve with 40 mL of water in a separa-

3 hours).

Loss on drying Not more than 0.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.5 g of Diclofenac Sodium,
previously dried, dissolve with 40 mL of water in a separa-
tor, add 2 mL of dilute hydrochloric acid, and extract the precipitate formed with 50 mL of chloroform. Extract again
with two 20-mL portions of chloroform, and filter the ex-
tract each time through a pledget of absorbent cotton mois-
tened with chloroform. Wash the tip of the separator and the absorbent cotton with 15 mL of chloroform, com-
bine the washing with the extracts, add 10 mL of a solution of
1 mol/L hydrochloric acid TS in ethanol (99.5) (1 in 100),
and titrate with 0.1 mol/L potassium hydroxide-ethanol
VS from the first equivalent point to the second equivalent point (potentiometric titration).

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 31.81 mg of C5H6Cl2N2O4S2.

Containers and storage Containers—Tight containers.

Diclofenamide

Dichlorphenamide

ジクロフェナミド

C6H6Cl2N2O4S2: 305.16
4,5-Dichlorobenzene-1,3-disulfonamide
[120-97-8]

Diclofenamide, when dried, contains not less than
98.0% of C6H6Cl2N2O4S2.

Description Diclofenamide occurs as a white, crystalline
powder. It is very soluble in N,N-dimethylformamide, soluble in
water (95), and very slightly soluble in ethanol.

Diclofenamide dissolves in sodium hydroxide TS.

Identification (1) Dissolve 0.01 g of Diclofenamide in 100
mL of 0.01 mol/L sodium hydroxide TS. To 10 mL of the
solution add 0.1 mL of hydrochloric acid. Determine the
absorption spectrum of the solution as directed under Ultra-
violet-visible Spectrophotometry <2.24>, and compare the
spectrum with the Reference Spectrum or the spectrum of a
solution of Diclofenamide RS prepared in the same manner
as the sample solution: both spectra exhibit similar intensi-
ties of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dico-
fenamide, as directed in the potassium bromide disk method
under Infrared Spectrophotometry <2.25>, and compare the
spectrum with the Reference Spectrum or the spectrum of Diclofenamide RS: both spectra exhibit similar intensities of
absorption at the same wave numbers.

Melting point 229–237°C

Purity (1) Chloride 1.07—Dissolve 0.10 g of Diclofen-
amide in 10 mL of N,N-dimethylformamide, and add 6 mL
of dilute nitric acid and water to make 50 mL. Perform the
test using this solution as the test solution. Prepare the con-

mL of 0.01 mol/L hydro-
chloric acid VS add 10 mL of N,N-dimethylformamide,
6 mL of dilute nitric acid and water to make 50 mL (not more than 0.160%).

(2) Selenium—To 0.10 g of Diclofenamide add 0.5 mL
of a mixture of perchloric acid and sulfuric acid (1:1) and 2
mL of nitric acid, and heat on a water bath until no more
brown gas evolves and the solution becomes to be a light yel-
low clear solution. After cooling, add 4 mL of nitric acid to
this solution, then add water to make exactly 50 mL, and use
this solution as the sample solution. Separately, pipet 3 mL
of Standard Selenium Solution, add 0.5 mL of a mixture of
perchloric acid and sulfuric acid (1:1) and 6 mL of nitric
acid, then add water to make exactly 50 mL, and use this
solution as the standard solution. Perform the test with the
sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to
the following conditions, and determine constant absor-
bances, A7 and A5, obtained on a recorder after rapid in-
creasing of the absorption: A7 is smaller than A5 (not more
than 30 ppm).

Perform the test by using a hydride generating system and
a thermal absorption cell. Lamp: A selenium hollow cathode lamp.
Wavelength: 196.0 nm.
Temperature of sample atomizer: When an electric fur-
nace is used, about 1000°C.
Carrier gas: Nitrogen or Argon.

(3) Heavy metals 1.07—Procede with 2.0 g of Diclo-
fenamide according to Method 2, and perform the test. Pre-
pare the control solution with 2.0 mL of Standard Lead Sol-
lution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Diclofen-
amide in 50 mL of the mobile phase, and use this solution as
the sample solution. Pipet 2 mL of the sample solution, add
the mobile phase to make exactly 100 mL, and use this solu-

tion as the standard solution. Perform the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of diclofenamide obtained from the sample solution is not larger than the peak area of diclofenamide from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of diclofenamide.

**System suitability**—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of diclofenamide obtained from 10 \( \mu L \) of this solution is equivalent to 3.5 to 6.5% of that from 10 \( \mu L \) of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diclofenamide is not more than 1.0%.

**Loss on drying** \(<2.41>\) Not more than 1.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 5 hours).

**Residue on ignition** \(<2.44>\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 50 mg each of Diclofenamide and Diclofenamide RS, previously dried, and dissolve each in 30 mL of the mobile phase. To each add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of diclofenamide to that of the internal standard, respectively.

\[
\text{Amount (mg) of } C_6H_6Cl_2N_2O_4S_2 = M_S \times \frac{Q_T}{Q_S}
\]

\( M_S \): Amount (mg) of Diclofenamide RS

**Internal standard solution**—A solution of butyl parahydroxy benzoate in the mobile phase (3 in 5000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadeccylsilanized silica gel for liquid chromatography (10 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of sodium phosphate TS and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of diclofenamide is about 7 minutes.

**System suitability**—

System performance: When the procedure is run with 10 \( \mu L \) of the standard solution under the above operating conditions, diclofenamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of diclofenamide to that of the internal standard is not more than 1.0%.

**Diclofenamide Tablets**

**Dichlorphenamide Tablets**

**ジクロフェナミド錠**

Diclofenamide Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of diclofenamide (\( C_6H_6Cl_2N_2O_4S_2 \): 305.16).

**Method of preparation** Prepare as directed under Tablets, with Diclofenamide.

**Identification** To a quantity of powdered Diclofenamide Tablets, equivalent to 0.2 g of Diclofenamide according to the labeled amount, add 20 mL of methanol, shake, and filter. Evaporate the filtrate on a water bath to dryness, and dissolve 0.01 g of the residue in 100 mL of 0.01 mol/L sodium hydroxide TS. To 10 mL of this solution add 0.1 mL of hydrochloric acid TS, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\): it exhibits maxima between 284 nm and 288 nm, and between 293 nm and 297 nm.

**Dissolution** \(<6.10D>\) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Diclofenamide Tablets is not less than 70%.

Start the test with 1 tablet of Diclofenamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \( \mu m \). Discard the first 10 mL of the filtrate, pipet \( V \) mL of the subsequent filtrate, add water to make exactly \( V \) mL so that each mL contains about 56 \( \mu g \) of diclofenamide (\( C_6H_6Cl_2N_2O_4S_2 \)) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 55 mg of Diclofenamide RS, previously dried under reduced pressure not exceeding 0.67 kPa at 100°C for 5 hours, dissolve in 10 mL of ethanol (95), and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), using water as the blank.

Dissolution rate (% with respect to the labeled amount of diclofenamide (\( C_6H_6Cl_2N_2O_4S_2 \))

\[
M_S = \frac{M_S}{A_T} = \frac{M_S}{A_S} \times \frac{1}{C} \times 90
\]

\( M_S \): Amount (mg) of Diclofenamide RS

\( C \): Labeled amount (mg) of diclofenamide (\( C_6H_6Cl_2N_2O_4S_2 \)) in 1 tablet.
**Dicloxacillin Sodium Hydrate**

**Description**

Dicloxacillin Sodium Hydrate occurs as a white to light yellowish white crystalline powder. It is odorless, and has an acid and bitter taste.

**Identification**

(1) Determine the absorption spectrum of a solution of Dicloxacillin Sodium Hydrate (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry \(\text{C22H25Cl2N3O3S.H2O}\), and compare the spectrum with the Reference Spectrum or the spectrum of Dicloxacillin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave lengths.

(2) Determine the infrared absorption spectrum of Dicloxacillin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry \(\text{C22H25Cl2N3O3S.H2O}\), and compare the spectrum with the Reference Spectrum or the spectrum of Dicloxacillin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dicloxacillin Sodium Hydrate responds to the Quantitative Tests \(\text{C22H25Cl2N3O3S.H2O}\) for sodium salt.

**Water**

Not less than 3.0% and not more than 4.5% (0.1 g, volumetric titration, direct titration).

**Assay**

Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics \(\text{C22H25Cl2N3O3S.H2O}\) according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium in (i) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.5 to 6.6 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Dicloxacillin Sodium RS equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C in a refrigerator, and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10 \(\mu\)g (potency) and 2.5 \(\mu\)g (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Dicloxacillin Sodium Hydrate equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10 \(\mu\)g (potency) and 2.5 \(\mu\)g (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

**Containers and storage**

Containers—Well-closed containers.

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**Diethylcarbamazine Citrate**

**Description**

Diethylcarbamazine Citrate occurs as a white, crystalline powder. It is odorless, and has an acid and bitter taste.

**Identification**

1. **Determination of Mass**
   - Weigh accurately an amount of Diethylcarbamazine Citrate, when dried, and use the amounts so that each mL contains 10 \(\mu\)g (potency) and 2.5 \(\mu\)g (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

2. **Determination of Potency**
   - Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics (0.1 g, volumetric titration, direct titration).

**Water**

Not less than 3.0% and not more than 4.5% (0.1 g, volumetric titration, direct titration).

**Assay**

Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics (0.1 g, volumetric titration, direct titration).

**Containers and storage**

Containers—Tight containers.
practically insoluble in acetone, in chloroform and in diethyl ether.

A solution of Diethylcarbamazine Citrate (1 in 20) is acid. It is hygroscopic.

**Identification** (1) Dissolve 0.5 g of Diethylcarbamazine Citrate in 2 mL of water, add 10 mL of sodium hydroxide TS, and extract with four 5-mL portions of chloroform. Wash the combined chloroform extracts with 10 mL of water, and evaporate the chloroform on a water bath. Add 1 mL of iodoethane to the residue, and boil gently under a reflux condenser for 5 minutes. Evaporate the excess iodoethane with the aid of a current of air, and dissolve the residue in 4 mL of ethanol (95). Cool the ethanol solution in an ice bath, with continuous stirring, add diethyl ether until precipitates are formed, and stir until crystallization is evident. Allow to stand in the ice bath for 30 minutes, and collect the precipitate. Dissolve the precipitate in 4 mL of ethanol (95), repeat the recrystallization in the same manner, then dry at 105°C for 4 hours: the crystals so obtained melt <2.60°C between 151°C and 155°C.

(2) Neutralize the remaining aqueous layer obtained in (1) with dilute sulfuric acid: the solution responds to the Qualitative Tests <1.00> (2) and (3) for citrate.

**Melting point** <2.60> 135.5 – 138.5°C

**Purity** Heavy metals <1.07>—Proceed with 2.0 g of Diethylcarbamazine Citrate according to Method 4, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <2.41> Not more than 1.0% (2 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.75 g of Diethylcarbamazine Citrate, previously dried, dissolve in 50 mL of acetic acid (100) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 39.14 mg of C_{10}H_{21}N_{3}O.C_{6}H_{8}O_{7}

**Containers and storage** Containers—Tight containers.

**Diethylcarbamazine Citrate Tablets**

ジエチルカルバマジンクエン酸塩錠

Diethylcarbamazine Citrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of diethylcarbamazine citrate (C\textsubscript{10}H\textsubscript{21}N\textsubscript{3}O.C\textsubscript{6}H\textsubscript{8}O\textsubscript{7}: 391.42).

**Method of preparation** Prepare as directed under Tablets, with Diethylcarbamazine Citrate.

**Identification** To a quantity of the powdered Diethylcarbamazine Citrate Tablets, equivalent to 0.1 g of Diethylcarbamazine Citrate according to the labeled amount, add 10 mL of water, shake well, and filter. To the filtrate add 1 mL of Reinecke salt TS: a light red precipitate is formed.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Diethylcarbamazine Citrate Tablets add 70 mL of the mobile phase, shake vigorously for 10 minutes, add the mobile phase to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet 5 mL of the subsequent filtrate, equivalent to about 2.5 mg of diethylcarbamazine citrate (C\textsubscript{10}H\textsubscript{21}N\textsubscript{3}O.C\textsubscript{6}H\textsubscript{8}O\textsubscript{7}), exactly 5 mL of the internal standard solution, and add this solution to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of diethylcarbamazine citrate (C\textsubscript{10}H\textsubscript{21}N\textsubscript{3}O.C\textsubscript{6}H\textsubscript{8}O\textsubscript{7})

\[ M_S = \frac{M_i \times Q_i}{Q_b} \times \frac{V_i}{V} \]

\[ M_S = \text{Amount (mg) of Diethylcarbamazine Citrate RS} \]

**Internal standard solution**—A solution of 2-aminobenzimidazole in the mobile phase (1 in 12,500).

**Assay** Weigh accurately about 25 mg of Diethylcarbamazine Citrate RS, previously dried at 105°C for 4 hours, dissolve in the mobile phase to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet 5 mL of the subsequent filtrate, and add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Diethylcarbamazine Citrate RS, previously dried at 105°C for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q\textsubscript{i} and Q\textsubscript{b}, of the peak area of diethylcarbamazine to that of the internal standard.

Amount (mg) of diethylcarbamazine citrate (C\textsubscript{10}H\textsubscript{21}N\textsubscript{3}O.C\textsubscript{6}H\textsubscript{8}O\textsubscript{7})

\[ M_S = \frac{M_i \times Q_i}{Q_b} \times 2 \]

\[ M_S = \text{Amount (mg) of Diethylcarbamazine Citrate RS} \]

**Internal standard solution**—A solution of 2-aminobenzimidazole in the mobile phase (1 in 12,500).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 0.05 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust the pH to 2.5. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of diethylcarbamazine is about 14 minutes.
JP XVI
Official Monographs / Diflucortolone Valerate  715

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, diethylcarbamazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of diethylcarbamazine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Difenidol Hydrochloride
ジフェニドール塩酸塩

\[
\text{C}_{21}\text{H}_{27}\text{NO}.\text{HCl}: 345.91
\]
1,1-Diphenyl-4-piperidino-1-butene hydrochloride [3254-89-5]

Difenidol Hydrochloride, when dried, contains not less than 98.5% of \( \text{C}_{21}\text{H}_{27}\text{NO}.\text{HCl} \).

Description Difenidol Hydrochloride occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in methanol, soluble in ethanol (95), sparingly soluble in water and in acetic acid (100), and practically insoluble in diethyl ether.

Melting point: about 217°C (with decomposition).

Identification (1) Dissolve 0.01 g of Difenidol Hydrochloride in 1 mL of sulfuric acid: an orange-red color develops. To this solution add carefully 3 drops of water: the solution becomes yellowish brown, and colorless on the addition of 10 mL of water.

(2) To 5 mL of a solution of Difenidol Hydrochloride (1 in 100) add 2 mL of Reinecke salt TS: a light red precipitate forms.

(3) To 10 mL of a solution of Difenidol Hydrochloride (1 in 100) add 2 mL of sodium hydroxide TS, and extract with two 15-mL portions of chloroform. Combine the extracts, wash with three 10-mL portions of water, evaporate with two 15-mL portions of chloroform. Combine the extracts, wash with three 10-mL portions of water, evaporate the chloroform on a water bath, and dry the residue in a desiccator (in vacuum, silica gel, 55°C) for 5 hours: the residue melts \( \approx 260°C \) between 103°C and 106°C.

(4) A solution of Difenidol Hydrochloride (1 in 100) responds to the Qualitative Tests \( <1.09 \) for chloride.

pH \( <2.44 \) Dissolve 1.0 g of Difenidol Hydrochloride in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.7 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Difenidol Hydrochloride in 10 mL of methanol: the solution is clear and colorless.

Diflucortolone Valerate
ジフルコルトロン吉草酸エステル

\[
\text{C}_{27}\text{H}_{36}\text{F}_{2}\text{O}_{5}: 478.57
\]
6α,9-Difluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-pentanate [59198-70-8]

Diflucortolone Valerate contains not less than 98.0% and not more than 102.0% of \( \text{C}_{27}\text{H}_{36}\text{F}_{2}\text{O}_{5} \), calculated on the dried basis.

Description Diflucortolone Valerate occurs as white crys-
tals or crystalline powder.

It is sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification (1)** Prepare the test solution by proceeding with 10 mg of Diflucortolone Valerate according to the Oxygen Flask Combustion Method \(<1.00\), using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid; the test solution responds to the Qualitative Tests \(<1.00\) for fluoride.

(2) Determine the absorption spectrum of a solution of Diflucortolone Valerate in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Diflucortolone Valerate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Diflucortolone Valerate as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\), and compare the spectrum with the Reference Spectrum or the spectrum of Diflucortolone Valerate RS: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation \(<2.49\) \([\alpha]_D^{20} + 110 – +115^\circ\) (0.1 g calculated on the dried basis, ethanol (99.5), 10 mL, 100 mm).

**Melting point \(<2.60\) 200 – 204°C**

**Purity (1)** Heavy metals \(<1.07\)—Proceed with 2.0 g of Diflucortolone Valerate in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm). Carbonize and incinerate as directed under Residue on Ignition \(<2.44\).

(2) Related substances—Use the sample solution obtained in the Assay as the sample solution. Perform the test with 10 \(\mu\)L of the sample solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions. Determine each peak area of sample solution by the automatic integration method, and calculate the amounts of these peaks by the area percentage method: the amount of each peak of flucortolone valerate, 12α diflucortolone valerate and 14 diflucortolone valerate, having the relative retention times of about 0.97, 1.03 and 1.05 with respect to diflucortolone valerate, respectively, is not more than 0.6%, respectively; the amount of the peak of clocortolone valerate, having the relative retention time of about 1.09, is not more than 0.3%; and the amount of each peak other than those mentioned above is not more than 0.1%. Furthermore, the total amount of the peaks other than the peak of diflucortolone valerate is not more than 2.0%.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with sulfonamide group bound to hexadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase A: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS, adjusted to pH 3.0 with phosphoric acid, and acetonitrile for liquid chromatography (11:9).
Mobile phase B: Acetonitrile for liquid chromatography.
Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>100 → 90</td>
<td>0 → 10</td>
</tr>
<tr>
<td>10 – 25</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>25 – 45</td>
<td>90 → 35</td>
<td>10 → 65</td>
</tr>
<tr>
<td>45 – 50</td>
<td>35</td>
<td>65</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute.

**System suitability—**

System performance: When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of diflucortolone valerate are not less than
Digitoxin

Digitoxin, when dried, contains not less than 90.0% of C_{41}H_{64}O_{13}.

Description  Digitoxin occurs as a white to light yellowish white, crystalline powder. It is odorless.

It is soluble in chloroform, sparingly soluble in methanol and in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification  (1) Transfer 1 mg of Digitoxin to a small test tube about 10 mm in inside diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids a brown ring develops slowly, and then fades.

(2) To 2 mg of Digitoxin add 25 mL of a freshly prepared solution of 1,3-dinitrobenzene in ethanol (95) (1 in 100), and dissolve by shaking. Take 2 mL of this solution, add 2 mL of a solution of tetramethylammonium hydroxide (100) (1 in 10,000), and dissolve by shaking. Take 2 mL of this solution, add 2 mL of a solution of cholesterol in ethanol (95) in a test tube, having the inner walls which are free from scratches, add 2 mL of a solution of cholesterol in ethanol (95) (1 in 200), mix gently, and allow to stand for 10 minutes: no turbidity is produced.

Optical rotation  <2.49> [α]_{D}^{19}: +16° - +18° (after drying, 0.5 g, chloroform, 20 mL, 200 mm).

Purity  Digitoxin—Dissolve 10 mg of Digitoxin in 2 mL of ethanol (95) in a test tube, having the inner walls which are free from scratches, add 2 mL of a solution of cholesterol in ethanol (95) (1 in 200), mix gently, and allow to stand for 10 minutes: no turbidity is produced.

Loss on drying  <2.41> Not more than 1.5% (0.5 g, in vacuum, 100°C, 2 hours).

Residue on ignition  <2.44> Not more than 0.5% (0.1 g).

Assay  Dissolve about 20 mg each of Digitoxin and Digitoxin RS, previously dried and accurately weighed, in methanol to make exactly 200 mL. Pipet 5 mL each of these solutions, add exactly 10 mL of the internal standard solution to each solution, add 12.5 mL of water, then add methanol to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q_{T} and Q_{S}, of the peak area of digitoxin to that of the internal standard, respectively.

\[ M_{5} = \frac{Q_{T} / Q_{S}}{M_{5}} \]

M_{5}: Amount (mg) of C_{41}H_{64}O_{13} = M_{5} \times Q_{T} / Q_{S}

Internal standard solution—A solution of acenaphthene in methanol (3 in 1,000,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of methanol and water (3:1). Flow rate: Adjust the flow rate so that the retention time of digitoxin is about 5 minutes.

Selection of column: Proceed with 50 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of digitoxin and the internal standard in this order with the resolution between these peaks being not less than 6.

Containers and storage  Containers—Light-resistant.

Storage—Light-resistant.
Digitoxin Tablets

Digitoxin Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of digitoxin (C41H64O13; 764.94).

Method of preparation Prepare as directed under Tablets, with Digitoxin.

Identification (1) Place a portion of powdered Digitoxin Tablets, equivalent to 2 mg of digitoxin (C41H64O13) according to the labeled amount, in a separator, shake with 30 mL of water, and shake vigorously with 30 mL of chloroform. Filter the chloroform extract with a funnel on which a small ing to the residue, and dissolve by shaking. Proceed with 2 mL of the solution as directed in the Identification (1) under Digitoxin.

(2) Evaporate 4 mL of the chloroform solution obtained in (1) to dryness, by warming under reduced pressure, add a freshly prepared solution of 1,3-dinitrobenzene in ethanol (95) (1 in 100) to the residue, and dissolve in 10 mL of chloroform. Transfer 5 mL of this solution to a small test tube about 10 mm in inside diameter, and evaporate to dryness on a water bath with the aid of a current of air. Proceed with the residue as directed in the Identification (1) under Digitoxin.

Uniformity of dosage units 6.02 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Digitoxin Tablets to a 50-mL beaker, add 0.5 mL of water to disintegrate the tablet, add 5 mL of acetonitrile, and warm on a water bath for 5 minutes, covering the beaker with a watch glass. After cooling, transfer the solution to separator A, rinse the beaker with 30 mL of chloroform and then with 20 mL of water, transfer the rinsings to separator A, and extract by vigorous shaking. Transfer the chloroform extract to separator B containing 5 mL of a solution of sodium hydrogen carbonate (1 in 100), and shake to wash. Filter the chloroform layer through a pledget of absorbent cotton, previously moistened with chloroform. Extract the water layer in separator A with two 30-mL portions of chloroform, wash the chloroform extract with a solution of sodium hydrogen carbonate (1 in 100) in separator B, filter in the same manner, and combine the filtrate with the first one. Evaporate this filtrate to dryness under reduced pressure by warming, add diluted ethanol (95) (4 in 5) to make exactly V mL of a solution containing 5 µg of digitoxin (C41H64O13) per mL. Shake vigorously for 20 minutes to dissolve, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Digitoxin RS, previously dried under reduced pressure at 100°C for 2 hours, and dissolve in ethanol (95) to make exactly 100 mL. Measure exactly 1 mL of this solution, add the dissolution medium to make exactly 500 mL, warm at 37 ± 0.5°C for 60 minutes, and filter through a membrane filter (less than 0.8 µm in pore size). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution.

Amount (mg) of digitoxin (C41H64O13)
\[ M_a \times \left( \frac{F_t - F_0}{F_0 - F_b} \right) \times \frac{V}{1000} \]
\[ M_a: \text{Amount (mg) of Digitoxin RS} \]

Dissolution 6.10D When the test is performed at 100 revolutions per minute according to the Basket method, using 500 mL of diluted hydrochloric acid (3 in 500) as the dissolution medium, the dissolution rates in 30 minutes and in 60 minutes of Digitoxin Tablets are not less than 60% and 85%, respectively. No retest requirement is applied to Digitoxin Tablets.

Start the test with 1 tablet of Digitoxin Tablets, withdraw α + 15 mL of the medium at the specified minute after starting the test, immediately add the same volume of fresh dissolution medium, previously warmed at 37 ± 0.5°C, to the vessel, and filter withdrawing medium through a membrane filter with a pore size not exceeding 0.8 µm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Measure exactly a mL of the sample solution, equivalent to about 2 µg of digitoxin (C41H64O13) according to the labeled amount, transfer to a glass-stoppered centrifuge tube Tα, and warm at 37 ± 0.5°C for 30 minutes. Further, at 60 minutes after starting the test, take α + 15 mL of the dissolved solution, proceed in the same manner as above, measure exactly a mL of the sample solution so obtained, and transfer to a glass-stoppered centrifuge tube Tα+15. Separately, weigh accurately 100 times the labeled amount of Digitoxin RS, previously dried under reduced pressure at 100°C for 2 hours, and dissolve in ethanol (95) to make exactly 100 mL. Measure exactly 1 mL of this solution, add the dissolution medium to make exactly 500 mL, warm at 37 ± 0.5°C for 60 minutes, and filter through a membrane filter (less than 0.8 µm in pore size). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the standard solution. Measure exactly a mL each of the standard solution and the dissolution medium, transfer to glass-stoppered centrifuge tubes Tα and Tα+15, respectively. Add exactly 7 mL of chloroform to each of the glass-stoppered centrifuge tubes Tα, Tα+15, Tα+30, Tα+60, Tα+90, and Tα+150, shake vigorously for 10 minutes and centrifuge. Discard the aqueous layer, measure exactly 5 mL of the chloroform layer, transfer to brown test tubes Tα, Tα+15, Tα+30, Tα+60, Tα+90, and Tα+150, evaporate the chloroform, add exactly 4 mL each of 0.05 g/dL L-ascorbic acid-hydrochloric acid solution, shake well, and allow to stand at a constant temperature between 25°C and 30°C for 45 minutes. Determine the fluorescence intensities, \( F_{α} \), \( F_{α+15} \), \( F_{α+30} \), \( F_{α+60} \), and \( F_{α+90} \) of these solutions at about 395 nm of the excitation wavelength and at about 560 nm of the fluorescence wavelength as directed under Fluorometry <2.22>.

Dissolution rate (%) with respect to the labeled amount of digitoxin (C41H64O13) for 30 minutes
\[ M_b \times \left( \frac{F_0 - F_b}{F_0 - F_t} \right) \times \frac{V}{150} \]
\[ M_b: \text{Amount (mg) of Digitoxin RS} \]
Dissolution rate (%) with respect to the labeled amount of digitoxin (C_{41}H_{64}O_{13}) for 60 minutes

\[ = MS \times \left( \frac{F_{60} - F_{0}}{F_{S} - F_{0}} + \frac{F_{30} - F_{0}}{F_{S} - F_{0}} \times a + 15 \right) \times 1/C \]

\( MS \): Amount (mg) of Digitoxin RS

\( C \): The labeled amount (mg) of digitoxin (C_{41}H_{64}O_{13}) in 1 tablet

\( a + 15 \): Measured volume (mL) of dissolved solution at the specified minute

**Assay**  
Weigh accurately and powder not less than 20 Digitoxin Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 mg of digitoxin (C_{41}H_{64}O_{13}), and shake with 12.5 mL of water for 10 minutes. Add exactly 10 mL of the internal standard solution, shake for 20 minutes, and add methanol to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Digitoxin RS, previously dried in vacuum at 100°C for 2 hours, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of the solution, add exactly 10 mL of the internal standard solution, add 12.5 mL of water, then methanol to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Digoxin.

\[ \text{Amount (mg) of digitoxin (C}_{41}\text{H}_{64}\text{O}_{13}) = MS \times Q_{T} / Q_{S} \times 1/40 \]

\( MS \): Amount (mg) of Digitoxin RS

**Internal standard solution**—A solution of acenaphthene in methanol (3 in 1,000,000).

**Containers and storage**  
Containers—Tight containers.  
Storage—Light-resistant.

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**Digoxin**

ジゴキシン

C_{41}H_{64}O_{14}: 780.94

3\(\beta\)-[2,6-Dideoxy-\(\beta\)-d-ribo-hexopyranosyl-(1\(\rightarrow\)4)-2,6-dideoxy-\(\beta\)-d-ribo-hexopyranosyl-(1\(\rightarrow\)4)-2,6-dideoxy-\(\beta\)-d-ribo-hexopyranosyl]-12\(\beta\),14-dihydroxy-5\(\beta\),14\(\beta\)-card-20(22)-enolide

[20830-75-5]

Digoxin, when dried, contains not less than 96.0% and not more than 106.0% of C_{41}H_{64}O_{14}.

**Description**  
Digoxin occurs as colorless or white crystals or a white crystalline powder.

It is freely soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in acetic acid (100), and practically insoluble in water.

**Identification**  
(1) Transfer 1 mg of Digoxin to a small test tube about 10 mm in inside diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids a brown ring free from a reddish color is produced, and the color of the upper layer near the contact zone changes to green through purple. Finally the entire acetic acid layer shows a green color through a deep blue color.

(2) Determine the infrared absorption spectrum of Digoxin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49>  
\[ \left[ \alpha \right]_{D}^{20} \approx +10.0 - +13.0^\circ \] (after drying, 0.20 g, dehydrated pyridine, 10 mL, 100 mm).

**Purity**  
(1) Clarity and color of solution—Dissolve 0.10 g of Digoxin in 15 mL of diluted ethanol (95) (4 in 5) by warming: the solution is clear and colorless.

(2) Related substances—Dissolve 25.0 mg of Digitoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add 10 mL of water and dilute ethanol to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve exactly 5.0 mg of Gitoxin RS, previously dried under
Digoxin Injection / Official Monographs

Digoxin Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 105.0% of the labeled amount of digoxin (C_{41}H_{64}O_{14}: 780.94).

Method of preparation Prepare as directed under Injections, with a solution of Digoxin in 10 to 50 vol% ethanol.

Description Digoxin Injection is a clear, colorless liquid.

Identification Dilute Digoxin Injection, if necessary, with methanol so that each mL contains about 0.25 mg of digoxin according to the labeled amount, and use this solution as the sample solution. In case where ingredients are suspected to affect the test, remove them by means of a solid-phase extraction. Separately, dissolve 0.5 mg of Digoxin RS in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.02. Spot 10 mL of each of the sample solution and standard solution on a plate of octadecylsilanized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and water (7:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of a solution of trichloroacetic acid in ethanol (99.5) (1 in 4) and a freshly prepared solution of sodium toluenesulfonchloramide trihydrate (3 in 100) (4:1) on the plate, heat at 110°C for 10 minutes, and examine under ultraviolet light (main wavelength: 366 nm): the RF values of the principal spots with the sample solution and the standard solution are not different each other.

Alcohol number <1.01> 0.8 - 1.2 (Method 1).
Purity  Related substances—To a volume of Digoxin Injection, equivalent to about 2.5 mg of Digoxin according to the labeled amount, add dilute ethanol to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than digoxin is not more than 5%.

Operating conditions—
Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of digoxin, beginning after the solvent peak.

System suitability—
Test for required detectability: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, add ethanol (95) to make 100 mL. To 10 mL of this solution add 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained from 10 μL of the solution for system suitability test

System performance: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Digoxin Tablets  ジゴキシン錠

Digoxin Tablets contain not less than 90.0% and not more than 105.0% of the labeled amount of digoxin (C41H64O14: 780.94).

Method of preparation  Prepare as directed under Tablets, with Digoxin.

Identification  To an amount of pulverized Digoxin Tablets, equivalent to 0.5 mg of Digoxin according to the labeled amount, add 2 mL of methanol, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 0.5 mg of Digoxin RS in 2 mL of methanol, and use this as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of octadecylsilanized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and water (7:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of a solution of trichloroacetic acid in ethanol (99.5) (1 in 4) and a freshly prepared solution of sodium tol-

Each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q1 and Q5, of the peak area of digoxin to that of the internal standard.

\[
M_S = \frac{M_S \times Q_1}{Q_5 \times 1/10}
\]

\[
Q_1 < 2.01
\]

\[
Q_5 < 6.05
\]

\[
Q_1 > 6.07
\]

\[
Q_5 > 2.03
\]

Storage—Light-resistant.
enesulfonchloramide trihydrate (3 in 100) (4:1) on the plate, heat at 110°C for 10 minutes, and examine under ultraviolet light (main wavelength: 366 nm): the Rf values of the principal spots with the sample solution and the standard solution are not different each other.

**Purity** Related substances—Powder not less than 20 Digoxin Tablets. Weigh a portion of the powder equivalent to 2.5 mg of Digoxin according to the labeled amount, add 30 mL of dilute ethanol, treat with ultrasonic waves for 20 minutes, and shake for 5 minutes. After cooling, add dilute ethanol to make 50 mL, filter, and use the filtrate as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amount of these peaks by the area percentage method: the total amount of the peaks other than digoxin is not more than 5%.

**Operating conditions**
- Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.
- Time span of measurement: About 4 times as long as the retention time of digoxin, beginning after the solvent peak.

**System suitability**—
- Test for required detectability: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained from 10 μL of this solution is equivalent to 0.07 to 0.13% of that of digoxin from 10 μL of the solution for system suitability test.
- System performance: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.
- System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5%.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Digoxin Tablets add 0.5 mL of water to disintegrate, then add exactly 0.5 mL of the internal standard solution, and add V mL of dilute ethanol so that each mL contains about 21 μg of digoxin (C41H64O14). Express this solution to ultrasonic waves for 20 minutes, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 25 mg of Digoxin RS, previously dried under reduced pressure at 105°C for 1 hour, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, and add ethanol (95) to make exactly 20 mL. Pipet 1 mL of this solution, add exactly 0.5 mL of the internal standard solution, then add 1.5 mL of water and (V − 2) mL of dilute ethanol, and use this as the standard solution. Proceed with the sample solution and standard solution as directed in the Assay.

**Amount (mg) of digoxin (C41H64O14)**

\[
M_S = \frac{M_F}{Q_F} \times 0.125
\]

**Mₙ:** Amount (mg) of Digoxin RS

**Internal standard solution**—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 40,000/V).

**Dissolution** <6.10>

When the test is performed at 100 revolutions per minute according to the Basket method, using 500 mL of diluted hydrochloric acid (3 in 500) as the dissolution medium, the dissolution rate in 60 minutes of Digoxin Tablets is not less than 65%. No retest requirement is applied to Digoxin Tablets.

Start the test with 1 tablet of Digoxin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Digoxin RS, previously dried in vacuum at 105°C for 1 hour, dissolve in a small portion of ethanol (95), and add a mixture of ethanol (95) and water (4:1) to make exactly 500 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 500 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution, the standard solution and the dissolution medium, and transfer to brown glass-stoppered test tubes. Add exactly 10 mL of 0.012 g/dL L-ascorbic acid-hydrochloric acid TS to these tubes, and shake. Immediately add exactly 1 mL of dilute hydrogen peroxide TS, shake well, and allow to stand at a constant temperature between 25°C and 30°C for 45 minutes. Determine the fluorescence intensities, F₁, F₂, and F₃, of these solutions at 360 nm of the excitation wavelength and at 485 nm of the fluorescence wavelength as directed under Fluorometry <2.22>, respectively.

**Dissolution rate (%) with respect to the labeled amount of digoxin (C₄₁H₆₄O₁₄)**

\[
M_S = \frac{M_F}{Q_F} \times (F_F - F_B)/(F_S - F_B) \times 1/C
\]

**Mₙ:** Amount (mg) of Digoxin RS

**C:** The labeled amount (mg) of digoxin (C₄₁H₆₄O₁₄) in 1 tablet

**Assay** Weigh accurately the mass of not less than 20 Digoxin Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 2.5 mg of digoxin (C₄₁H₆₄O₁₄), add 30 mL of dilute ethanol, exposure to ultrasonic waves for 20 minutes, and shake for 5 minutes. Add exactly 5 mL of the internal standard solution and dilute ethanol to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of Digoxin RS, previously dried under reduced pressure at 105°C for 1 hour, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the standard solution. Perform
the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of digoxin to that of the internal standard.

\[
\text{Amount (mg) of digoxin (C}_{41}\text{H}_{64}\text{O}_{14}) = M_S \times Q_2 / Q_1 \times 1/10
\]

M_S: Amount (mg) of Digoxin RS

**Internal standard solution**—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust the flow rate so that the retention time of digoxin is about 10 minutes.

**System suitability**—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, digoxin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

**Dihydrocodeine Phosphate**

ジヒドロコデインリン酸塩

C_{18}N_{23}O_{32}·H_3PO_4·3·9·9·38·8·9·38 (5R,6S)-4,5-Epoxy-3-methoxy-17-methylmorphinan-6-ol monophosphate [24204-13-5]

Dihydrocodeine Phosphate contains not less than 98.0% of C_{18}H_{32}NO_3·H_3PO_4, calculated on the dried basis.

**Description** Dihydrocodeine Phosphate occurs as a white to yellowish white, crystalline powder.

It is freely soluble in water and in acetic acid (100), slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Dihydrocodeine Phosphate (1 in 10) is between 3.0 and 5.0.
It is affected by light.

**Identification** (1) Determine the absorption spectrum of a solution of Dihydrocodeine Phosphate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Dihydrocodeine Phosphate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Dihydrocodeine Phosphate (1 in 20) responds to the Qualitative Tests <1.99> (1) for phosphate.

**Purity** (1) Chloride <1.07>—Perform the test with 0.5 g of Dihydrocodeine Phosphate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(2) Sulfate <1.14>—Perform the test with 0.20 g of Dihydrocodeine Phosphate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

(2) Related substances—Dissolve 0.20 g of Dihydrocodeine Phosphate in 10 mL of diluted ethanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.62>. The plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spots from the standard solution.

**Loss on drying <2.41>** Not more than 1.0% (0.5 g, 105°C, 4 hours).

**Assay** Weigh accurately about 0.5 g of Dihydrocodeine Phosphate, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

= 39.94 mg of C_{18}H_{32}NO_3·H_3PO_4

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.
1% Dihydrocodeine Phosphate Powder

1% Dihydrocodeine Phosphate Powder contains not less than 0.90% and not more than 1.10% of dihydrocodeine phosphate \((C_{18}H_{23}NO_3.H_3PO_4: 399.38)\).

**Method of preparation**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g)</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrocodeine Phosphate</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Lactose Hydrate</td>
<td>a sufficient</td>
<td></td>
</tr>
<tr>
<td></td>
<td>To make</td>
<td>1000 g</td>
</tr>
</tbody>
</table>

Prepare as directed under Granules or Powders, with the above ingredients.

**Identification** Determine the absorption spectrum of a solution of 1% Dihydrocodeine Phosphate Powder (1 in 100) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\): it exhibits a maximum between 281 nm and 285 nm.

**Assay** Weigh accurately about 5 g of 1% Dihydrocodeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of dihydrocodeine phosphate for assay (previously determine the loss on drying \(<2.4>\) (105°C, 4 hours)), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of dihydrocodeine to that of the internal standard.

\[
\text{Amount (mg) of dihydrocodeine phosphate (C_{18}H_{23}NO_3.H_3PO_4)} \times M_S = M_S \times Q_T / Q_S
\]

\(M_S\): Amount (mg) of dihydrocodeine phosphate for assay, calculated on the dried basis

**Internal standard solution**—A solution of ethylefrine hydrochloride (3 in 10,000).

**Operating conditions**—

- Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran.
- Flow rate: Adjust the flow rate so that the retention time of dihydrocodeine is about 9 minutes.

**System suitability**—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, dihydrocodeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

**System repeatability:** When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydrocodeine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

10% Dihydrocodeine Phosphate Powder

10% Dihydrocodeine Phosphate Powder contains not less than 9.3% and not more than 10.7% of dihydrocodeine phosphate \((C_{18}H_{23}NO_3.H_3PO_4: 399.38)\).

**Method of preparation**

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<td></td>
</tr>
<tr>
<td></td>
<td>To make</td>
<td>1000 g</td>
</tr>
</tbody>
</table>

Prepare as directed under Powders, with the above ingredients.

**Identification** Determine the absorption spectrum of a solution of 10% Dihydrocodeine Phosphate Powder (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\): it exhibits a maximum between 281 nm and 285 nm.

**Assay** Weigh accurately about 2.5 g of 10% Dihydrocodeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of dihydrocodeine phosphate for assay (previously determine the loss on drying \(<2.4>\) (105°C, 4 hours)), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of dihydrocodeine to that of the internal standard.

\[
\text{Amount (mg) of dihydrocodeine phosphate (C_{18}H_{23}NO_3.H_3PO_4)} \times M_S = M_S \times Q_T / Q_S \times 5
\]

\(M_S\): Amount (mg) of dihydrocodeine phosphate for assay, calculated on the dried basis

**Internal standard solution**—A solution of ethylefrine hydrochloride (3 in 10,000).

**Operating conditions**—

- Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of dihydrocodeine is about 9 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, dihydrocodeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydrocodeine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Dihydroergotamine Mesilate
ジヒドロエルゴタミンメシル酸塩

C₃₃H₃₇N₅O₅.CH₄O₃S: 679.78
(5'S,10R)-5'-Benzy1-12'-hydroxy-2'-methyl-9,10-dihydroergotaman-3',6',18-trione monomethanesulfonate [6190-39-2]

Dihydroergotamine Mesilate contains not less than 97.0% of C₃₃H₃₇N₅O₅.CH₄O₃S, calculated on the dried basis.

Description—Dihydroergotamine Mesilate occurs as a white to yellowish white or grayish white to reddish white powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol and in chloroform, slightly soluble in water and in ethanol (95), and practically insoluble in acetic anhydride and in diethyl ether.

It is gradually colored by light.

Melting point: about 214°C (with decomposition).

Identification (1) Dissolve 1 mg of Dihydroergotamine Mesilate in 5 mL of a solution of L-tartaric acid (1 in 100). To 1 mL of this solution add 2 mL of 4-dimethylaminobenzaldehyde-ferric chloride TS, and shake: a blue color develops.

(2) To 0.1 g of Dihydroergotamine Mesilate add 0.4 g of sodium hydroxide, stir well, and incinerate by gradual ignition. After cooling, add 10 mL of water to the residue, heat to boiling, cool, and filter. To the filtrate add 0.5 mL of hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for sulfate. Separately, to 0.1 g of Dihydroergotamine Mesilate add 5 mL of dilute hydrochloric acid, shake for 5 minutes, filter, and to the filtrate add 1 mL of barium chloride TS: the solution is clear.

(3) Determine the absorption spectrum of a solution of Dihydroergotamine Mesilate in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Dihydroergotamine Mesilate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D = -16.7° ± 22.7° [0.5 g, calculated on the dried basis, a mixture of ethanol (99.5), chloroform and ammonia solution (28) (10:10:1), 20 mL, 100 mm].

pH <2.54> Dissolve 0.05 g of Dihydroergotamine Mesilate in 50 mL of water: the pH of this solution is between 4.4 and 5.4.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Dihydroergotamine Mesilate in 0.1 mL of a solution of methanesulfonic acid (7 in 100) and 50 mL of water: the solution is clear, and has no more color than the following control solutions [1] or [2].

Control solution [1]: Pipet 0.6 mL of Iron (III) Chloride CS and 0.15 mL of Cobalt (II) Chloride CS, mix, and add dilute hydrochloric acid (1 in 40) to make exactly 100 mL.

Control solution [2]: Pipet 0.6 mL of Iron (III) Chloride CS, 0.25 mL of Cobalt (II) Chloride CS and 0.1 mL of Copper (II) Sulfate CS, mix, and add dilute hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Dihydroergotamine Mesilate in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add a mixture of chloroform and methanol (9:1) to make exactly 25 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:6:1) to a distance of about 15 cm, and dry the plate with cold wind within 1 minute. Develop the plate again immediately with a freshly prepared mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:6:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and dry the plate with warm wind: the spots other than the principal spot from the sample solution are not more intense than the spot from the stand-
ard solution (1), and the spots, which are more intense than the spot from the standard solution (2), are not more than two.

**Loss on drying** Not more than 4.0% (0.5 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 6 hours).

**Assay** Weigh accurately about 0.2 g of Dihydroergotamine Mesilate, dissolve in 170 mL of a mixture of acetic anhydride and acetic acid (100) (10:1), and titrate with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS = 13.60 mg of \( C_{13}H_{17}N_2O_5 \cdot CH_4O_3S \)

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Dihydroergotoxine Mesilate**

ジヒドロエルゴトキシンメシル酸塩

Dihydroergocornine Mesilate

\( C_{13}H_{17}N_2O_5 \cdot CH_4O_3S: \) 659.79

\((5'S,10R)-12'-Hydroxy-2',5'-bis(1-methylethyl)-9,10-dihydroergotaman-3',6',18-trione monomethanesulfonate\)

Dihydro-\( \alpha \)-ergocryptine Mesilate

\( C_{13}H_{17}N_2O_5 \cdot CH_4O_3S: \) 673.82

\((5'S,10R)-12'-Hydroxy-2'-((1-methylethyl)-5'-((2-methylpropyl)-9,10-dihydroergotaman-3',6',18-trione monomethanesulfonate\)

Dihydro-\( \beta \)-ergocryptine Mesilate

\( C_{13}H_{17}N_2O_5 \cdot CH_4O_3S: \) 707.84

\((5'S,10R)-5'-Benzyl-12'-hydroxy-2'-((1-methylethyl)-9,10-dihydroergotaman-3',6',18-trione monomethanesulfonate\)

Dihydroergocristine Mesilate

\( C_{13}H_{17}N_2O_5 \cdot CH_4O_3S: \) 8067-24-1, Dihydroergotoxine Mesilate

Dihydroergotoxine Mesilate contains not less than 97.0% and not more than 103.0% of dihydroergotoxine mesilate [as a mixture of dihydroergocornine mesilate \( (C_{13}H_{17}N_2O_5 \cdot CH_4O_3S) \), dihydro-\( \alpha \)-ergocryptine mesilate \( (C_{13}H_{17}N_2O_5 \cdot CH_4O_3S) \), dihydro-\( \beta \)-ergocryptine mesilate \( (C_{13}H_{17}N_2O_5 \cdot CH_4O_3S) \) and dihydroergocristine mesilate \( (C_{13}H_{17}N_2O_5 \cdot CH_4O_3S) \)], calculated on the anhydrous basis. The relative contents of dihydroergocornine mesilate \( (C_{13}H_{17}N_2O_5 \cdot CH_4O_3S) \), dihydroeryderocryptine mesilate \( (C_{13}H_{17}N_2O_5 \cdot CH_4O_3S) \) and dihydroergocristine mesilate \( (C_{13}H_{17}N_2O_5 \cdot CH_4O_3S) \) are 30.3–36.3% each, and the content ratio of dihydro-\( \alpha \)-ergocryptine mesilate and dihydro-\( \beta \)-ergocryptine mesilate is 1.5–2.5:1.

**Description** Dihydroergotoxine Mesilate occurs as a white
to pale yellow powder.

It is soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in water, in acetonitrile and in chloroform, and practically insoluble in diethyl ether.

**Identification** Determine the infrared absorption spectrum of Dihydroergotoxine Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2,49> $[\alpha]_{D}^{20} + 11.0 - + 15.0^\circ$ (0.2 g, calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

**Purity (1)** Clarity and color of solution—Dissolve 0.10 g of Dihydroergotoxine Mesilate in 20 mL of water: the solution is clear and the color of the solution is not more intense than that of the following control solution.

Control solution: To a mixture of 1.0 mL of Cobalt (II) Chloride CS, 0.4 mL of Copper (II) Sulfate CS and 2.4 mL of Iron (III) Chloride CS add diluted hydrochloric acid (1 in 40) to make exactly 200 mL.

(2) Heavy metals <1,07>—Proceed with 1.0 g of Dihydroergotoxine Mesilate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately 0.100 g of Dihydroergotoxine Mesilate, dissolve it in a mixture of chloroform and methanol (9:1) to make exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately 10 mg of dihydroergocristine mesilate for thin-layer chromatography, and dissolve in a mixture of chloroform and methanol (9:1) to make exactly 100 mL. Pipet 6 mL, 4 mL and 2 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 10 mL, respectively, and use these solutions as the standard solutions (1), (2) and (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2,01> without putting the filter paper in the developing vessel. Spot 5 µL each of the sample solution and the standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography.

Develop the plate with a mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:3:1) to a distance of about 15 cm, and dry the plate with the aid of a cool air stream. Immediately after that, develop the plate again with a newly prepared mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:3:1) to a distance of about 15 cm, and dry the plate within 1 minute with the aid of a cool air stream. Spray evenly $p$-dimethylaminobenzaldehyde-hydrochloric acid TS on the plate, dry the plate within 2 minutes with the aid of a cool air stream, and heat it at 40°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), not more than 2 spots are more intense than that from the standard solution (2), and not more than 4 spots are more intense than that from the standard solution (3).

**Water** <2,48> Not more than 5.0% (0.2 g, volumetric titration, direct titration).

**Residue on ignition** <2,44> Not more than 0.1% (1 g).

**Assay (1)** Dihydroergotoxine mesilate—Weigh accurately about 30 mg each of Dihydroergotoxine Mesilate and Dihydroergotoxine Mesilate RS, and dissolve them separately in a suitable amount of a mixture of water and acetonitrile (3:1). To these solutions add exactly 10 mL of the internal standard solution and an amount of a mixture of water and acetonitrile (3:1) to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 µL of the sample solution and standard solution as directed under Liquid Chromatography <2,01> according to the following conditions, and calculate the ratios of the peak areas of dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine to the peak area of the internal standard of these solutions.

**Amount (mg) of dihydroergotoxine mesilate**

$$\text{Amount} = M_S \times \left( \frac{Q_{TA} + Q_{TB} + Q_{TC} + Q_{TD}}{Q_{SA} + Q_{SB} + Q_{SC} + Q_{SD}} \right)$$

$M_S$: Amount (mg) of Dihydroergotoxine Mesilate RS, calculated on the anhydrous basis

$Q_{TA}$: Ratio of the peak area of dihydroergocornine to that of the internal standard of the sample solution $\times$ 659.80

$Q_{TB}$: Ratio of the peak area of dihydro-α-ergocryptine to that of the internal standard of the sample solution $\times$ 673.83

$Q_{TC}$: Ratio of the peak area of dihydroergocristine to that of the internal standard of the sample solution $\times$ 707.85

$Q_{TD}$: Ratio of the peak area of dihydro-β-ergocryptine to that of the internal standard of the sample solution $\times$ 727

$Q_{SA}$: Ratio of the peak area of dihydroergocornine to that of the internal standard of the standard solution $\times$ 659.80

$Q_{SB}$: Ratio of the peak area of dihydro-α-ergocryptine to that of the internal standard of the standard solution $\times$ 673.83

$Q_{SC}$: Ratio of the peak area of dihydroergocristine to that of the internal standard of the standard solution $\times$ 707.85

$Q_{SD}$: Ratio of the peak area of dihydro-β-ergocryptine to that of the internal standard of the standard solution $\times$ 727

**Internal standard solution**—Dissolve 0.04 g of chloramphenicol in a mixture of water and acetonitrile (3:1) to make 250 mL.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and triethylamine (30:10:1).

Flow rate: Adjust the flow rate so that the retention time of chloramphenicol is about 5 minutes.

**System suitability**—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the internal standard, dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocrypt-
tine are eluted in this order with the resolution between the peaks of dihydro-α-ergocryptine and dihydroergocristine being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine to that of the internal standard is not more than 0.5%.

(2) Relative contents of dihydroergocornine mesilate, dihydroergocornine mesilate and dihydroergocristine mesilate—Calculate the relative amounts of dihydroergocornine mesilate, dihydroergocryptine mesilate (dihydro-α-ergocryptine mesilate and dihydro-β-ergocryptine mesilate) and dihydroergocristine mesilate from the chromatogram obtained in Assay (1) for the sample solution using the following equations:

\[
\text{Relative amount (\%) of dihydroergocornine mesilate} = \frac{Q_{TA}}{Q_{TA} + Q_{TB} + Q_{TC} + Q_{TD}} \times 100
\]

\[
\text{Relative amount (\%) of dihydroergocryptine mesilate} = \frac{Q_{TB} + Q_{TD}}{Q_{TA} + Q_{TB} + Q_{TC} + Q_{TD}} \times 100
\]

\[
\text{Relative amount (\%) of dihydroergocristine mesilate} = \frac{Q_{TC}}{Q_{TA} + Q_{TB} + Q_{TC} + Q_{TD}} \times 100
\]

(3) Ratio of the content of dihydro-α-ergocryptine mesilate to dihydro-β-ergocryptine mesilate—Calculate the ratio of the amount of dihydro-α-ergocryptine mesilate to dihydro-β-ergocryptine mesilate from the chromatogram obtained in the Assay (1) for the sample solution using the following equations:

\[
\text{Ratio of the content of dihydro-α-ergocryptine mesilate to dihydro-β-ergocryptine mesilate} = \frac{Q_{TB}}{Q_{TD}}
\]

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Dilazep Hydrochloride Hydrate

Dilazep Hydrochloride Hydrate,

\[
\text{C}_{33}\text{H}_{44}\text{N}_{2}\text{O}_{10} \cdot \text{2HCl} \cdot \text{H}_{2}\text{O}: 695.63
\]

3,3’-(1,4-Diazepane-1,4-diyl)dipropyl bis(3,4,5-trimethoxybenzoate) dihydrochloride monohydrate

\[20153-98-4, \text{anhydride}\]

Dilazep Hydrochloride Hydrate contains not less than 98.0% of dilazep hydrochloride (\(\text{C}_{33}\text{H}_{44}\text{N}_{2}\text{O}_{10} \cdot \text{2HCl}: 677.62\)), calculated on the dried basis.

Description Dilazep Hydrochloride Hydrate occurs as a white, crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in chloroform, soluble in water, slightly soluble in ethanol (95) and in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: 200 – 204°C Immense the sample in a bath of 110°C, and raise the temperature at the rate of about 3°C per minute from 140°C to 150°C, about 10°C per minute from 160°C to 195°C and about 1°C per minute from 195°C.

Identification (1) To 1 mL of a solution of Dilazep Hydrochloride Hydrate (1 in 100) add 0.1 mL of a solution of hydroxylammonium chloride (1 in 10) and 0.1 mL of 8 mol/L potassium hydroxide TS, and warm in a water bath of 70°C for 10 minutes. After cooling, add 0.5 mL of dilute hydrochloric acid and 0.1 mL of iron (III) chloride TS: a purple color develops.

(2) To 5 mL of a solution of Dilazep Hydrochloride Hydrate (3 in 500) add 0.3 mL of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Dilazep Hydrochloride Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Dilazep Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH 2.54 – 3.4 – Dissolve 1.0 g of Dilazep Hydrochloride Hydrate in 100 mL of water: the pH of this solution is between 3.0 and 4.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dilazep Hydrochloride Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Sulfate 1.14 – Perform the test with 0.5 g of Dilazep Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals 1.07 – Proceed with 2.0 g of Dilazep Hydrochloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic 1.11 – Prepare the test solution with 1.0 g of Dilazep Hydrochloride Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.40 g of Dilazep Hydrochloride Hydrate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, ethyl acetate, dichloromethane and hydrochloric acid (500:200:100:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragenoff’s TS for spraying on the plate: the spots other than the principal spot from the sample
solution are not more intense than the spot from the standard solution.

Loss on drying $<2.4\%$ 2.0–3.0% (1 g, 105°C, 3 hours).

Residue on ignition $<2.4\%$ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Dilazep Hydrochloride Hydrate, dissolve in 40 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 33.88 mg of C$_2$H$_{12}$N$_2$O$_8$.2HCl

**Containers and storage** Containers—Tight containers.

## Diltiazem Hydrochloride

ジルチアゼム塩酸塩

C$_{22}$H$_{26}$N$_2$O$_4$S.HCl: 450.98

(25,35)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate monohydrochloride [33286-22-5]

Diltiazem Hydrochloride, when dried, contains not less than 98.5% of C$_{22}$H$_{26}$N$_2$O$_4$.2HCl.

**Description** Diltiazem Hydrochloride occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in formic acid, freely soluble in water, in methanol and in chloroform, sparingly soluble in acetonitrile, slightly soluble in acetic anhydride and in ethanol (99.5), and practically insoluble in diethyl ether.

**Identification** (1) Dissolve 0.05 g of Diltiazem Hydrochloride in 1 mL of 1 mol/L hydrochloric acid TS, add 2 mL of ammonium thiocyanate-cobalt (II) nitrate TS and 5 mL of chloroform, shake well, and allow to stand: a blue color develops in the chloroform layer.

(2) Proceed as directed under Oxygen Flask Combustion Method $<1.06>$ with 0.03 g of Diltiazem Hydrochloride, using 20 mL of water as the absorbing liquid, and prepare the test solution: the test solution responds to the Qualitative Tests $<1.09>$ (1) for sulfate.

(3) Dissolve 0.01 g of Diltiazem Hydrochloride in 0.01 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of the solution add 0.01 mol/L hydrochloric acid TS to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry $<2.24>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Diltiazem Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25>$: it exhibits absorption at the wave numbers of about 1741 cm$^{-1}$, 1678 cm$^{-1}$, 1252 cm$^{-1}$ and 1025 cm$^{-1}$.

(5) A solution of Diltiazem Hydrochloride (1 in 50) responds to the Qualitative Tests $<1.09>$ (2) for chloride.

**Optical rotation** $<2.49>$ $[\alpha]_D^15:+115-+120$° (after drying, 0.20 g, water, 20 mL, 100 mm).

**Melting point** $<2.60>$ 210–215°C (with decomposition).

**pH** $<2.54>$ Dissolve 1.0 g of Diltiazem Hydrochloride in 100 mL of water: the pH of this solution is between 4.3 and 5.3.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Diltiazem Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Sulfate $<1.14>$—Perform the test with 1.0 g of Diltiazem Hydrochloride. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals $<1.07>$—Proceed with 2.0 g of Diltiazem Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic $<1.11>$—Place 1.0 g of Diltiazem Hydrochloride in a decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the neck of the flask, and heat cautiously until white fumes are evolved. After cooling, add 2 mL of nitric acid, heat, and repeat this procedure twice, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution becomes colorless to pale yellow. After cooling, add 2 mL of saturated solution of ammonium oxalate monohydrate, and heat again until white fumes are evolved. After cooling, add water to make 5 mL, use this solution as the test solution, and perform the test: the test solution has no more color than the following control solution (not more than 2 ppm).

Control solution: Proceed in the same manner as the test solution without Diltiazem Hydrochloride, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and proceed in the same manner as the test solution.

(5) Related substances—Dissolve 50 mg of Diltiazem Hydrochloride in 50 mL of diluted ethanol (4 in 5), and use this solution as the sample solution. Measure exactly 1 mL of the sample solution, add diluted ethanol (4 in 5) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 mL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.07>$ according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total peak area of peaks other than the peak of diltiazem obtained from the sample solution is not larger than 3/5 times the peak area of diltiazem obtained from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diame-
ter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 8 g of sodium acetate trihydrate and 1.5 g of d-camphorsulfonic acid in 500 mL of water, and filter using a membrane filter (0.4 μm in pore size). Add 250 mL each of acetonitrile and methanol to the filtrate, and adjust the solution to a pH of 6.6 by adding sodium acetate trihydrate.

Flow rate: Adjust the flow rate so that the retention time of diltiazem is about 9 minutes.

Time span of measurement: About twice as long as the retention time of diltiazem beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add diluted ethanol (4 in 5) to make exactly 10 mL. Confirm that the peak area of diltiazem obtained from this solution is equivalent to 15 to 25% of that from 20 μL of the standard solution.

System performance: Dissolve 0.03 g of Diltiazem Hydrochloride, 0.02 g of d-3-hydroxy-cis-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepin-4-(5H)-one hydrochloride and 0.02 g of phenylbenzoate in 160 mL of ethanol (99.5), and add water to make 200 mL. When this solution is run with 20 μL of this solution under the above operating conditions, 3,5-dihydroxy-cis-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepin-4-(5H)-one, diltiazem and phenyl benzoate are eluted in this order with the resolutions between the peaks of each and diltiazem and between the peaks of diltiazem and vare between 2.50 and 2.49, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diltiazem is not more than 2.0%.

Loss on drying <2.40> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Diltiazem Hydrochloride, previously dried, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.56> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 45.10 mg of C22H26N2O4S.HCl

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dimemorfan Phosphate

ジメモルファンリン酸塩

C18H22N.H3PO4: 353.39
(95,135,145)-3,17-Dimethylmorphinan monophosphate [36304-84-4]

Dimemorfan Phosphate, when dried, contains not less than 98.5% of C18H22N.H3PO4.

Description Dimemorfan Phosphate occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 265°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Dimemorfan Phosphate (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dimemorfan Phosphate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 2 mL of a solution of Dimemorfan Phosphate (1 in 100) add 2 to 3 drops of silver nitrate TS: a yellow precipitate is formed, and it dissolves on the addition of dilute nitric acid.

Optical rotation <2.49> [α]D²⁰: +25 to +27° (after drying, 1 g, methanol, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Dimemorfan Phosphate in 100 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Dimemorfan Phosphate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 1.0 g of Dimemorfan Phosphate according to Method 3, and perform the test. Use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).
Dimenhydrinate

Dimenhydrinate, when dried, contains not less than 53.0% and not more than 55.5% of diphenhydramine (C₁₇H₂₁NO): 255.36, and not less than 44.0% and not more than 47.0% of 8-chlorotheophylline (C₁₇H₂₁NO·C₇H₇ClN₄O₂: 214.61).

Description

Dimenhydrinate occurs as a white, crystalline powder. It is odorless, and has a bitter taste. It is very soluble in chloroform, freely soluble in ethanol (95), and slightly soluble in water and in diethyl ether.

Identification (1)

Dissolve 0.5 g of Dimenhydrinate in 30 mL of dilute ethanol, add 30 mL of water, and use this solution as the sample solution. Transfer 30 mL of the sample solution to a separator, and add 2 mL of ammonia solution (28). Extract with two 10-mL portions of diethyl ether, combine the diethyl ether extracts, and wash the combined diethyl ether extracts with 5 mL of water, and then extract the combined extracts with 15 mL of dilute hydrochloric acid (1 in 100). With this acid extract perform the following tests.

(i) To 5 mL of this acid extract add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(ii) To 10 mL of this acid extract add 10 mL of 2,4,6-trinitrophenol TS dropwise, and allow to stand for 30 minutes. Collect the precipitate by filtering, recrystallize from dilute ethanol, and dry at 105°C for 30 minutes: the crystals melt <2.60°C between 128°C and 133°C.

(2) To 30 mL of the sample solution obtained in the Identification (1) add 2 mL of dilute sulfuric acid, and cool for 30 minutes. Scratch the inside wall of the container frequently to facilitate crystallization. Filter, and wash the white crystals with a small amount of ice-cooled water. Dry the crystals for 1 hour at 105°C: the crystals melt <2.60°C between 300°C and 305°C with decomposition.

(3) To 0.01 g of the crystals obtained in the Identification (2) add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue shows a yellow-red color. When the dish containing the residue is held over a vessel containing 2 to 3 drops of ammonia TS, the color changes to red-purple, which is discharged on the addition of 2 to 3 drops of sodium hydroxide TS.

(4) Mix well 0.05 g of the crystals obtained in the Identification (2) with 0.5 g of sodium peroxide in a nickel crucible, and heat until the mass melts. Cool, dissolve the melted mass in 20 mL of water, and acidify with dilute nitric acid: the solution responds to the Qualitative Tests <1.09r for chloride.

Melting point 102 – 107°C

Purity (1) Chloride <1.03—Transfer 50 mL of the filtrate obtained in the Assay (2) to a Nessler tube, add 1 mL of nitric acid, and allow to stand for 5 minutes: the turbidity of the solution is not greater than that of the following control solution.

Control solution: Dilute 0.25 mL of 0.01 mol/L hydrochloric acid VS with 6 mL of dilute nitric acid and with water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes (not more than 0.044%).

(2) Bromide and iodide—Place 0.10 g of Dimenhydrinate in a glass-stoppered test tube, and add 0.05 g of sodium nitrite, 10 mL of chloroform and 10 mL of dilute hydrochloric acid. Stopper, shake well, and allow to stand: the chlorofom layer remains colorless.

Loss on drying <2.4r—Not more than 0.5% (3 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition <2.4r—Not more than 0.3% (1 g).

Assay (1) Diphenhydramine—Weigh accurately about 0.5 g of Dimenhydrinate, previously dried, transfer to a 250-mL separator, and add 50 mL of water, 3 mL of ammonia TS and 10 g of sodium chloride. Extract with six 15-mL portions of diethyl ether with shaking, combine the diethyl ether extracts, and wash the combined diethyl ether extracts with three 50-mL portions of water. To the diethyl ether extracts add exactly 25 mL of 0.05 mol/L sulfuric acid VS, and add 25 mL of water. Shake thoroughly, and evaporate the diethyl ether gently. Cool, and titrate the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L sulfuric acid VS = 25.54 mg C₁₇H₂₁NO

(2) 8-Chlorotheophylline—Weigh accurately about 0.8 g of Dimenhydrinate, previously dried, transfer to a 200-mL volumetric flask, add 50 mL of water, 3 mL of ammonia TS and 6 mL of a solution of ammonium nitrate (1 in 10), and heat on a water bath for 5 minutes. Add exactly 25 mL of 0.1 mol/L silver nitrate VS, heat on a water bath for 15 minutes with occasional shaking, cool, and add water to make exactly 200 mL. Allow to stand overnight to settle the

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precipitate, and filter through a dry filter paper, discarding the first 20 mL of the filtrate. Measure exactly 100 mL of the subsequent filtrate, acidify with nitric acid, add 3 mL of nitric acid, and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
\[= 21.46 \text{ mg of C}_7\text{H}_7\text{ClN}_4\text{O}_2\]

**Containers and storage** Containers—Well-closed containers.

## Dimenhydrinate Tablets

Dimenhydrinate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of dimenhydrinate \((\text{C}_{17}\text{H}_{21}\text{NO} \cdot \text{C}_7\text{H}_7\text{ClN}_4\text{O}_2)\) according to the labeled amount, with 25 mL of warm ethanol (95), and filter. Dilute the filtrate with 40 mL of water, and filter again. Use the filtrate as the sample solution. Transfer 30 mL of the sample solution to a separator, and proceed as directed in the Identification (1) under Dimenhydrinate.

(2) With 30 mL of the sample solution obtained in (1), proceed as directed in the Identification (2), (3) and (4) under Dimenhydrinate.

### Method of Preparation

Prepare as directed under Tablets, with Dimenhydrinate.

### Dissolution [6.10]

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Dimenhydrinate Tablets is not less than 85%.

Start the test with 1 tablet of Dimenhydrinate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains about 28 μg of dimenhydrinate \((\text{C}_{17}\text{H}_2\text{NO} \cdot \text{C}_7\text{H}_7\text{ClN}_4\text{O}_2)\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of dimenhydrinate for assay, previously dried in vacuum over phosphorus (V) oxide for 24 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_1\) and \(A_3\), of the sample solution and standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry [2.24]

Dissolution rate (% with respect to the labeled amount of dimenhydrinate \((\text{C}_{17}\text{H}_2\text{NO} \cdot \text{C}_7\text{H}_7\text{ClN}_4\text{O}_2)\)
\[= M_S \times \frac{A_1}{A_S} \times \frac{V}{V'} \times \frac{1}{C} \times 90\]

\(M_S\): Amount (mg) of dimenhydrinate for assay
\(C\): Labeled amount (mg) of dimenhydrinate \((\text{C}_{17}\text{H}_2\text{NO} \cdot \text{C}_7\text{H}_7\text{ClN}_4\text{O}_2)\) in 1 tablet

### Assay

Weigh accurately, and powder not less than 20 Dimenhydrinate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of dimenhydrinate \((\text{C}_{17}\text{H}_2\text{NO} \cdot \text{C}_7\text{H}_7\text{ClN}_4\text{O}_2)\), transfer to a flask, add 40 mL of ethanol (95), and heat with swirling on a water bath until the solution just boils. Continue to heat for 30 seconds, and filter through a glass filter (G4). Wash the filter with warm ethanol (95), transfer the filtrate and washings to a flask, and evaporate the ethanol on a water bath to make 5 mL. Add 50 mL of water, 3 mL of ammonia TS and 6 mL of a solution of ammonium nitrate (1 in 10), heat the mixture on a water bath for 5 minutes, add exactly 25 mL of 0.1 mol/L silver nitrate VS, and heat on a water bath for 15 minutes with occasional shaking. Transfer the mixture to a 200-mL volumetric flask, using water to rinse the flask, cool, add water to make exactly 200 mL, and proceed as directed in the Assay (2) under Dimenhydrinate.

Each mL of 0.1 mol/L silver nitrate VS
\[= 47.00 \text{ mg of C}_7\text{H}_7\text{ClN}_4\text{O}_2\]

### Dimercaprol

Dimercaprol is a colorless or pale yellow liquid. It has a mercaptan-like, disagreeable odor. It is miscible with methanol and with ethanol (99.5) It is soluble in peanut oil, and sparingly soluble in water. It shows no optical rotation.

**Identification (1)** Add 1 drop of Dimercaprol to a mixture of 1 drop of a solution of cobalt (II) chloride hexahydrate (1 in 200) and 5 mL of water: a yellow-brown color develops.

(2) Determine the infrared absorption spectrum of Dimercaprol as directed in the liquid film method under Infrared Spectrophotometry [2.25], and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Refractive index** [2.45]

\(\eta_{D}^{20} : 1.570 - 1.575\)

**Specific gravity** [2.56]

\(d_{20}^{20} : 1.238 - 1.248\)

### Purity (1)

Clarity and color of solution—Dissolve 1.0 mL of Dimercaprol in 20 mL of peanut oil: the solution is clear and colorless to pale yellow.

(2) Bromide—To 2.0 g of Dimercaprol add 25 mL of
dilute potassium hydroxide-ethanol TS, and heat in a water bath under a reflux condenser for 2 hours. Evaporate the ethanol in a current of warm air, add 20 mL of water, and cool. Add a mixture of 10 mL of strong hydrogen peroxide and 40 mL of water, boil gently under a reflux condenser for 10 minutes, and filter rapidly after cooling. Wash the residue with two 10-mL portions of water, combine the washings with the filtrate, add 10 mL of dilute nitric acid and exactly 5 mL of 0.1 mol/L silver nitrate VS, and titrate ≤2.50 with the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination: not more than 1.0 mL of 0.1 mol/L silver nitrate VS is consumed.

Perform the test immediately with 0.05 mol/L iodine VS until a pale yellow color is produced. Perform a blank determination: not more than 1.0 mL of 0.1 mol/L silver nitrate VS is consumed.

(3) Heavy metals

Proceed with 1.0 g of Dimercaprol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Assay**

Weigh accurately about 0.15 g of Dimercaprol into a glass-stoppered flask, dissolve in 10 mL of methanol, and titrate ≤2.50 immediately with 0.05 mol/L iodine VS until a pale yellow color is produced. Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 6.212 mg of C$_2$H$_3$O$_2$S

**Containers and storage**

Containers—Tight containers.

Storage—Not exceeding 5°C.

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**Dimercaprol Injection**

ジメルカプロール注射液

Dimercaprol Injection is an oily solution for injection. It contains not less than 95.0% and not more than 105.0% of the labeled amount of dimercaprol (C$_2$H$_3$O$_2$S: 124.23).

**Method of preparation**

Prepare as directed under Injections, with Dimercaprol. Benzyl Benzoate or Benzyl Alcohol may be added to increase the solubility.

**Description**

Dimercaprol Injection is a clear, colorless or light yellow liquid. It has an unpleasant odor.

**Identification**

Measure a volume of Dimercaprol Injection, equivalent to 30 mg of Dimercaprol according to the labeled amount, and proceed as directed in the Identification (1) under Dimercaprol.

**Extractable volume**

≤6.0% It meets the requirement.

**Foreign insoluble matter**

≤6.0% Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter**

≤6.0% Perform the test according to Method 2: it meets the requirement.

**Sterility**

≤4.0% Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay**

Pipet a volume of Dimercaprol Injection, equivalent to about 0.1 g of dimercaprol (C$_2$H$_3$O$_2$S), into a flask, and rinse the pipet several times with a mixture of methanol and diethyl ether (3:1), adding the rinsings to the flask. Add the mixture of methanol and diethyl ether (3:1) to make 50 mL, and titrate ≤2.50 with 0.05 mol/L iodine VS until a yellow color persists. Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 6.212 mg of C$_2$H$_3$O$_2$S

**Dimorpholamine**

ジモルホラミン

C$_{20}$H$_{38}$N$_4$O$_4$: 398.54
N,N’-Ethylenebis(N-butylmorpholine-4-carboxamide) [119-48-2]

Dimorpholamine, when dried, contains not less than 98.0% and not more than 101.0% of C$_{20}$H$_{38}$N$_4$O$_4$.

**Description**

Dimorpholamine is a white to light yellow, crystalline powder, masses or syrupy liquid. It is very soluble in ethanol (99.5) and in acetic anhydride, and soluble in water. The pH of a solution prepared by dissolving 1.0 g of Dimorpholamine in 10 mL of water is between 6.0 and 7.0. It is hygroscopic.

**Identification**

(1) Determine the absorption spectrum of a solution of Dimorpholamine (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ≤2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dimorpholamine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry ≤2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of Dimorpholamine in 50 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride ≤1.0%—To 20 mL of the solution obtained in (1) add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Sulfate ≤1.4%—To 10 mL of the solution obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.096%).

(4) Heavy metals ≤1.0%—Proceed with 2.0 g of Dimorpholamine according to Method 2, and perform the test.
Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.20 g of Dimorpholamine in 10 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>.

Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5) and water (4:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 10 minutes: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying <2.41>** Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 8 hours).

**Residue on ignition <2.44>** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.6 g of Dimorpholamine, previously dried, dissolve in 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>.

Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5) and water (4:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 10 minutes: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

**Containers and storage** Containers—Hermetic containers.

**Storage**—Light-resistant.

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### Dimorpholamine Injection

ジモルホラミン注射液

Dimorpholamine Injection is a clear, colorless liquid.

- **pH:** 3.0 – 5.5

**Identification**

1. **(1)** To a volume of Dimorpholamine Injection, equivalent to 0.1 g of Dimorpholamine according to the labeled amount, add 3 drops of Dragendorff’s TS: an orange color develops.

2. **(2)** To a volume of Dimorpholamine Injection, equivalent to 50 mg of Dimorpholamine according to the labeled amount, add 1 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 2 mL of hydrochloric acid, boil for 10 minutes under a reflux condenser, and evaporate to dryness on a water bath. Dissolve the residue with 1 mL of water, neutralize with sodium hydroxide TS, and add 0.2 mL of a solution of acetaldehyde (1 in 20), 0.1 mL of sodium pentacyanonitrosyl ferrate (III) TS and 0.5 mL of sodium carbonate TS: a blue color develops.

**Bacterial endotoxins <4.01>** Less than 5.0 EU/mg. Perform the test with the sample diluted to 0.15 w/v% with water for bacterial endotoxins test.

**Extractable volume <6.05>** It meets the requirement.

**Foreign insoluble matter <6.06>** Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter <6.07>** Perform the test according to Method 1: it meets the requirement.

**Sterility <4.06>** Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Measure exactly a volume of Dimorpholamine Injection, equivalent to about 30 mg of dimorpholamine \( \text{C}_{20}\text{H}_{38}\text{N}_{4}\text{O}_4 \), and add water to make exactly 200 mL. Pipet 1 mL of this solution, shake with exactly 4 mL of the internal standard solution for 5 minutes, and use this solution as the standard solution. Separately, weigh accurately about 0.15 g of dimorpholamine for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 8 hours, and dissolve in water to make exactly 1000 mL. Pipet 1 mL of this solution, shake with exactly 4 mL of the internal standard solution for 5 minutes, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_t \) and \( Q_s \), of the peak area of dimorpholamine to that of the internal standard.

\[
\text{Amount (mg) of dimorpholamine (C}_{20}\text{H}_{38}\text{N}_{4}\text{O}_4) = M_S \times \frac{Q_t}{Q_s} \times \frac{1}{5}
\]

- **Amount:** (mg) of dimorpholamine for assay

**Internal standard solution**—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 25,000).

**Operating conditions**—

- **Detector:** An ultraviolet absorption photometer (wavelength: 216 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature:** A constant temperature of about 40°C.
- **Mobile phase:** A mixture of water and acetonitrile (1:1).
- **Flow rate:** Adjust the flow rate so that the retention time of dimorpholamine is about 4 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, dimorpholamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dimorpholamine to that of the internal standard is not more than 1.0%

**Containers and storage** Containers—Hermetic containers.
Dinoprost

Prostaglandin F2α

ジノプロスト

C_{20}H_{34}O_5: 354.48


Dinoprost contains not less than 98.5% of C_{20}H_{34}O_5, calculated on the anhydrous basis.

Description

Dinoprost occurs as white, waxy masses or powder, or a clear, colorless to light yellow and viscous liquid. It is odorless.

It is very soluble in N,N-dimethylformamide, freely soluble in methanol, in ethanol (99.5) and in diethyl ether, and very slightly soluble in water.

Identification

(1) To 5 mg of Dinoprost add 2 mL of sulfuric acid, and dissolve by shaking for 5 minutes: a dark red color develops. To this solution add 30 mL of sulfuric acid: an orange color develops with a green fluorescence.

(2) Dissolve 1 mg of Dinoprost in 50 mL of diluted sulfuric acid (7 in 10), and warm in a water bath heated at 50°C for 40 minutes. After cooling, determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Warm Dinoprost at 40°C to effect a liquid, and determine the infrared absorption spectrum of the liquid as directed in the liquid film method under Infrared Spectroscopy <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation

<2.49> [α]_D^20: +24° to +31° (0.2 g, ethanol (99.5), 10 mL, 100 mm).

Purity

(1) Clarity and color of solution—Dissolve 0.20 g of Dinoprost in 5 mL of ethanol (99.5): the solution is clear and colorless to pale yellow.

(2) Related substances—Dissolve 10 mg of Dinoprost in 2 mL of methanol, add water to make 10 mL, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add diluted methanol (1 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than the peak of dinoprost from the sample solution is not larger than the peak area of dinoprost from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogenphosphate TS and acetonitrile (5:2).

Flow rate: Adjust the flow rate so that the retention time of dinoprost is about 20 minutes.

Selection of column: Dissolve 0.01 g each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 2 mL of methanol, and add water to make 10 mL. To 1 mL of this solution add diluted methanol (1 in 5) to make 30 mL, proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 2.5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of dinoprost from the standard solution composes 5% to 15% of the full scale.

Time span of measurement: About 1.5 times as long as the retention time of dinoprost beginning after the solvent peak.

Water<2.48> Not more than 0.5% (0.3 g, volumetric titration, direct titration).

Assay

Weigh accurately about 50 mg of Dinoprost, dissolve in 30 mL of N,N-dimethylformamide, and titrate<2.50> with 0.02 mol/L tetramethylammonium hydroxide VS under a stream of nitrogen (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L tetramethylammonium hydroxide VS = 7.090 mg of C_{20}H_{34}O_5

Containers and storage

Containers—Tight containers.

Storage—Light-resistant, and in a place not exceeding 5°C.

Diphenhydramine

ジフェンヒドラミン

C_{17}H_{21}NO: 255.35

2-(Diphenylmethoxy)-N,N-dimethylethylamine [58-73-1]

Diphenhydramine contains not less than 96.0% of C_{17}H_{21}NO.

Description

Diphenhydramine is a clear, light yellow to
yellow liquid. It has a characteristic odor, and has a burning taste at first, followed by a slight sensation of numbness on the tongue.

It is miscible with acetic anhydride, with acetic acid (100), with ethanol (95) and with diethyl ether.

It is very slightly soluble in water.

Boiling point: about 162°C (in vacuum, 0.67 kPa).

Refractive index \( n_\text{D}^\text{20} \approx 1.55 \)

It is gradually affected by light.

**Identification (1)**

To 0.05 g of Diphenhydramine add 2 mL of sulfuric acid: an orange-red precipitate is produced immediately, and its color changes to red-brown on standing. Add carefully 2 mL of water to this solution: the intensity of the color changes, but the color tone does not change.

(2) Dissolve 0.1 g of Diphenhydramine in 10 mL of dilute ethanol, add an excess of a saturated solution of 2,4,6-trinitrophenol in dilute ethanol with stirring, and cool in ice. Collect the produced crystals, recrystallize from dilute ethanol, and dry at 105°C for 30 minutes: the crystals melt \(<2.60^\circ\text{C}\) between 128°C and 135°C.

**Specific gravity** \( <2.50 \quad d_\text{20}^\approx 1.013 – 1.020 \)

**Purity (1)**

- \( \beta \)-Dimethylaminoethanol—Dissolve 1.0 g of Diphenhydramine in 20 mL of diethyl ether, and extract with two 10-mL portions of water with thorough shaking. Combine the water extracts, and add 2 drops of phenolphthalein TS and 1.0 mL of 0.05 mol/L sulfuric acid VS: no red color develops.

- Benzohydrol—Transfer 1.0 g of Diphenhydramine to a separator, dissolve in 20 mL of diethyl ether, and extract with two 25-mL portions of diluted hydrochloric acid (1 in 15) with thorough shaking. Separate the diethyl ether layer, evaporate slowly on a water bath, and dry in a desiccator (in vacuum, silica gel) for 2 hours: the mass of the residue is not more than 20 mg.

- Heavy metals \(<1.07^\circ\text{C}\)—Proceed with 1.0 g of Diphenhydramine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Residue on ignition** \( <2.44 \quad \text{Not more than 0.1%} \ (1 \text{g}) \)

**Assay**

Weigh accurately about 0.5 g of Diphenhydramine, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate \( <2.50 \) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 25.54 mg of C₁₇H₂₁NO

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant, and almost well-filled.

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**Diphenhydramine and Bromovalerylurea Powder**

**Method of preparation**

| Diphenhydramine Tannate | 90 g |
| Bromovalerylurea | 500 g |
| Starch, Lactose Hydrate, or their mixture | a sufficient quantity |

To make 100 g

Prepare as directed under Powders, with the above ingredients.

**Description**

Diphenhydramine and Bromovalerylurea Powder occurs as a slightly grayish white powder.

**Identification (1)**

To 0.1 g of Diphenhydramine and Bromovalerylurea Powder add 5 mL of dilute hydrochloric acid, 1 mL of ethanol (95) and 10 mL of water, shake, and filter. To the filtrate add 10 mL of sodium hydroxide TS, and extract with 10 mL of chloroform. Separate the chloroform layer, add 1 mL of bromphenol blue TS, and shake: a yellow color develops in the chloroform layer (diphenhydramine tannate).

(2) Shake 0.02 g of Diphenhydramine and Bromovalerylurea Powder with 10 mL of diethyl ether, filter, and evaporate the filtrate on a water bath. Dissolve the residue in 2 mL of sodium hydroxide TS, and add 5 mL of dimethylglyoxime-thiosemicarbazide TS, and heat on a water bath for 30 minutes: a red color develops (bromovalerylurea).

(3) Shake 0.3 g of Diphenhydramine and Bromovalerylurea Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Dissolve 0.15 of bromovalerylurea and 0.03 g of diphenhydramine tannate in 5 mL each of methanol, and use the solutions as standard solution (1) and standard solution (2). Perform the test as directed under Thin-layer Chromatography \(<2.03\) with these solutions. Spot 5 \( \mu \text{L} \) each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate in a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm. Air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): 3 spots from the sample solution and the corresponding spot from standard solutions (1) and (2) show the same \( R_f \) value. Spray Dragendorff’s TS for spraying evenly on the plate: the spot from the standard solution (2) and the corresponding spot from the sample solution reveal an orange color.

**Containers and storage**

Containers—Well-closed containers.
Diphenhydramine Hydrochloride

ジフェンヒドラミン塩酸塩

C₁₇H₂₁NO.HCl: 291.82
2-(Diphenylmethoxy)-N,N-dimethylethylamine monohydrochloride

Diphenhydramine Hydrochloride, when dried, contains not less than 98.0% of C₁₇H₂₁NO.HCl.

**Description** Diphenhydramine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste, followed by a sensation of numbness on the tongue.

It is very soluble in methanol and in acetic acid (100), freely soluble in water and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is gradually affected by light.

**Identification (1)** Determine the absorption spectrum of a solution of Diphenhydramine Hydrochloride in methanol (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Diphenhydramine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Diphenhydramine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

**pH** <2.40> Dissolve 1.0 g of Diphenhydramine Hydrochloride in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

**Melting point** <2.60> 166–170°C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Diphenhydramine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Diphenhydramine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Diphenhydramine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate, methanol and ammonia solution (28) (10:4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iodine TS on the plate: the spots other than the principal spot and the spot on the original point from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.47> Not more than 0.5% (2 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Diphenhydramine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <2.30> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 29.18 mg of C₁₇H₂₁NO.HCl

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

Diphenhydramine, Phenol and Zinc Oxide Liniment

ジフェンヒドラミン・フェノール・亜鉛華リニメント

**Method of preparation**

| Diphenhydramine | 20 g |
| Phenol and Zinc Oxide Liniment | 980 g |

To make 1000 g

Dissolve and mix the above ingredients.

**Description** Diphenhydramine, Phenol and Zinc Oxide Liniment is a white to whitish, pasty mass. It has a slight odor of phenol.

**Identification (1)** To 3 g of Diphenhydramine, Phenol and Zinc Oxide Liniment add 20 mL of hexane, shake well, and separate the hexane layer. Shake thoroughly the hexane solution with 10 mL of 0.2 mol/L hydrochloric acid. Separate the aqueous layer, and adjust with sodium hydroxide TS to a pH of 4.6. Add 1 mL of bromphenol blue-potassium biphthalate TS and 10 mL of chloroform, and shake: a yellow color develops in the chloroform layer (diphenhydramine).

(2) Place 1 g of Diphenhydramine, Phenol and Zinc Oxide Liniment in a porcelain crucible, gradually raise the temperature by heating until the mass is charred, and ignite strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. Add 2 to 3 drops of potassium hexacyanoferrate (II) TS to the filtrate: a white precipitate is produced (zinc oxide).

(3) Shake 0.5 g of Diphenhydramine, Phenol and Zinc Oxide Liniment with 1 mL of water and 5 mL of chloroform, filter, and use the filtrate as the sample solution. Dissolve 0.01 g each of diphenhydramine and phenol in 5 mL each of chloroform, and use these solutions as standard solution (1) and standard solution (2). Perform the test as di-
Diphenhydramine Tannate

Diphenhydramine Tannate is a compound of diphenhydramine and tannic acid, and contains not less than 25.0% and not more than 35.0% of diphenhydramine (C_{17}H_{21}NO: 255.35).

**Description** Diphenhydramine Tannate occurs as a grayish white to light brown powder. It is odorless or has a slight, characteristic odor. It is tasteless. It is slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

**Identification (1)** To 1 g of Diphenhydramine Tannate add 15 mL of water and 0.3 mL of dilute hydrochloric acid, shake thoroughly for 1 minute, filter, and use this filtrate as the sample solution. Transfer 10 mL of the sample solution to a separator, extract with two 20-mL portions of chloroform, combine the chloroform extracts, and evaporate on a water bath to dryness. To 5 mL of a solution of the residue (1 in 100) add 5 drops of Reinecke salt TS: a light red precipitate is produced.

**Heavy metals** Not more than 7.0 mg/kg.

**Assay** Transfer about 1.7 g of Diphenhydramine Tannate, accurately weighed, to a separator, dissolve in 20 mL of water and 3.0 mL of dilute hydrochloric acid with thorough shaking, add 20 mL of a solution of sodium hydroxide (1 in 10) and exactly 25 mL of isooctane, shake vigorously for 5 minutes, dissolve 2 g of sodium chloride with shaking, and allow to stand. To 20 mL of the isooctane layer add exactly 80 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 25.54 mg of C_{17}H_{21}NO

**Containers and storage** Containers—Tight containers.

**Description** Freeze-dried Diphtheria Antitoxin, Equine, is a preparation for injection which is dissolved before use. It contains diphtheria antitoxin in immunoglobulin of horse origin.

**Freeze-dried Diphtheria Antitoxin, Equine**

Freeze-dried Diphtheria Antitoxin, Equine, is a preparation for injection which is dissolved before use. It contains diphtheria antitoxin in immunoglobulin of horse origin.

**Description** Freeze-dried Diphtheria Antitoxin, Equine, becomes a colorless or light yellow-brown, clear liquid or a slightly whitish turbid liquid on addition of solvent.

Diphtheria Toxoid

Diphtheria Toxoid is a liquid for injection containing diphtheria toxoid prepared by treating diphtheria toxin with formaldehyde by a method involving no appreciable loss of the immunogenicity.

**Description** Diphtheria Toxoid is a clear, colorless to light yellow-brown liquid.

**Adsorbed Diphtheria Toxoid for Adult Use**

Adsorbed Diphtheria Toxoid for Adult Use is a liquid for injection containing diphtheria toxoid prepared by treating diphtheria toxin with formaldehyde by a method involving no appreciable loss of the immunogenicity and very few antigenic substances other than toxoid, and rendered insoluble with aluminum salt.

**Description** Adsorbed Diphtheria Toxoid for Adult Use in the Minimum Requirements for Biological Products.
Requirements of Biological Products.

**Description** Adsorbed Diphtheria Toxoid for Adult Use becomes a homogeneous, whitish turbid liquid on shaking.

### Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine

Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine is a liquid for injection consisting of a liquid containing the protective antigen of *Bordetella pertussis*, Diphtheria Toxoid and a liquid containing tetanus toxoid obtained by detoxifying the tetanus toxin with formaldehyde solution without impairing its immunogenicity, to which aluminum is added to make the antigen and the toxoids insoluble.

It conforms to the requirements of Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine in the Minimum Requirements for Biological Products.

**Description** Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine becomes a homogeneous, white turbid liquid on shaking.

### Diphtheria-Tetanus Combined Toxoid

Diphtheria-Tetanus Combined Toxoid is a liquid for injection containing diphtheria toxoid and tetanus toxoid which are prepared by treating diphtheria toxin and tetanus toxin, respectively, with formaldehyde by a method involving no appreciable loss of the immunogenicity.

It conforms to the requirements of Diphtheria-Tetanus Combined Toxoid in the Minimum Requirements for Biological Products.

**Description** Diphtheria-Tetanus Combined Toxoid is a colorless or light yellow-brown, clear liquid.

### Adsorbed Diphtheria-Tetanus Combined Toxoid

Adsorbed Diphtheria-Tetanus Combined Toxoid is a liquid for injection containing diphtheria toxoid and tetanus toxoid which are prepared by treating diphtheria toxin and tetanus toxin, respectively, with formaldehyde by a method involving no appreciable loss of the immunogenicity and rendered insoluble by adding aluminum salt.

It conforms to the requirements of Adsorbed Diphtheria-Tetanus Combined Toxoid in the Minimum Requirements for Biological Products.

**Description** Adsorbed Diphtheria-Tetanus Combined Toxoid becomes a homogeneous, whitish turbid liquid on shaking.

### Dipyridamole

Dipyridamole, when dried, contains not less than 98.5% of C_{24}H_{36}N_{8}O_{4}.

**Description** Dipyridamole occurs as yellow crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water and in diethyl ether.

**Identification**

1. Dissolve 5 mg of Dipyridamole in 2 mL of sulfuric acid, add 2 drops of nitric acid, and shake: a deep purple color develops.

2. Determine the absorption spectrum of a solution of Dipyridamole in a mixture of methanol and hydrochloric acid (99:1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

3. Determine the infrared absorption spectrum of Dipyridamole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.0D> 165 – 169°C

**Purity**

1. Clarity and color of solution—Dissolve 0.5 g of Dipyridamole in 10 mL of chloroform: the solution is clear, and shows a yellow color.

2. Heavy metals <1.07>—Proceed with 2.0 g of Dipyridamole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

3. Arsenic <1.11>—Prepare the test solution with 1.0 g of Dipyridamole according to Method 3, and perform the
test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Diprydamole in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of diprydamole from the sample solution is not larger than the peak area of diprydamole from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 0.2 g of potassium dihydrogen phosphate in 200 mL of water, and add 800 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of diprydamole is about 4 minutes.
Time span of measurement: About 5 times as long as the retention time of diprydamole.

System suitability—
Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of diprydamole obtained from 20 μL of this solution is equivalent to 15 to 25% of that of diprydamole obtained from 20 μL of the standard solution.
System performance: Dissolve 7 mg of Diprydamole and 3 mg of terphenyl in 50 mL of methanol. When the procedure is run with 20 μL of this solution under the above operating conditions, diprydamole and terphenyl are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diprydamole is not more than 1.0%.

Loss on drying <2.41> Not more than 0.2% (1 g, 105°C, 3 hours).
Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Diprydamole, previously dried, dissolve in 70 mL of methanol, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.
Each mL of 0.1 mol/L perchloric acid VS = 50.46 mg of C₂₁H₂₉N₃O.

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Disopyramide
ジソピラミド

C₂₁H₂₉N₃O: 339.47
(2RS)-4-Bis(1-methylethyl)amino-2-phenyl-2-(pyridin-2-yl)butanamide

[3737-09-5]

Disopyramide contains not less than 98.5% of C₂₁H₂₉N₃O, calculated on the dried basis.

Description Disopyramide occurs as white crystals or crystalline powder.
It is very soluble in methanol and in ethanol (95), freely soluble in acetic anhydride, in acetic acid (100) and in diethyl ether, and slightly soluble in water.

Identification (1) To 1 mL of a solution of Disopyramide in ethanol (95) (1 in 20) add 10 mL of 2,4,6-trinitrophenol TS, and warm: a yellow precipitate is formed. Filter this precipitate, wash with water, and dry at 105°C for 1 hour: the residue melts <2.60> between 172°C and 176°C.

(2) Determine the absorption spectrum of a solution of Disopyramide in 0.05 mol/L sulfuric acid-methanol TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Disopyramide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> E₁% (cm) (269 nm): 194 – 205 (10 mg, 0.05 mol/L sulfuric acid-methanol TS, 500 mL).

Purity (1) Heavy metals <1.07>—Dissolve 1.0 g of Disopyramide in 10 mL of ethanol (95), and add 2 mL of dilute acetic acid (31) and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 10 mL of ethanol (95), 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Disopyramide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.40 g of Disopyramide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 400 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>, Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water
and ammonia solution (28) (45:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.4\) Not more than 0.5% (0.5 g, in vacuum, 80°C, 2 hours).

**Residue on ignition** \(<2.4\) Not more than 0.20% (1 g).

**Assay** Weigh accurately about 0.25 g of Disopyramide, dissolve in 30 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 16.97 mg of C₂₁H₂₉N₃O

**Containers and storage** Containers—Tight containers.

### Distigmine Bromide

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![Distigmine Bromide](image)

C₂₂H₃₂Br₂N₄O₄: 576.32
3,3'-[Hexamethylenebis(methyliminocarbonyloxy)]bis(1-methylpyridinium) dibromide [15876-67-2]

Distigmine Bromide contains not less than 98.5% of C₂₂H₃₂Br₂N₄O₄, calculated on the anhydrous basis.

**Description** Distigmine Bromide occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in methanol, in ethanol (95) and in acetic acid (100), and slightly soluble in acetic anhydride.

The pH of a solution of Distigmine Bromide (1 in 100) is between 5.0 and 5.5.

It is slightly hygroscopic.

It is gradually colored by light.

Melting point: about 150°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Distigmine Bromide (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Distigmine Bromide as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Distigmine Bromide (1 in

10) add 2 mL of dilute nitric acid: the solution responds to the Qualitative Tests \(<1.09\) (1) for bromide.

**Purity** (1) Clarity and color of solution—Dissolve 0.25 g of Distigmine Bromide in 5 mL of water: the solution is clear and colorless.

(2) Sulfate \(<1.14\)—Perform the test with 0.40 g of Distigmine Bromide. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals \(<1.07\)—Proceed with 2.0 g of Distigmine Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 40 mg of Distigmine Bromide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.93\). Spot 10 μL each of the sample solution and standard solution on a plate of cellulose with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, ethanol (99.5) and acetic acid (100) (8:3:2:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Spray evenly Dragendorff’s TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Water** \(<2.48\) Not more than 1.0% (1 g, volumetric titration, direct titration).

**Residue on ignition** \(<2.44\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Distigmine Bromide, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (8:1), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration with platinum electrode) Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 28.82 mg of C₂₂H₃₂Br₂N₄O

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

### Distigmine Bromide Tablets

ジスチグミン臭化物錠

Distigmine Bromide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of distigmine bromide (C₂₂H₃₂Br₂N₄O₄: 576.32).

**Method of preparation** Prepare as directed under Tablets, with Distigmine Bromide.

**Identification** Determine the absorption spectrum of the sample solution obtained in the Assay, as directed under Ultraviolet-visible Spectrophotometry \(<2.24\): it exhibits a
maximum between 268 nm and 272 nm, and a minimum between 239 nm and 243 nm.

**Uniformity of dosage units** 
6.02 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Distigmine Bromide Tablets add 30 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, and add 0.1 mol/L hydrochloric acid TS to make exactly V’ mL so that each mL contains about 30 µg of distigmine bromide (C₂₂H₃₂Br₂N₄O₄), and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of distigmine bromide (C₂₂H₃₂Br₂N₄O₄) = M₅ × (A₁₂₂ − A₁₁)/((Aₛ₂ − Aₛ₁) × V’/V × 1/20

M₅: Amount (mg) of distigmine bromide for assay, calculated on the anhydrous basis

**Dissolution** 6.10 When the test is performed at 75 revolutions per minute to the Paddle method, using 500 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Distigmine Bromide Tablets is not less than 80%.

Start the test with 1 tablet of Distigmine Bromide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 µm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V’ mL so that each mL contains about 10 µg of distigmine bromide (C₂₂H₃₂Br₂N₄O₄) according to the labeled amount, and use this solution as the sample solution. Separa
tely, weigh accurately about 50 mg of distigmine bromide for assay (separately, determine the water <2.48% in the same manner as Distigmine Bromide), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 500 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24, and determine the absorbances, A₁₁ and A₃₁, at 270 nm, and A₁₂ and A₃₂, at 350 nm.

Dissolution rate (%) with respect to the labeled amount of distigmine bromide (C₂₂H₃₂Br₂N₄O₄) = M₅ × (A₁₂₂ − A₁₁)/(Aₛ₂ − Aₛ₁) × V’/V × 1/C × 10

M₅: Amount (mg) of distigmine bromide for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of distigmine bromide (C₂₂H₃₂Br₂N₄O₄) in 1 tablet

**Assay** Weigh accurately and powder not less than 20 tablets of Distigmine Bromide Tablets. Weigh accurately a portion of the powder, equivalent to about 15 mg of Distigmine Bromide (C₂₂H₃₂Br₂N₄O₄), add 30 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and filter. Discard the first 20 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of distigmine bromide for assay (previously determine the water <2.48% in the same manner as Distigmine Bromide), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution, A₁₂ and A₃₂, at 270 nm and, A₁₁ and A₃₁, at 241 nm as directed under Ultraviolet-visible Spectrophotometry 2.24, respectively.

Amount (mg) of distigmine bromide (C₂₂H₃₂Br₂N₄O₄) = M₅ × (A₁₂₂ − A₁₁)/(Aₛ₂ − Aₛ₁) × 1/2

M₅: Amount (mg) of distigmine bromide for assay, calculated on the anhydrous basis

**Containers and storage** Containers—Tight containers.

**Disulfiram**

ジスルフィラム

\[
\text{C}_{10}\text{H}_{20}\text{N}_{2}\text{S}_{4}: 296.54
\]

Tetraethylthiuram disulfide [97-77-8]

Disulfiram, when dried, contains not less than 99.0% of C₁₀H₂₀N₂S₄.

**Description** Disulfiram occurs as white to yellowish white crystals or crystalline powder. It is freely soluble in acetone and in toluene, sparingly soluble in methanol and in ethanol (95), and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Disulfiram in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Disulfiram, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.60° 70 – 73°C

**Purity** (1) Heavy metals <1.07—Proceed with 2.0 g of Disulfiram according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.17—Prepare the test solution with 1.0 g of Disulfiram according to Method 4, and perform the test (not more than 2 ppm).

(3) Diethylthiocarbamic acid—Dissolve 0.10 g of Disulfiram in 10 mL of toluene, and shake with 10 mL of diluted sodium carbonate TS (1 in 20). Discard the toluene layer, wash the water layer with 10 mL of toluene, shake with 5 drops of a solution of cupric sulfate (1 in 250) and 2
mL of toluene, and allow to stand: no light yellow color develops in the toluene layer.

(4) Related substances—Dissolve 50 mg of Disulfiram in 40 mL of methanol, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of disulfiram from the sample solution is not larger than the peak area of disulfiram from the standard solution.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of methanol and water (7:3).
Flow rate: Adjust the flow rate so that the retention time of disulfiram is about 8 minutes.
Selection of column: Dissolve 50 mg of Disulfiram and 50 mg of benzenophenone in 40 mL of methanol, and add water to make 50 mL. To 1 mL of this solution add the mobile phase to make 200 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of benzenophenone and disulfiram in this order with the resolution between these peaks being not less than 4.
Detection sensitivity: Adjust the detection sensitivity so that the peak height of disulfiram obtained from 10 μL of the standard solution is 15–30 mm.

Time span of measurement: About 3.5 times of the retention time of disulfiram.

**Loss on drying** <2.41> Not more than 0.20% (2 g, silica gel, 24 hours).

**Residue on ignition** <2.44> Not more than 0.1% (2 g).

**Assay** Weigh accurately about 0.2 g of Disulfiram, previously dried, in an iodine bottle, dissolve in 20 mL of acetone, add 1.5 mL of water and 1.0 g of potassium iodide, and dissolve by shaking thoroughly. To this solution add 3.0 mL of hydrochloric acid, stopper the bottle tightly, shake, and allow to stand in a dark place for 3 minutes. Add 70 mL of water, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS = 14.83 mg of C₁₀H₂₀N₂S₄

**Containers and storage** Containers—Tight containers.

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**Dobutamine Hydrochloride**

**ドブタミン塩酸塩**

C₁₈H₂₃NO₃.HCl: 337.84
4-[2-[(1RS)-3-(4-Hydroxyphenyl)-1-methylpropylamino]ethyl]benzene-1,2-diol monohydrochloride [49745-95-1]

Dobutamine Hydrochloride, when dried, contains not less than 98.0% of C₁₈H₂₃NO₃.HCl.

**Description** Dobutamine Hydrochloride occurs as white to very pale orange crystalline powder or grains. It is freely soluble in methanol, sparingly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Dobutamine Hydrochloride (1 in 100) shows no optical rotation.

**Identification** (1) Determine the infrared absorption spectra of Dobutamine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Dobutamine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Dobutamine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

**pH** <2.54> Dissolve 1.0 g of Dobutamine Hydrochloride in 100 mL of water: the pH of this solution is between 4.0 and 5.5.

**Melting point** <2.60> 188–191°C

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Dobutamine Hydrochloride in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.67>—Dissolve 1.0 g of Dobutamine Hydrochloride in 40 mL of water by warming, cool, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Dobutamine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

**Related substances**—Dissolve 10 mL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and formic acid (78:22:5) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 5 minutes in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.
Loss on drying \(<2.4\)% Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition \(<2.4\)% Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g each of Dobutamine Hydrochloride and Dobutamine Hydrochloride RS, each previously dried, dissolve separately in exactly 10 mL of the internal standard solution, add diluted methanol (1 in 2) to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\)> according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of dobutamine to that of the internal standard, respectively.

\[
\text{Amount (mg) of } C_{18}H_{23}NO_3\cdot HCl = M_S \times \frac{Q_T}{Q_S}
\]

\(M_S\): Amount (mg) of Dobutamine Hydrochloride RS

Internal standard solution—A solution of salicylamide in diluted methanol (1 in 2) (1 in 125).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of tartrate buffer solution, pH 3.0 and methanol (7:3).

Flow rate: Adjust the flow rate so that the retention time of dobutamine is about 7 minutes.

**System suitability—**

System performance: When the procedure is run with 5 \(\mu\)L of the standard solution under the above operating conditions, dobutamine and internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dobutamine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

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**Domperidone**

\(\text{C}_{22}\text{H}_{24}\text{ClN}_5\text{O}_2\): 425.91

5-Chloro-1-[1-[3-(2-oxo-2,3-dihydro-1H-benzoimidazol-1-yl)propyl]piperidin-4-yl]-1,3-dihydro-2H-benzoimidazol-2-one

[57808-66-9]

Domperidone, when dried, contains not less than 99.0% and not more than 101.0% of \(\text{C}_{22}\text{H}_{24}\text{ClN}_5\text{O}_2\).

**Description** Domperidone occurs as a white to pale yellow, crystalline powder or powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (99.5), very slightly soluble in 2-propanol, and practically insoluble in water.

Melting point: about 243°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Domperidone in a mixture of 2-propanol and 0.1 mol/L hydrochloric acid TS (9:1) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Related substances**—Dissolve 30 mg of Domperidone in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu\)L of each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\>\) according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than domperidone obtained from the sample solution is not larger than 1/2 times the peak area of domperidone from the standard solution. Furthermore, the total area of the peaks other than domperidone is not larger than the peak area of domperidone from the standard solution.

**Purity (1)** Heavy metals \(<1.07\>—Proceed with 2.0 g of Domperidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wave-
Donepezil Hydrochloride

\[ \text{C}_{24}\text{H}_{29}\text{NO}_3\cdot\text{HCl}; 415.95] \\
(2RS)-2-\{(1-Benzylpiperidin-4-yl)methyl\}-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one monohydrochloride

\[120011-70-3]\

Donepezil Hydrochloride contains not less than 98.0% and not more than 102.0% of \( \text{C}_{24}\text{H}_{29}\text{NO}_3\cdot\text{HCl} \), calculated on the anhydrous basis.

**Description**

Donepezil Hydrochloride occurs as a white crystalline powder.

It is soluble in water, and slightly soluble in ethanol (99.5).

A solution of Donepezil Hydrochloride (1 in 100) shows no optical rotation.

**Identification (1)**

Determine the absorption spectrum of a solution of Donepezil Hydrochloride (1 in 50,000) as directed in Ultraviolet-visible Spectrophotometry \(2.24\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Donepezil Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Determine the infrared absorption spectrum of Donepezil Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry \(2.25\), and compare the spectrum with the Reference Spectrum or the spectrum of Donepezil Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the RS according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

A solution of Donepezil Hydrochloride (1 in 50) responds to the Qualitative Tests \(1.09\) (2) for chloride.

**Purity (1)**

Heavy metals \(1.07\)—To 1.0 g of Donepezil Hydrochloride in a porcelain or platinum crucible add 5 mL of sulfuric acid, incinerate by heating gradually, then incinerate by ignition between 500 and 600°C. If a carbonized residue still retains, moisten the residue with a little amount of sulfuric acid, and incinerate again by ignition between 500 and 600°C. After cooling, dissolve the residue with 3 mL of hydrochloric acid, then evaporate to dryness on a water bath or hot plate, and dissolve the residue with 10 mL of water by warming. Then, proceed as directed in Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

Related substances—Dissolve 50 mg of Donepezil Hydrochloride in 25 mL of the mobile phase. To 10 mL of this solution add the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to
make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than donepezil obtained from the sample solution is not larger than the peak area of donepezil from the standard solution.

Operating conditions —
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of donepezil, beginning after the solvent peak.

System suitability —
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Containers and storage — Containers—Well-closed containers.

**Donepezil Hydrochloride Fine Granules**

Donepezil Hydrochloride Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of donepezil hydrochloride (C_{24}H_{29}NO_{3}.HCl: 415.95).

Method of preparation — Prepare as directed under Granules, with Donepezil Hydrochloride.

Identification — To 2.5 mL of the sample solution obtained in the Assay add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 228 nm and 232 nm, between 269 nm and 273 nm, and between 313 nm and 317 nm.

Uniformity of dosage units — Perform the test according to the following method: the Donepezil Hydrochloride Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Donepezil Hydrochloride Fine Granule add exactly V mL of 0.1 mol/L hydrochloric acid TS so that each mL contains about 0.2 mg of donepezil hydrochloride (C_{24}H_{29}NO_{3}.HCl), disperse the particles with the aid of ultrasonic waves with occasional shaking, and treat with ultrasonic waves for a further 10 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg each of Donepezil Hydrochloride and Donepezil Hydrochloride RS (separately determine the water <2.48> in the same manner as Donepezil Hydrochloride); dissolve them in the mobile phase to make exactly 25 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the donepezil peak areas, A_F and A_S, of both solutions.

Amount (mg) of donepezil hydrochloride (C_{24}H_{29}NO_{3}.HCl) = M_S × A_F/A_S

M_S: Amount (mg) of Donepezil Hydrochloride RS, calculated on the anhydrous basis

Operating conditions —
Detector: An ultraviolet absorption photometer (wavelength: 271 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeccysilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: Dissolve 2.5 g of sodium 1-decansulfonate in 650 mL of water, and add 350 mL of acetonitrile and 1 mL of perchloric acid.
Flow rate: Adjust the flow rate so that the retention time of donepezil is about 11 minutes.

System suitability —
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.
System suitability—

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

**Dissolution**

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Donepezil Hydrochloride Fine Granules is not less than 80%.

Start the test with an accurately weighed amount of Donepezil Hydrochloride Fine Granules, equivalent to about 3 mg of donepezil hydrochloride \( (C_{24}H_{29}NO_3.HCl) \) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu m \). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 55 mg of Donepezil Hydrochloride RS (separately determine the water \(< 2.48% \) in the same manner as Donepezil Hydrochloride), and dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid (3:1) to make exactly 50 mL. Pipet 3 mL of this solution, add the dissolution medium to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium at the specified minute after starting the test, and treat with ultrasonic waves with occasional shaking, and treat with ultrasonic waves for a further 15 minutes. Add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Donepezil Hydrochloride RS (separately determine the water \(< 2.48% \) in the same manner as Donepezil Hydrochloride), and dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 25 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \(< 2.07% \) according to the following conditions, and determine the peak areas, \( A_1 \) and \( A_3 \), of donepezil from each solution.

Amount (mg) of donepezil hydrochloride \( (C_{24}H_{29}NO_3.HCl) \) = \( \frac{M_S \times A_1}{A_3} \times \frac{1/C}{27/5} \)

**Operating conditions**

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

Mobile phase: A mixture of water, acetonitrile and perchloric acid (650:350:1).

Flow rate: Adjust the flow rate so that the retention time of donepezil is about 4 minutes.

System suitability—

System performance: When the procedure is run with 50 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

**Particle size**

It meets the requirements of Fine granules.

**Assay**

Powder Donepezil Hydrochloride Fine Granules, if necessary. Weigh accurately a portion of the powder, equivalent to about 20 mg of donepezil hydrochloride \( (C_{24}H_{29}NO_3.HCl) \), add 30 mL of 0.1 mol/L hydrochloric acid TS, disperse into the fine particles with the aid of ultrasonic waves with occasional shaking, and treat with ultrasonic waves for a further 15 minutes. Add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Donepezil Hydrochloride RS (separately determine the water \(< 2.48% \) in the same manner as Donepezil Hydrochloride), and dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 25 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \(< 2.07% \) according to the following conditions, and determine the peak areas, \( A_1 \) and \( A_3 \), of donepezil from each solution.

Amount (mg) of donepezil hydrochloride \( (C_{24}H_{29}NO_3.HCl) \) = \( \frac{M_S \times A_1}{A_3} \times \frac{1/C}{27/5} \)

**Operating conditions**

Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

**System suitability**

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

**Containers and storage**

Containers—Well-closed containers.

Storage—Light-resistant.

**Donepezil Hydrochloride Tablets**

Donepezil Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of donepezil hydrochloride \( (C_{24}H_{29}NO_3.HCl) = 415.95 \) g.

**Method of preparation**

Prepare as directed under Tablets, with Donepezil Hydrochloride.

**Identification**

To 2.5 mL of the sample solution obtained in the Assay add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultravi-
Uniformity of dosage units (6.02) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Donepezil Hydrochloride Tablets add exactly \( V \) mL of a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) so that each mL contains about 0.2 mg of donepezil hydrochloride (C\(_{24}\)H\(_{29}\)NO\(_3\)HCl), disperse with the aid of ultrasonic waves. Shake until the tablet is disintegrated, and treat with ultrasonic waves for a further 10 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Donepezil Hydrochloride RS (separately, determine the water (2.48) in the same manner as Donepezil Hydrochloride), dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the donepezil peak areas, \( A_T \) and \( A_S \), of both solutions.

Amount (mg) of donepezil hydrochloride (C\(_{24}\)H\(_{29}\)NO\(_3\)HCl) = \( M_S \times A_T/A_S \times V/250 \)

\( M_S \) : Amount (mg) of Donepezil Hydrochloride RS, calculated on the anhydrous basis

Operating conditions —
Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

System suitability —
System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Assay Accurately weigh the mass of not less than 20 Donepezil Hydrochloride Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of donepezil hydrochloride (C\(_{24}\)H\(_{29}\)NO\(_3\)HCl), add 30 mL of a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1), disperse with the aid of ultrasonic waves, and add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Donepezil Hydrochloride RS (separately, determine the water (2.48) in the same manner as Donepezil Hydrochloride), dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add the dissolution medium to make exactly 50 mL. Further, pipet 3 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of donepezil from each solution.

Dissolution rate (%) with respect to the labeled amount of donepezil hydrochloride (C\(_{24}\)H\(_{29}\)NO\(_3\)HCl) = \( M_S \times A_T/A_S \times V/V \times 1/C \times 27/5 \)

\( M_S \) : Amount (mg) of Donepezil Hydrochloride RS, calculated on the anhydrous basis

C: Labeled amount (mg) of donepezil hydrochloride (C\(_{24}\)H\(_{29}\)NO\(_3\)HCl) in 1 tablet

Operating conditions —
Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

Mobil phase: A mixture of water, acetonitrile and perchloric acid (650:350:1). Flow rate: Adjust the flow rate so that the retention time of donepezil is about 4 minutes.

System suitability —
System performance: When the procedure is run with 50 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Assay Accurately weigh the mass of not less than 20 Donepezil Hydrochloride Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of donepezil hydrochloride (C\(_{24}\)H\(_{29}\)NO\(_3\)HCl), add 30 mL of a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1), disperse with the aid of ultrasonic waves, and add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Donepezil Hydrochloride RS (separately, determine the water (2.48) in the same manner as Donepezil Hydrochloride), dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add the dissolution medium to make exactly 50 mL. Further, pipet 3 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of donepezil from each solution.

Dissolution rate (%) with respect to the labeled amount of donepezil hydrochloride (C\(_{24}\)H\(_{29}\)NO\(_3\)HCl) = \( M_S \times A_T/A_S \times V/V \times 1/C \times 27/5 \)

\( M_S \) : Amount (mg) of Donepezil Hydrochloride RS, calculated on the anhydrous basis

C: Labeled amount (mg) of donepezil hydrochloride (C\(_{24}\)H\(_{29}\)NO\(_3\)HCl) in 1 tablet

Operating conditions —
Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

Mobil phase: A mixture of water, acetonitrile and perchloric acid (650:350:1). Flow rate: Adjust the flow rate so that the retention time of donepezil is about 4 minutes.

System suitability —
System performance: When the procedure is run with 50 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.
Operating conditions—

Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Containers and storage  Containers—Well-closed containers.

Dopamine Hydrochloride

Dopamine Hydrochloride Injection

Dopamine Hydrochloride injection is an aqueous solution for injection. It contains not less than 97.0% and not more than 103.0% of the labeled amount of dopamine hydrochloride (C₈H₁₁NO₂.HCl: 189.64).

Method of preparation  Prepare as directed under Injections, with Dopamine Hydrochloride.

Description  Dopamine Hydrochloride Injection occurs as a clear, colorless liquid.

Identification  To a volume of Dopamine Hydrochloride Injection, equivalent to 0.04 g of Dopamine Hydrochloride according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL. To 5 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 278 nm and 282 nm.

pH  <2.54>  3.0 – 5.0

The total content of Donepezil Hydrochloride in the sample is not less than 97.0% of the labeled amount. The total content of dopamine hydrochloride is not more than 1.0% of the labeled amount.

Containers and storage  Containers—Well-closed containers.

Loss on drying  <7.2.4>  Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition  <7.4.4>  Not more than 0.1% (1 g).

The solution is clear and colorless.
Bacterial endotoxins <4.0I> Less than 4.2 EU/mg.

Extractable volume <6.0I> It meets the requirement.

Foreign insoluble matter <6.0I> Perform the test according to Method 1; it meets the requirement.

Insoluble particulate matter <6.0I> It meets the requirement.

Sterility <4.0I> Perform the test according to the Membrane filtration method; it meets the requirement.

Assay To an exact volume of Dopamine Hydrochloride Injection, equivalent to about 30 mg of dopamine hydrochloride (C8H11NO2.HCl), add the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add exactly 2.5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of dopamine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add exactly 2.5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0I> according to the following conditions, and calculate the ratios, Qs and Qs5, of the peak area of dopamine to that of the internal standard.

\[ Q_s = \frac{A_s}{A_{s5}} \]

Amount (mg) of dopamine hydrochloride (C8H11NO2.HCl) = \( M_s \times Q_s/Q_{s5} \)

\( M_s \): Amount (mg) of dopamine hydrochloride for assay

Internal standard solution—A solution of uracil in the mobile phase (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Disodium hydrogen phosphate-citric acid buffer solution, pH 3.0.

Flow rate: Adjust the flow rate so that the retention time of dopamine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and dopamine are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of peak area of dopamine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

Doxapram Hydrochloride Hydrate

Doxapram Hydrochloride Hydrate contains not less than 98.0% of doxapram hydrochloride (C24H30N2O2.HCl: 414.97), calculated on the anhydrous basis.

Description Doxapram Hydrochloride Hydrate occurs as white crystals or crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in water, in ethanol (95) and in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Doxapram Hydrochloride Hydrate (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxapram Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Doxapram Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.0I> for chloride.

pH <2.5I> Dissolve 1.0 g of Doxapram Hydrochloride Hydrate in 50 mL of water: the pH of this solution is between 3.5 and 5.0.

Melting point <2.6I> 218 – 222°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Doxapram Hydrochloride Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14I>—Perform the test with 1.0 g of Doxapram Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals <1.0I>—Proceed with 2.0 g of Doxapram Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.1I>—Prepare the test solution with 1.0 g of Doxapram Hydrochloride Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.5 g of Doxapram Hydrochloride Hydrate in 10 mL of methanol, and use this so-
lution as the sample solution. Pipet 3 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 6 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, formic acid, ethyl formate and methanol (8:3:3:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 3.5 - 4.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.3% (1 g).

**Assay** Weigh accurately about 0.8 g of Doxapram Hydrochloride Hydrate, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 41.50 mg of C₂₄H₃₀N₂O₂.HCl

**Containers and storage** Containers—Tight containers.

**Doxazosin Mesilate**

Doxazosin Mesilate, when dried, contains not less than 98.0% and not more than 102.0% of C₂₃H₂₅N₅O₅.CH₄O₃S.

**Description** Doxazosin Mesilate occurs as a white to yellowish white crystalline powder. It is freely soluble in dimethylsulfoxide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5%).

A solution of Doxazosin Mesilate in dimethylsulfoxide solution (1 in 20) shows no optical rotation.

Melting point: about 272°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Doxazosin Mesilate in 0.01 mol/L hydrochloric acid-methanol TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.26>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Doxazosin Mesilate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxazosin Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.27>, and compare the spectrum with the Reference Spectrum or the spectrum of Doxazosin Mesilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) 30 mg of Doxazosin Mesilate responds to the Qualitative Tests <1.09> (2) for mesilate.

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Doxazosin Mesilate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Doxazosin Mesilate in 5 mL of a mixture of methanol and acetic acid (100) (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and acetic acid (100) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of methanol and acetic acid (100) (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with an upper layer of a mixture, prepared by adding 1 volume of water and 1 volume of acetic acid (100) to 2 volumes of 4-methyl-2-pentanone and shaking, to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot at the Rf value about 0.15 obtained from the sample solution is not more intense than the spot from the standard solution, and no spots other than the principal spot and other than the spots mentioned above appear from the sample solution.

(3) Residual solvent—Being specified separately.

**Loss on drying <2.41>** Not more than 1.0% (1 g, 105°C, 4 hours).

**Residue on ignition <2.44>** Not more than 0.2% (1 g).

**Assay** Weigh accurately about 25 mg each of Doxazosin Mesilate and Doxazosin Mesilate RS, previously dried, dissolve separately in methanol to make exactly 50 mL. Pipet 3 mL each of these solutions, add the mobile phase to make exactly 100 mL. Pipet 5 µL of this solution, add a mixture of methanol and acetic acid (100) (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₃, of doxazosin in each solution.

Amount (mg) of doxazosin mesilate (C₂₃H₂₅N₅O₅.CH₄O₃S) = M₅ × A₁/A₃

M₅: Amount (mg) of Doxazosin Mesilate RS

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 246 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecysiliclanized silica
gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0, methanol and acetonitrile (12:8:3).

Flow rate: Adjust the flow rate so that the retention time of doxazosin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of doxazosin are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxazosin is not more than 1.0%.

Containers and storage  Containers—Tight containers.

Doxazosin Mesilate Tablets

Dokisyosun Mesumeshi Akusatai

Doxazosin Mesilate Tablets contain not less than 95.0% and not more than 105.0% of doxazosin (C23H25N5O5): 451.48.

Method of preparation  Prepare as directed under Tablets, with Doxazosin Mesilate.

Identification  To a quantity of powdered Doxazosin Mesilate Tablets, equivalent to 5 mg of doxazosin (C23H25N5O5) according to the labeled amount, add 100 mL of 0.01 mol/L hydrochloric acid-methanol TS, shake vigorously, and centrifuge. To 4 mL of the supernatant liquid add 0.01 mol/L hydrochloric acid-methanol TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 244 nm and 248 nm.

Uniformity of dosage units <6.02>  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Doxazosin Mesilate Tablets add 1 mL of water, disintegrate the tablet by shaking, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL, and shake for 30 minutes. Centrifuge, pipet 5 mL of the supernatant liquid, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 10 mL so that each mL contains about 5 μg of doxazosin (C23H25N5O5), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Doxazosin Mesilate RS, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL. Then, pipet 2 mL of this solution, add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography: it exhibits a maximum between 244 nm and 248 nm.

Dissolution <6.10>  When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 as the dissolution medium, the dissolution rate in 15 minutes of Doxazosin Mesilate Tablets is not less than 75%.

Start the test with 1 tablet of Doxazosin Mesilate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, and add the dissolution medium to make exactly 5 mL so that each mL contains about 0.56 μg of doxazosin (C23H25N5O5) according to the labeled amount. Pipet 5 mL of this solution, add exactly 5 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of Doxazosin Mesilate RS, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL. Then, pipet 2 mL of this solution, add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the dissolution medium, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography: it exhibits a maximum between 244 nm and 248 nm.

Operating conditions—

Detector: An ultraviolet absorption spectrophotometer (wavelength: 246 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate in 500 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10). To 450 mL of this solution add 550 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of doxazosin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of doxazosin are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxazosin is not more than 2.0%.

Assay  Weigh accurately the mass of not less than 20 Doxazosin Mesilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of doxazosin (C23H25N5O5), add 0.01 mol/L hydrochloric acid-
methanol TS to make exactly 100 mL, and stir for 30 minutes. Centrifuge, pipet 4 mL of the supernatant liquid, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 24 mg of Doxazosin Mesilate RS, previously dried at 105°C for 4 hours, dissolve in 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 246 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Amount (mg) of doxazosin (C}_{23}\text{H}_{32}\text{N}_{2}\text{O}_{3}) = \frac{M_S \times A_T/A_S}{1/4 \times 0.825}
\]

**Doxifluridine**

Doキシフルリジン

\[
\text{C}_{9}\text{H}_{11}\text{FN}_{2}\text{O}_{5}: 246.19
\]

5'-Deoxy-5-fluorouridine

\[\text{C}_9\text{H}_{10}\text{FN}_{2}\text{O}_5 \cdot \text{H}_{2}\text{O} \quad \text{M}: \text{293.25} \]

Doxifluridine, when dried, contains not less than 98.5% and not more than 101.0% of \( \text{C}_9\text{H}_{11}\text{FN}_{2}\text{O}_5 \).

**Description**

Doxifluridine occurs as a white crystalline powder.
It is freely soluble in \( \text{N},\text{N}-\text{dimethylformamide} \), soluble in water and in methanol, and slightly soluble in ethanol (99.5).
It dissolves in 0.1 mol/L hydrochloric acid TS and in 0.01 mol/L sodium hydroxide TS.
Melting point: about 191°C (with decomposition).

**Identification**

(1) Determine the absorption spectrum of a solution of Doxifluridine in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxifluridine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.4> \([\alpha]_{\text{D}}^20 +160 – +174^\circ\) (after drying, 0.1 g, water, 10 mL, 100 mm).

\( \text{PH} <2.54> \) The pH of a solution obtained by dissolving 0.10 g of Doxifluridine in 10 mL of water is between 4.2 and 5.2.

**Purity** (1) Fluoride—Dissolve 0.10 g of Doxifluridine in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution into a 20-mL volumetric flask, add 5 mL of a mixture of acetone and lanthanum-alizarin complexone TS (2:1) and water to make 20 mL, allow to stand for 1 hour, and use this solution as the sample solution. Separately, put 1.0 mL of Standard Fluorine Solution in a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) and 5 mL of the mixture of acetone and alizarin complexone TS (2:1), then proceed in the same manner as for preparation of the sample solution, and use the solution so obtained as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 620 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution obtained in the same way with 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) as a blank: \( A_T \) is not larger than \( A_S \).

(2) Chloride <1.03>—Perform the test with 0.30 g of Doxifluridine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.035%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Doxifluridine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 20 mg of Doxifluridine in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 25 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100) and water (17:2:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot with the sample solution is not more than three, and they are not more intense than the spot with the standard solution.

**Loss on drying** <2.4> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.4> Not more than 0.1% (1 g, platinum crucible).

**Assay**
Weigh accurately about 0.25 g of Doxifluridine, previously dried, dissolve in 50 mL of \( \text{N},\text{N}-\text{dimethylformamide} \) and titrate <2.30> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.
Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 24.62 mg of \( \text{C}_9\text{H}_{11}\text{FN}_{2}\text{O}_5 \).

**Containers and storage**
Containers—Tight containers.
Doxifluridine Capsules

Doxifluridine Capsules contain not less than 95.0% and not more than 105.0% of doxifluridine (C9H11FN2O5: 246.19).

Method of preparation  Prepare as directed under Capsules, with Doxifluridine.

Identification  (1)  Dissolve an amount of the contents of Doxifluridine Capsules, equivalent to 20 mg of Doxifluridine according to the labeled amount, in 0.1 mol/L hydrochloric acid TS to make 100 mL, and filter. To 1 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 0.1 mol/L hydrochloric acid TS as the blank: it exhibits a maximum between 267 nm and 271 nm.

(2)  To an amount of powdered contents of Doxifluridine Capsules, equivalent to 20 mg of Doxifluridine according to the labeled amount, add 2 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg of doxifluridine in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100) and water (17:2:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot with the sample solution and standard solution show a dark purple color and the f values are the same.

Uniformity of dosage units  <6.02>  It meets the requirements of the Mass variation test.

Dissolution  <6.10>  When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Doxifluridine Capsules is not less than 85%.

Start the test with 1 capsule of Doxifluridine Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V mL so that each mL contains about 13 μg of doxifluridine (C9H11FN2O5) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 26 mg of doxifluridine for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A3, of the sample solution and standard solution at 269 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of doxifluridine (C9H11FN2O5)

\[ M_S \times \frac{A_1}{A_S} \times \frac{V}{V'} \times \frac{1}{C} \times 45 \]

M_S: Amount (mg) of doxifluridine for assay
C: Labeled amount (mg) of doxifluridine (C9H11FN2O5) in 1 capsule

Assay  Weigh accurately the mass and powder the contents of not less than 20 Doxifluridine Capsules. Weigh accurately a portion of the powder, equivalent to about 50 mg of doxifluridine (C9H11FN2O5), add 40 mL of water, shake for about 10 minutes, add water to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, then add a mixture of water and methanol (5:3) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of doxifluridine for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mixture of water and methanol (5:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q1 and Q5, of the peak height of doxifluridine to that of the internal standard.

Amount (mg) of doxifluridine (C9H11FN2O5)

\[ M_S \times \frac{Q_1}{Q_5} \]

M_S: Amount (mg) of doxifluridine for assay

Internal standard solution—A solution of anhydrous caffeine (1 in 1000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of water and methanol (13:7).
Flow rate: Adjust the flow rate so that the retention time of doxifluridine is about 2.5 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, doxifluridine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of doxifluridine to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Tight containers.
Doxorubicin Hydrochloride

Doxorubicin Hydrochloride is the hydrochloride of a derivative of daunorubicin.

It contains not less than 980 μg (potency) and not more than 1080 μg (potency) per mg, calculated on the anhydrous basis. The potency of Doxorubicin Hydrochloride is expressed as mass (potency) of doxorubicin hydrochloride (C27H29NO11.HCl).

**Description**

Doxorubicin Hydrochloride occurs as a red-orange crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetone.

**Identification** (1) Determine the absorption spectrum of a solution of Doxorubicin Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.4>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Doxorubicin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxorubicin Hydrochloride as directed in the potassium dichromate disk method under Infrared Spectrophotometry <2.5>, and compare the spectrum with the Reference Spectrum or the spectrum of Doxorubicin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Doxorubicin Hydrochloride (1 in 200) responds to the Qualitative Tests <1.09> (1) for chloride.

**Optical rotation** <2.4> [α]D: +240 – +290° (20 mg calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

**pH** <2.54> The pH of a solution obtained by dissolving 50 mg of Doxorubicin Hydrochloride in 10 mL of water is between 4.0 and 5.5.

**Purity** (1) Clarity and color of solution—Dissolve 50 mg of Doxorubicin Hydrochloride in 10 mL of water: the solution is clear and red.

(2) Related substances—Dissolve 25 mg of Doxorubicin Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than doxorubicin obtained from the sample solution is not larger than 1/4 times the peak area of doxorubicin from the standard solution, and the total area of the peaks other than doxorubicin is not larger than the peak area of doxorubicin from the standard solution.

**Operating conditions—**

- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: Dissolve 3 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (7 in 5000), and add 1000 mL of acetonitrile.
- Flow rate: Adjust the flow rate so that the retention time of doxorubicin is about 8 minutes.
- Time span of measurement: About 3 times as long as the retention time of doxorubicin.

**System suitability—**

Test for required detectability: Measure 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of doxorubicin obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the standard solution.

System performance: Dissolve 5 mg of Doxorubicin Hydrochloride in 20 mL of water, add 1.5 mL of phosphoric acid, and allow to stand at room temperature for 30 minutes. Adjust the pH of this solution to 2.5 with 2 mol/L sodium hydroxide TS. When the procedure is run with 20 μL of this solution under the above operating conditions, doxorubicinone, having the relative retention time of about 0.6 with respect to doxorubicin, and doxorubicin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxorubicin is not more than 2.0%.

**Water** <2.48> Not more than 3.0% (0.3 g, volumetric titration, direct titration).

**Assay**

Weigh accurately an amount of Doxorubicin Hydrochloride and Doxorubicin Hydrochloride RS, equivalent to about 10 mg (potency), dissolve each in water to make exactly 25 mL. Pipet 5 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances at 495 nm, A1 and A2, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Amount [μg (potency)] of } \text{C}_{27}\text{H}_{29}\text{NO}_{11}\cdot\text{HCl} = \frac{M_s \times A_1}{A_2} \times 1000
\]

\[M_s: \text{Amount [μg (potency)] of Doxorubicin Hydrochlo-}\]
Doxorubicin Hydrochloride for Injection

注射用ドキソルビシン塩酸塩

Doxorubicin Hydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of doxorubicin hydrochloride (C27H29NO11.HCl: 579.98).

Method of preparation Prepare as directed under Injections, with Doxorubicin Hydrochloride.

Description Doxorubicin Hydrochloride for Injection occurs as red-orange, powder or masses.

Identification Dissolve an amount of Doxorubicin Hydrochloride for Injection, equivalent to 10 mg (potency) of Doxorubicin Hydrochloride according to the labeled amount, in methanol to make 100 mL. To 5 mL of this solution add methanol to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 231 nm and 235 nm, between 250 nm and 254 nm, between 477 nm and 481 nm, and between 493 nm and 497 nm, and exhibits a shoulder between 528 nm and 538 nm.

pH (2.54) The pH of a solution, prepared by dissolving an amount of Doxorubicin Hydrochloride for Injection equivalent to 10 mg (potency) of Doxorubicin Hydrochloride according to the labeled amount in 2 mL of water, is 5.0 to 6.0.

Purity Clarity and color of solution—Dissolve an amount of Doxorubicin Hydrochloride for Injection, equivalent to 50 mg (potency) of Doxorubicin Hydrochloride according to the labeled amount, in 10 mL of water: the solution is clear and red.

Water (2.48) Not more than 4.0% (0.25 g, volumetric titration, direct titration).

Bacterial endotoxins (4.01) Less than 2.50 EU/mg (potency).

Uniformity of dosage units (6.02) It meets the requirement of the Mass variation test.

Foreign insoluble matter (6.06) Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter (6.07) It meets the requirement.

Sterility (4.06) Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Doxorubicin Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Doxorubicin Hydrochloride, add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use the solution as the sample solution. Separately, weigh accurately an amount of Doxorubicin Hydrochloride RS, equivalent to 10 mg (potency), add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01), and calculate the ratios, Q1 and Q2, of the peak area of doxorubicin to that of the internal standard.

\[
M_S = \frac{Q_1}{Q_2} \times M_S
\]

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (7 in 5000). To this solution add 1000 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of doxorubicin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, doxorubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5, and the symmetry factor of the peak of doxorubicin is between 0.8 and 1.2.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of doxorubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.
**Doxycycline Hydrochloride Hydrate**

ドキシサイクリン塩酸塩水和物

\[
\text{C}_{22}\text{H}_{24}\text{N}_{2}\text{O}_{8}\cdot\text{HCl} \cdot \frac{1}{2}\text{C}_{2}\text{H}_{2}\text{O} \cdot \frac{1}{2}\text{H}_{2}\text{O} : 512.94
\]

(4S,4aR,5S,5aR,6R,12aS)-4-Dimethylamino-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydratetraene-2-carboxamide monohydrochloride hemihydrate

[564-25-0, Doxycycline]

Doxycycline Hydrochloride Hydrate is the hydrochloride of a derivative of oxytetracycline.

It contains not less than 880 μg (potency) and not more than 943 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of ethanol. The potency of Doxycycline Hydrochloride Hydrate is expressed as mass (potency) of doxycycline (C\textsubscript{22}H\textsubscript{24}N\textsubscript{2}O\textsubscript{8}: 444.43).

**Description**

Doxycycline Hydrochloride Hydrate occurs as yellow to dark yellow, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (99.5).

**Identification**

(1) Determine the infrared absorption spectrum of Doxycycline Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.255>, and compare the spectrum with the Reference Spectrum or the spectrum of Doxycycline Hydrochloride Hydrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 10 mg of Doxycycline Hydrochloride Hydrate in 10 mL of water, and add silver nitrate TS: a white turbidity is produced.

**Absorbance**

\(<2.24\) \(E_{1%}^{10}\) (349 nm): 285 – 315 (10 mg, 0.01 mol/L hydrochloric acid-methanol TS, 500 mL).

**Optical rotation**

\(<2.49\) \(\left[\alpha\right]_{D}\): –105 – –120° (0.25 g calculated on the anhydrous basis and corrected by the amount of ethanol, 0.01 mol/L hydrochloric acid-methanol TS, 25 mL, 100 mm). Determine within 5 minutes after the sample solution is prepared.

**Purity**

(1) Heavy metals \(<1.07\)—Proceed with 1.0 g of Doxycycline Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 5.0 mL of Standard Lead Solution (not more than 50 ppm).

(2) Related substance—Dissolve 20 mg of Doxycycline Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 6-epidoxycycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 6-epidoxycycline hydrochloride stock solution. Separately, dissolve 20 mg of metacycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as metacycline hydrochloride stock solution. Pipet 2 mL each of 6-epidoxycycline hydrochloride stock solution and metacycline hydrochloride stock solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.015> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of metacycline and 6-epidoxycycline obtained from the sample solution are not larger than the peak areas of them obtained from the standard solution, respectively, and the areas of the two peaks, appeared between the solvent peak and metacycline and behind of doxycycline, obtained from the sample solution are not larger than 1/4 times the peak area of 6-epidoxycycline from the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Mix 125 mL of 0.2 mol/L potassium dihydrogen phosphate TS, 117 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 500 mL. To 400 mL of this solution add 50 mL of a solution of tetrabutylammonium hydrogen sulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetracetate dihydrate (1 in 25), 60 g of t-butanol and 200 mL of water, adjust the pH to 8.0 with 2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of doxycycline is about 19 minutes.

Time span of measurement: About 2.4 times as long as the retention time of doxycycline beginning after the solvent peak.

**System suitability**

Test for required detectability: Measure exactly 1 mL of the standard solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak areas of 6-epidoxycycline and metacycline obtained from 20 μL of this solution are equivalent to 3.5 to 6.5% of them obtained from 20 μL of the standard solution, respectively.

System performance: To 8 mL of the sample solution, 3 mL of 6-epidoxycycline hydrochloride stock solution and 2 mL of metacycline hydrochloride stock solution add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, metacycline, 6-epidoxycycline and doxycycline are eluted in this order with the resolutions between the peaks, metacycline and 6-epidoxycycline, and 6-epidoxycycline and doxycycline, being not less than 1.3 and not less than 2.0, respectively, and the symmetry factor of the peak of doxycycline is not more than 1.3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of metacycline and 6-epidoxycycline are not more than
amount of ethanol is not less than 4.3% and not more than 6.0%.

\[ \text{Amount (％) of ethanol} = \frac{M_S}{M_T} \times \frac{Q_I}{Q_S} \]

- \( M_S \): Amount (mg) of ethanol (99.5)
- \( M_T \): Amount (mg) of the sample

**Internal standard solution—A solution of 1-propanol (1 in 2000).**

**Operating conditions—**
- Detector: An hydrogen flame-ionization detector.
- Column: A glass column 3.2 mm in inside diameter and 1.5 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (0.0075 \( \mu \text{m} \) in average pore size, 500 – 600 \( \mu \text{m}^2/g \) in specific surface area) (150 – 180 \( \mu \text{m} \) in particle diameter).
- Column temperature: A constant temperature of about 135°C.
- Carrier gas: Nitrogen.
- Flow rate: Adjust the flow rate so that the retention time of ethanol is about 5 minutes.

**System suitability—**
- System performance: When the procedure is run with 1 \( \mu \text{L} \) of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.
- System repeatability: When the test is repeated 5 times with 1 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethanol to that of the internal standard is not more than 2.0%.

**Water** \( <2.48 \) Not less than 1.4% and not more than 2.8% (0.6 g, volumetric titration, direct titration).

**Residue on ignition** \( <2.44 \) Not more than 0.3% (1 g).

**Assay**
- Weigh accurately an amount of Doxycycline Hydrochloride Hydrate and Doxycycline Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in water to make exactly 10 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 \) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of doxycycline.

\[
\text{Amount [\mu g (potency)] of doxycycline (C}_{22}\text{H}_{22}\text{FN}_{3}\text{O}_{2}} = \frac{M_S}{M_T} \times A_T/A_S \times 1000
\]

- \( M_S \): Amount [\mu g (potency)] of Doxycycline Hydrochlo-

**Operating conditions—**
- Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
- Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 \( \mu \text{m} \) in particle diameter).
- Column temperature: A constant temperature of about 30°C.
- Mobile phase: Dissolve 7.0 g of sodium dihydrogen phosphate dihydrate in 450 mL of water, add 553 mL of a mixture of methanol and N,N-dimethyl-n-octylamine (550:3), and adjust the pH to 8.0 with a solution of sodium hydroxide (43 in 200).
- Flow rate: Adjust the flow rate so that the retention time of doxycycline is about 6 minutes.

**System suitability—**
- System performance: When the procedure is run with 10 \( \mu \text{L} \) of the standard solution under the above operating conditions, the theoretical plates and the symmetry factor of the peak of doxycycline are not less than 1000 and not more than 2.0, respectively.
- System repeatability: When the test is repeated 6 times with 10 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxycycline is not more than 1.0%.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Droperidol**

The description of Droperidol is provided with a chemical structure and additional information including its molecular weight, CAS number, and various properties such as solubility, description, and identification. The text also includes the identification of droperidol, its solubility in various solvents, and its preparation for analysis. The instruction for preparation includes dissolving droperidol in a brown volumetric flask and transferring it to a volumetric flask to reach a specific concentration. This preparation is followed by the addition of hydrochloric acid to adjust the pH to a specific value, which is crucial for the subsequent analysis.
make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Droperidol, previously dried, in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25> \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Droperidol in acetone, evaporate the acetone, dry the residue in a desiccator (in vacuum, silica gel, 70°C) for 4 hours, and perform the test with the residue.

**Purity**

(1) Heavy metals \( <1.07> \)—Produce with 1.0 g of Droperidol in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Droperidol in 5 mL of dichloromethane, and use this solution as the standard solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.03> \). Spot 10 mL of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, chloroform, methanol and acetic acid-sodium acetate buffer solution, pH 4.7, (54:23:18:5) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \( <2.41> \) Not more than 3.0% (0.5 g, in vacuum, silica gel, 70°C, 4 hours).

**Residue on ignition** \( <2.44> \) Not more than 0.2% (1 g, platinum crucible).

**Assay**

Weigh accurately about 0.5 g of Droperidol, previously dried, dissolve in 50 mL of acetic acid (100), and titrate \( <2.50> \) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 37.94 mg of \( \text{C}_2\text{H}_2\text{F}_7\text{N}_2\text{O}_2 \)

**Containers and storage**

Containers—Tight containers. Storage—Light-resistant.

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**Droxidopa**

Droxidopa occurs as white to light brown crystals or crystalline powder. It is slightly soluble in water and practically insoluble in ethanol (99.5). It dissolves in 0.1 mol/L hydrochloric acid TS.

**Identification**

(1) Determine the absorption spectrum of a solution of Droxidopa in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Droxidopa as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25> \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \( <2.49> \) \( [\alpha]_D^{20} = -38 - -43^\circ \) (after drying, 0.1 g, 0.1 mol/L hydrochloric acid TS, 20 mL, 100 mm).

**Purity**

(1) Chloride \( <1.03> \) Dissolve 0.40 g of Droxidopa in 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals \( <1.07> \) Proceed with 2.0 g of Droxidopa according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic \( <1.11> \) Prepare the test solution with 1.0 g of Droxidopa according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—To 0.10 g of Droxidopa add 50 mL of 0.1 mol/L hydrochloric acid TS, dissolve by shaking while cooling in an ice bath, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01> \) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than d Roxidopa obtained from the sample solution is not larger than the peak area of d Roxidopa from the standard solution.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (3 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 1.0 g of sodium 1-heptanesulfonate and 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.0 with phosphoric acid. To 930 mL of this solution add 70 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of droxidopa is about 5 minutes.
Time span of measurement: About 12 times as long as the retention time of droxidopa, beginning after the solvent peak.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of droxidopa are not less than 10,000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of droxidopa is not more than 2.0%.
(5) Residual solvent—Being specified separately.
Loss on drying <2.4> Not more than 0.1% (1 g, in vacuum, 60°C, 3 hours).
Residue on ignition <2.4> Not more than 0.2% (1 g).
Assay Weigh accurately about 0.3 g of Droxidopa, previously dried, dissolve in exactly 20 mL of 0.1 mol/L perchloric acid VS, add 50 mL of acetic acid (100), and titrate <2.5> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.
Each mL of 0.1 mol/L perchloric acid VS = 21.32 mg of C₉H₁₁NO₅
Containers and storage Containers—Well-closed containers.

Droxidopa Capsules
ドロキシドパカプセル

Droxidopa Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of droxidopa (C₉H₁₁NO₅: 213.19).
Method of preparation Prepare as directed under Capsules, with Droxidopa.
Identification (1) To an amount of the contents of Droxidopa Capsules, equivalent to 50 mg of Droxidopa according to the labeled amount, add 20 mL of diluted acetic acid (100) (1 in 500), shake for 10 minutes, and filter. To 1 mL of the filtrate add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to light brown.
(2) To an amount of the contents of Droxidopa Capsules, equivalent to 50 mg of Droxidopa according to the labeled amount, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, and to 2 mL of the subsequent filtrate add 0.1 mol/L hydrochloric acid TS to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 278 nm and 282 nm.
Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.
To the contents of 1 capsule of Droxidopa Capsules, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake well, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.5 mg of droxidopa (C₉H₁₁NO₅). Filter this solution, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of droxidopa for assay, previously dried in vacuum at 60°C for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 280 nm.

\[
\text{Amount (mg) of droxidopa (C₉H₁₁NO₅)} = \frac{M_S \times A_T}{A_S} \times \frac{1}{100}
\]
\[
M_S: \text{Amount (mg) of droxidopa for assay}
\]

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Droxidopa Capsules is not less than 70%.
Start the test with 1 capsule of Droxidopa Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 56 μg of droxidopa (C₉H₁₁NO₅) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of droxidopa for assay, previously dried in vacuum at 60°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 280 nm, and A_T and A_S, at 350 nm.

sules, equivalent to 20 mg of Droxidopa according to the labeled amount, add 20 mL of diluted acetic acid (100) (1 in 500), shake for 10 minutes, and filter. To 1 mL of the filtrate add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to light brown.
Dissolution rate (%) with respect to the labeled amount of droxidopa (C₉H₁₁NO₅)

\[ M_s = M_s \times \frac{A_{T1}}{A_S} \]

Mₕ: Amount (mg) of droxidopa for assay
C: Labeled amount (mg) of droxidopa (C₉H₁₁NO₅) in 1 capsule

**Assay**

Take out the contents of not less than 20 Droxidopa Capsules, weigh accurately the mass of the contents, and mix uniformly. Weigh accurately an amount equivalent to about 50 mg of droxidopa (C₉H₁₁NO₅), add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of droxidopa for assay, previously dried in vacuum at 60°C for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A₁ and Aₕ, at 280 nm.

\[ \text{Amount (mg) of droxidopa (C₉H₁₁NO₅)} = M_s \times \frac{A_{T1}}{A_S} \]

Mₕ: Amount (mg) of droxidopa for assay

**Containers and storage**

Containers—Tight containers.

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**Droxidopa Fine Granules**

Droxidopa Fine Granules contain not less than 93.0% and not more than 107.0% of droxidopa (C₉H₁₁NO₅: 213.19).

**Method of preparation**

Prepare as directed under Granules, with Droxidopa.

**Identification**

(1) To a quantity of powdered Droxidopa Fine Granules, equivalent to 50 mg of Droxidopa according to the labeled amount, add 50 mL of water, shake for 10 minutes, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, heat in a water bath for 3 minutes: a blue-purple color develops.

(2) To a quantity of powdered Droxidopa Fine Granules, equivalent to 20 mg of Droxidopa according to the labeled amount, add 20 mL of dilute acetic acid (100) (1 in 500), shake for 10 minutes, and filter. To 1 mL of the filtrate add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to light brown.

(3) To a quantity of powdered Droxidopa Fine Granules, equivalent to 50 mg of Droxidopa according to the labeled amount, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make 100 mL, and filter. Discard the first 10 mL of the filtrate, to 2 mL of the subsequent filtrate add 0.1 mol/L hydrochloric acid TS to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 278 nm and 282 nm.

**Dissolution<6.10>**

When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Droxidopa Fine Granules is not less than 70%.

Start the test with an accurately weighed amount of Droxidopa Fine Granules, equivalent to about 0.1 g of droxidopa (C₉H₁₁NO₅), according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of water, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of droxidopa for assay, previously dried in vacuum at 60°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A₁ and Aₕ, at 280 nm, and A₁ and Aₕ, at 350 nm.

\[ \text{Amount (mg) of droxidopa (C₉H₁₁NO₅)} = M_s \times \frac{A_{T1}}{A_S} \]

Mₕ: Amount (mg) of droxidopa for assay
Mₚ: Amount (g) of Droxidopa Fine Granules
C: Labeled amount (mg) of droxidopa (C₉H₁₁NO₅) in 1 g

**Particle size <6.03>**

It meets the requirements of Fine granules.

**Assay**

Powder not less than 20 g of Droxidopa Fine Granules. Weigh accurately a portion of the powder, equivalent to about 50 mg of droxidopa (C₉H₁₁NO₅), add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of droxidopa for assay, previously dried in vacuum at 60°C for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A₁ and Aₕ, at 280 nm.

\[ \text{Amount (mg) of droxidopa (C₉H₁₁NO₅)} = M_s \times \frac{A_{T1}}{A_S} \]

Mₕ: Amount (mg) of droxidopa for assay

**Containers and storage**

Containers—Tight containers.
Dydrogesterone

Dydrogesterone occurs as white to light yellowish white crystals or crystalline powder. It is odorless.

**Description**  
Dydrogesterone is freely soluble in chloroform, soluble in acetonitrile, sparingly soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

**Identification (1)**  
To 5 mg of Dydrogesterone add 5 mL of 4-methoxybenzaldehyde-acetic acid TS and 2 to 3 drops of sulfuric acid, and heat in a water bath for 2 minutes: an orange-red color develops.

(2) Determine the absorption spectrum of a solution of Dydrogesterone in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave lengths.

(3) Determine the infrared absorption spectrum of Dydrogesterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation (2.49)**  
[a]_D^20: -470° to -500° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

**Melting point (2.60)**  
167° to 171°C

**Purity (1)**  
Heavy metals (1.07)—Proceed with 1.0 g of Dydrogesterone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 10 mg of Dydrogesterone in 200 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of peaks other than the peak of dydrogesterone from the sample solution is not larger than the peak area of dydrogesterone from the standard solution.

**Operating conditions—**  
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Dydrogesterone Tablets

Dydrogesterone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of dydrogesterone (C_{21}H_{28}O_{2}: 312.45).

**Method of preparation**  
Prepare as directed under Tablets, with Dydrogesterone.

**Identification (1)**  
To a quantity of powdered Dydrogesterone Tablets, equivalent to 0.05 g of Dydrogesterone according to the labeled amount, add 50 mL of methanol, shake well, and filter. Evaporate 5 mL of the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Dydrogesterone.

(2) To 1 mL of the filtrate obtained in (1) add methanol to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits a maximum between 284 nm and 288 nm.

**Uniformity of dosage units (6.02)**  
Perform the test accord-
ing to the following method: it meets the requirement of the Content uniformity test.

Crush 1 tablet of Dydrogesterone Tablets, and add methanol to make exactly 100 mL. Shake until the tablet is completely disintegrated, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 20 mL of the filtrate, pipet the subsequent V mL of the filtrate, add methanol to make exactly V mL so that each mL contains about 5 μg of dydrogesterone (C21H28O2), and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of dydrogesterone (C}_2\text{H}_{28}\text{O}_2) = M_S \times A_T / A_S \times V'/V \times 1/20
\]

\[
M_S: \text{Amount (mg) of dydrogesterone for assay}
\]

**Dissolution** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Dydrogesterone Tablets is not less than 80%.

Start the test with 1 tablet of Dydrogesterone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 56 μg of dydrogesterone (C21H28O2) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of dydrogesterone for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), of the sample solution and standard solution at 296 nm as directed under Ultraviolet-visible Spectrophotometry.<2.24>, using water as the control.

\[
\text{Dissolution rate} = M_S \times A_T / A_S \times V'/V \times 1/C \times 9
\]

\[
M_S: \text{Amount (mg) of dydrogesterone for assay}
\]

\[
C: \text{Labeled amount (mg) of dydrogesterone (C}_2\text{H}_{28}\text{O}_2) \text{ in 1 tablet}
\]

**Assay** Weigh accurately and powder not less than 20 Dydrogesterone Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of dydrogesterone (C21H28O2), shake with 50 mL of methanol, and add methanol to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of dydrogesterone for assay, previously dried in vacuum for 24 hours using phosphorus (V) oxide as a desiccant, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL, and use the solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), of the sample solution and standard solution at 286 nm as directed under Ultraviolet-visible Spectrophotometry.<2.24>

\[
\text{Amount (mg) of dydrogesterone (C}_2\text{H}_{28}\text{O}_2) = M_S \times A_T / A_S
\]

\[
M_S: \text{Amount (mg) of dydrogesterone for assay}
\]

**Containers and storage** Containers—Tight containers.

**Ebastine**

エバスチン

\[
\begin{align*}
\text{C}_2\text{H}_{28}\text{NO}_2: & \quad 469.66 \\
1-\{1,1-\text{Dimethylethyl}\}\{\text{phenyl}\} & \quad 4-\{\text{diphenylmethoxy}\}\{\text{piperidin-1-yl}\}\{\text{butan-1-one}\} \\
& \quad [90729-43-4]
\end{align*}
\]

Ebastine, when dried, contains not less than 99.0% and not more than 101.0% of C21H28NO2.

**Description** Ebastine occurs as white crystals or crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

It gradually becomes yellowish white on exposure to light.

**Identification** (1) Dissolve 20 mg of Ebastine in 5 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of sodium hydroxide TS, and allow to stand: the color of the solution is purple to red-purple, which gradually changes to blained.

(2) Determine the absorption spectrum of a solution of Ebastine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry.<2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Ebastine as directed in the potassium bromide disk method under Infrared Spectrophotometry.<2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** 84 – 87°C

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Ebastine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm). A platinum crucible may be used.

(2) Related substances—Dissolve 0.10 g of Ebastine in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10μL each of the sample solution and standard solution as directed under Liquid Chromatography.<2.01>
according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than ebastine obtained from the sample solution is not larger than the peak area of ebastine from the standard solution, and the total area of the peaks other than ebastine from the sample solution is not larger than 4 times the peak area of ebastine from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 5), and add water to make 1000 mL. To 375 mL of this solution add 625 mL of acetonitrile for liquid chromatography, and dissolve 0.72 g of sodium lauryl sulfate in this solution.
Flow rate: Adjust the flow rate so that the retention time of ebastine is about 9 minutes.
Time span of measurement: About twice as long as the retention time of ebastine, beginning after the solvent peak.
System suitability—
Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ebastine obtained with 10 μL of this solution is equivalent to 35 to 65% of that with 10 μL of the standard solution.
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ebastine are not less than 6000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ebastine is not more than 2.0%.

Residual on drying <2.42> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 2 hours).
Residue on ignition <2.44> Not more than 0.1% (1 g).
Assay Weigh accurately about 0.5 g of Ebastine, previously dried, dissolve in 60 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.
Each mL of 0.1 mol/L perchloric acid VS = 46.97 mg of C32H39NO2

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Ebastine Orally Disintegrating Tablets

Ebastine Orally Disintegrating Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of ebastine (C32H39NO2: 469.66).

Method of preparation Prepare as directed under Tablets, with Ebastine.

Identification Pulverize Ebastine Orally Disintegrating Tablets. To a portion of the powder, equivalent to 30 mg of Ebastine according to the labeled amount, add 100 mL of methanol, shake for 10 minutes, then add methanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 251 nm and 255 nm.

Purity Related substances—Pulverize Ebastine Orally Disintegrating Tablets. To a portion of the powder, equivalent to 50 mg of Ebastine according to the labeled amount, add 30 mL of methanol for liquid chromatography, shake for 10 minutes, and add the mobile phase to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ebastine obtained from the sample solution is not larger than 75% of that with 10 μL of the standard solution.

Operating conditions—
Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Time span of measurement: About 3 times as long as the retention time of ebastine, beginning after the solvent peak.
System suitability—
Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of ebastine obtained with 10 μL of this solution is equivalent to 15 to 25% of that with 10 μL of the standard solution.
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ebastine are not less than 6000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ebastine is not more than 2.0%.
area of ebastine is not more than 2.0%.

**Uniformity of dosage units**<sup>6.02</sup> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ebastine Orally Disintegrating Tablets add 10 mL of 0.1 mol/L hydrochloric acid TS, and disperse the particles with the aid of ultrasonic waves with occasional shaking. Add 3 mL of methanol, shake for 10 minutes, then add methanol to make exactly 50 mL so that each mL contains about 0.1 mg of ebastine (C\textsubscript{32}H\textsubscript{39}NO\textsubscript{2}), and centrifuge. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of ebastine (C\textsubscript{32}H\textsubscript{39}NO\textsubscript{2})

\[ M_s = M_x \times \frac{Q_s}{Q_x} \times \frac{V}{500} \]

\( M_s \): Amount (mg) of ebastine for assay

**Internal standard solution**—A solution of diphenyl in the mobile phase (1 in 40,000).

**Disintegration** Being specified separately.

**Dissolution**<sup>6.10</sup> When the test is performed at 50 revolutions per minute according to the paddle method, using 900 mL of the 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Ebastine Orally Disintegrating Tablets is not less than 80%.

Start the test with 1 tablet of Ebastine Orally Disintegrating Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the dissolution medium to make exactly 50 mL so that each mL contains about 5.6 μg of ebastine (C\textsubscript{32}H\textsubscript{39}NO\textsubscript{2}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of ebastine for assay, previously dried at 60°C under reduced pressure with phosphorous (V) oxide for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_1 \) and \( A_2 \), of the sample solution and standard solution at 258 nm as directed under Liquid Chromatography<sup>2.24</sup>, using the dissolution medium as the blank.

Dissolution rate (% with respect to the labeled amount of ebastine (C\textsubscript{32}H\textsubscript{39}NO\textsubscript{2})

\[ D = M_x \times \frac{A_1}{A_2} \times \frac{V}{V} \times \frac{1}{C} \times 18 \]

\( D \): Dissolution rate (%)

\( M_x \): Amount (mg) of ebastine for assay

\( C \): Labeled amount (mg) of ebastine (C\textsubscript{32}H\textsubscript{39}NO\textsubscript{2}) in 1 tablet

**Assay** Weigh accurately the mass of not less than 20 Ebastine Orally Disintegrating Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of ebastine (C\textsubscript{32}H\textsubscript{39}NO\textsubscript{2}), add 20 mL of 0.1 mol/L hydrochloric acid TS, and disperse the particles with the aid of ultrasonic waves. Add 120 mL of methanol, shake for 10 minutes, then add methanol to make exactly 200 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of ebastine for assay, previously dried at 60°C under reduced pressure with phosphorous (V) oxide for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of 0.1 mol/L hydrochloric acid TS, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography<sup>2.01</sup> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of ebastine to that of the internal standard.

Amount (mg) of ebastine (C\textsubscript{32}H\textsubscript{39}NO\textsubscript{2})

\[ M_s = M_x \times \frac{Q_1}{Q_2} \times \frac{2}{5} \]

\( M_s \): Amount (mg) of ebastine for assay

**Internal standard solution**—A solution of diphenyl in the mobile phase (1 in 40,000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 5), and add water to make 1000 mL. To 375 mL of this solution add 625 mL of acetonitrile for liquid chromatography, and dissolve 0.72 g of sodium lauryl sulfate in this solution.

Flow rate: Adjust the flow rate so that the retention time of ebastine is about 9 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ebastine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ebastine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Ebastine Tablets**

**エバスチン錠**

Ebastine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of ebastine (C\textsubscript{32}H\textsubscript{39}NO\textsubscript{2}: 469.66).

**Method of preparation** Prepare as directed under Tablets, with Ebastine.

**Identification** Powder Ebastine Tablets. To a portion of the powder, equivalent to 30 mg of Ebastine according to the
labeled amount, add 70 mL of methanol, shake for 10 minutes, then add methanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, it exhibits a maximum between 251 nm and 255 nm.

**Purity** Related substances—Powder Ebastine Tablets. To a portion of the powder, equivalent to 50 mg of Ebastine according to the labeled amount, add 30 mL of methanol for liquid chromatography, shake for 10 minutes, and add the mobile phase to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ebastine obtained from the sample solution is not larger than the peak area of ebastine from the standard solution, and the total area of the peaks other than ebastine from the sample solution is not larger than 2 times the peak area of ebastine from the standard solution.

**Operating conditions**—
Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Time span of measurement: About 3 times as long as the retention time of ebastine, beginning after the solvent peak.

**System suitability**—
Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of ebastine obtained with 10 μL of this solution is equivalent to 15 to 25% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ebastine are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ebastine is not more than 2.0%.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ebastine Tablets add V/10 mL of 0.1 mol/L hydrochloric acid TS, and disperse the particles with the aid of ultrasonic waves with occasional shaking. Add 3V/5 mL of methanol, shake for 10 minutes, then add methanol to make exactly V mL so that each mL contains about 0.1 mg of ebastine (C32H39NO2), and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
M_S = \frac{M \times Q_T}{Q_S} \times \frac{V}{500}
\]

\[
M_S: \text{Amount (mg) of ebastine for assay}
\]

**Internal standard solution**—A solution of diphenyl in the mobile phase (1 in 40,000).

**Dissolution** <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Ebastine Tablets is not less than 75%.

Start the test with 1 tablet of Ebastine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 5.6 μg of ebastine (C32H39NO2) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of ebastine for assay, previously dried at 60°C under reduced pressure with phosphorous (V) oxide for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A and A9, of the sample solution and standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the blank.

\[
\text{Dissolution rate (\%): } \frac{A - A9}{A} \times 100
\]

\[
M_S: \text{Amount (mg) of ebastine for assay}
\]

**Assay** Weigh accurately the mass of not less than 20 Ebastine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of ebastine (C32H39NO2), add 20 mL of 0.1 mol/L hydrochloric acid TS, and disperse the particles with the aid of ultrasonic waves. Add 120 mL of methanol, shake for 10 minutes, add methanol to make exactly 200 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of ebastine for assay, previously dried at 60°C under reduced pressure with phosphorous (V) oxide for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of 0.1 mol/L hydrochloric acid TS, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of ebastine to that of the internal standard.

\[
\text{Amount (mg) of ebastine (C32H39NO2)} = \frac{M_S \times Q_T}{Q_S} \times \frac{V}{500}
\]

\[
M_S: \text{Amount (mg) of ebastine for assay}
\]
Internal standard solution—A solution of diphenyl in the mobile phase (1 in 40,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 5), and add water to make 1000 mL. To 375 mL of this solution add 625 mL of acetonitrile for liquid chromatography, and dissolve 0.72 g of sodium lauryl sulfate in this solution.
Flow rate: Adjust the flow rate so that the retention time of ebastine is about 9 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ebastine are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ebastine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ecabet Sodium Hydrate

エカベトナトリウム水和物

C₂₁H₂₇NaO₅S·5H₂O: 492.56
(1R,4aS,10aS)-1,4a-Dimethyl-7-(1-methylethyl)-6-sodiosulfonato-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid pentahydrate [219773-47-4]

Ecabet Sodium Hydrate contains not less than 98.5% and not more than 101.5% of ecabet sodium (C₂₁H₂₇NaO₅S: 402.48), calculated on the anhydrous basis.

Description Ecabet Sodium Hydrate is white crystals.
It is freely soluble in methanol, and slightly soluble in water and in ethanol (99.5).
It dissolves in sodium hydroxide TS.
Dissolve 1.0 g of Ecabet Sodium Hydrate in 200 mL of water: the pH of the solution is about 3.5.

Identification (1) Determine the absorption spectrum of a solution of Ecabet Sodium Hydrate in dilute sodium hydroxide TS (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ecabet Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Place 1 g of Ecabet Sodium Hydrate in a porcelain crucible, and carbonize. After cooling, add 0.5 mL of nitric acid, heat gradually to incinerate, and dissolve the residue in 10 mL of water: the solution responds to the Qualitative Tests <1.09> for sodium salt.

Optical rotation (2) <2.49> [α]D: +69°–+76° (0.25 g calculated on the anhydrous basis, methanol, 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Ecabet Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 10 mg of Ecabet Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than ecabet obtained from the sample solution is not larger than the peak area of ecabet from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 225 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To 0.1 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust the pH to 3.0. To 730 mL of this solution add 270 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of ecabet is about 8 minutes.
Time span of measurement: About 2 times as long as the retention time of ecabet, beginning after the solvent peak.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ecabet are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ecabet is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water <2.48> 17.3 – 19.2% (0.2 g, volumetric titration, direct titration).
Assay Weigh accurately about 1.2 g of Ecabet Sodium Hydrate, dissolve in 30 mL of methanol, add 30 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 4 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 40.25 mg of C$_{20}$H$_{27}$NaO$_5$S

Containers and storage Containers—Well-closed containers.

**Ecabet Sodium Granules**

Ecabet Sodium Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of ecabet sodium hydrate (C$_{20}$H$_{27}$NaO$_5$S.5H$_2$O: 492.56).

**Method of preparation** Prepare as directed under Granules, with Ecabet Sodium Hydrate.

**Identification** To a quantity of Ecabet Sodium Granules, equivalent to 50 mg of Ecabet Sodium Hydrate according to the labeled amount, add 25 mL of dilute sodium hydroxide TS, shake, and filter. Discard the first 10 mL of the filtrate, and to 3 mL of the subsequent filtrate add dilute sodium hydroxide TS to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 269 nm and 273 nm, and between 278 nm and 282 nm.

**Uniformity of dosage units**<6.02> Perform the test according to the following method: Ecabet Sodium Granules in single-unit containers meet the requirement of the Content uniformity test.

Take out the total amount of the content of 1 container of Ecabet Sodium Granules, add 70 mL of dilute sodium hydroxide TS, treat with ultrasonic waves for 5 minutes with occasional shaking, add dilute sodium hydroxide TS to make exactly V mL so that each mL contains about 10 mg of ecabet sodium hydrate (C$_{20}$H$_{27}$NaO$_5$S.5H$_2$O), and filter. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of ecabet sodium hydrate for assay (separately, determine the water <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 4 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 40.25 mg of C$_{20}$H$_{27}$NaO$_5$S

**Dissolution**<6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Ecabet Sodium Granules is not less than 80%.

Start the test with an accurately weighed amount of Ecabet Sodium Granules, equivalent to about 1 g (potency) of Ecabet Sodium Hydrate according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of ecabet sodium hydrate for assay (separately, determine the water <2.48> in the same manner as Ecabet Sodium Hydrate), dissolve in 1 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A$_T$ and A$_S$, at 271 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of ecabet sodium hydrate (C$_{20}$H$_{27}$NaO$_5$S.5H$_2$O) = M$_S$/M$_T$ × (A$_T$ - A$_S$) × 1/C × 4500 × 1.224

M$_S$: Amount (mg) of ecabet sodium hydrate for assay, calculated on the anhydrous basis

M$_T$: Amount (g) of Ecabet Sodium Granules

C: Labeled amount (mg) of ecabet sodium hydrate (C$_{20}$H$_{27}$NaO$_5$S.5H$_2$O) in 1 g

**Assay** Weigh accurately an amount of Ecabet Sodium Granules, equivalent to about 30 mg of ecabet sodium hydrate (C$_{20}$H$_{27}$NaO$_5$S.5H$_2$O), add exactly 5 mL of the internal standard solution, add 25 mL of diluted methanol (1 in 2), shake vigorously for 20 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, to 3 mL of the subsequent filtrate add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of ecabet sodium hydrate for assay (separately, determine the water <2.48> in the same manner as Ecabet Sodium Hydrate), dissolve in 5 mL of the internal standard solution, add 5 mL of the internal standard solution, and dissolve in dilute methanol (1 in 2) to make 30 mL. To 3 mL of this solution add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q$_T$ and Q$_S$, of the peak area of ecabet to that of the internal standard.

Amount (mg) of ecabet sodium hydrate (C$_{20}$H$_{27}$NaO$_5$S.5H$_2$O) = M$_S$ × Q$_T$/Q$_S$ × 1.224

M$_S$: Amount (mg) of ecabet sodium hydrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (1 in 2) (3 in 400).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diame-
Ecothiopate Iodide

エコチオパートヨウ化物

\[
\text{C}_9\text{H}_{23}\text{INO}_3\text{PS} : 383.23
\]

2-(Diethoxyphosphorylsulfanyl)-N,N,N-trimethylethylaminium iodide

[513-10-0]

Ecothiopate Iodide contains not less than 95.0% of C₉H₂₃INO₃PS, calculated on the dried basis.

**Description** Ecothiopate Iodide occurs as white crystals or crystalline powder.

It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

**Identification (1)** Dissolve 0.1 g of Ecothiopate Iodide in 2 mL of water, and add 1 mL of nitric acid: a brown precipitate is formed. To 1 drop of the turbid solution containing this precipitate add 1 mL of hexane, and shake: a light red color develops in the hexane layer.

(2) Heat the suspension of the precipitate obtained in (1) until it becomes colorless, cool, add 10 mL of water, and use this solution as the sample solution. Two mL of the sample solution responds to the Qualitative Tests \(<1.09>\) (2) for phosphate.

(3) Two mL of the sample solution obtained in (2) responds to the Qualitative Tests \(<1.09>\) for sulfate.

**pH** \(<2.54>\) Dissolve 0.1 g of Ecothiopate Iodide in 40 mL of water: the pH of this solution is between 3.0 and 5.0.

**Melting point** \(<2.60>\) 116 - 122°C

**Purity (1)** Clarity and color of solution—Dissolve 0.5 g of Ecothiopate Iodide in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals \(<1.07>\)—To 1.0 g of Ecothiopate Iodide in a Kjeldahl flask add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat. Repeat this procedure twice, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution becomes colorless, and white fumes are evolved. After cooling, transfer the solution together with a small quantity of water to a Nessler tube, and add water to make about 20 mL. Adjust the solution with ammonia solution (28) and ammonia TS to a pH between 3.0 and 3.5, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: proceed in the same manner as the preparation of the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Ecothiopate Iodide in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03>\). Spot 10 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.41>\) Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 50°C, 3 hours).

**Assay** Weigh accurately about 0.125 g of Ecothiopate Iodide, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add 30 mL of water, then add exactly 10 mL of phosphate buffer solution, pH 12, stopper the container, and allow to stand at 25 ± 3°C for 20 minutes. To this solution add quickly 2 mL of acetic acid (100), and titrate \(<2.50>\) with 0.002 mol/L iodine VS (potentiometric titration). Perform the test in the same manner without phosphate buffer solution, pH 12, and make any necessary correction.

Each mL of 0.002 mol/L iodine VS = 1.533 mg of C₉H₂₃INO₃PS

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, and not exceeding 0°C.

"JP XVI"
Edrophonium Chloride

エドロホニウム塩化物

\[
\text{C}_{10}\text{H}_{16}\text{ClNO} : 201.69
\]
\[\text{N-Ethyl-3-hydroxy-N,N-dimethylanilinium chloride}\]

[116-38-1]

**Edrophonium Chloride**

Edrophonium Chloride, when dried, contains not less than 98.0% of \(\text{C}_{10}\text{H}_{16}\text{ClNO}\).

**Description** Edrophonium Chloride occurs as white crystals or crystalline powder. It is odorless. It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

It is hygroscopic.

It is gradually colored by light.

**Identification**

1. To 5 mL of a solution of Edrophonium Chloride (1 in 100) add 1 drop of iron (III) chloride TS: a light red-purple color develops.

2. Determine the absorption spectrum of a solution of Edrophonium Chloride in 0.1 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Edrophonium Chloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

3. A solution of Edrophonium Chloride (1 in 50) responds to the Qualitative Tests <1.00> for chloride.

**pH** <2.54> Dissolve 1.0 g of Edrophonium Chloride in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

**Melting point** >2.60° 166 – 171°C (with decomposition).

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Edrophonium Chloride in 10 mL of water: the solution is clear and colorless.

2. Heavy metals <1.07>—Proceed with 1.0 g of Edrophonium Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

3. Arsenic <1.11>—Prepare the test solution with 1.0 g of Edrophonium Chloride according to Method 1, and perform the test (not more than 2 ppm).

4. Related substances—Dissolve 0.50 g of Edrophonium Chloride in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 100 mL. Pipet 3 mL of this solution, add ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonia solution (28) (16:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.20% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Edrophonium Chloride, previously dried, and dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 20.17 mg of \(\text{C}_{10}\text{H}_{16}\text{ClNO}\)

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant.

**Edrophonium Chloride Injection**

エドロホニウム塩化物注射液

Edrophonium Chloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of edrophonium chloride (\(\text{C}_{10}\text{H}_{16}\text{ClNO}\): 201.69).

**Method of preparation** Prepare as directed under Injections, with Edrophonium Chloride.

**Description** Edrophonium Chloride Injection is a clear and colorless liquid.

**Identification**

1. To a volume of Edrophonium Chloride Injection, equivalent to 0.04 g of Edrophonium Chloride, add 1 drop of iron (III) chloride TS: a light red-purple color develops.

2. Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet–visible Spectrophotometry <2.24>: it exhibits a maximum between 272 nm and 276 nm.

**pH** <2.54> 6.5 – 8.0

**Bacterial endotoxins** <4.01> Less than 15 EU/mg.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Conduct this procedure without exposure to daylight, using light-resistant containers. Measure exactly a volume of Edrophonium Chloride Injection, equivalent to about 50 mg of edrophonium chloride (\(\text{C}_{10}\text{H}_{16}\text{ClNO}\), place
in a chromatographic column prepared by pouring 10 mL of weakly basic DEAE-bridged dextran anion exchanger (Cl type) (50 to 150 \( \mu \)m in particle diameter) into a chromatographic tube about 2 cm in inside diameter and about 10 cm in length, add 25 mL of water, and elute at the flow rate of 1 to 2 mL per minute. Wash the column with two 25-mL portions of water at the flow rate of 1 to 2 mL per minute. Combine the washings with above effluent solutions, and add water to make exactly 100 mL. Measure exactly 10 mL of this solution, and add 10 mL of phosphate buffer solution, pH 8.0, and 5 g of sodium chloride. Wash this solution with four 20-mL portions of a mixture of diethyl ether and hexane (1:1), collect the water layer, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Edrophonium Chloride RS, previously dried to constant weight under Ultraviolet-visible Spectrophotometry \(<2.24\). Amount (mg) of edrophonium chloride (C\(_{10}\)H\(_{16}\)ClNO) = \( M_S \times \frac{A_T}{A_S} \),
\( M_S \): Amount (mg) of Edrophonium Chloride RS

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

**Elcatonin**

エルカトニン

![Elcatonin](Image 58x316 to 283x367)

Elcatonin contains not less than 5000 Elcatonin Units and not more than 7000 Elcatonin Units per 1 mg of peptide, calculated on the dehydrated and deacetic acid basis.

**Description** Elcatonin is a white powder.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

The pH of its solution (1 in 500) is between 4.5 and 7.0.

**Identification** Dissolve 5 mg of Elcatonin in 5 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Constituent amino acids** Put about 1 mg of Elcatonin in a test tube for hydrolysis, add phenol-hydrochloric acid TS to dissolve, replace the air inside with Nitrogen, seal the tube under reduced pressure, and heat at 110 ± 2°C for 24 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, dissolve the residue in 1 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh exactly 1.33 mg of L-aspartic acid, 1.19 mg of L-threonine, 1.05 mg of L-serine, 1.47 mg of L-glutamic acid, 1.15 mg of L-proline, 0.75 mg of glycine, 0.89 mg of L-alamine, 1.17 mg of L-valine, 1.89 mg of L-2-aminosubaric acid, 1.31 mg of L-leucine, 1.81 mg of L-tyrosine, 1.83 mg of L-lysine hydrochloride, 2.10 mg of L-histidine hydrochloride monohydrate and 2.11 mg of L-arginine hydrochloride, dissolve them in 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu \)L of each the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions: 14 peaks of amino acids appear on the chromatogram obtained from the sample solution, and their respective molar ratios against alanine are 1.7 – 2.2 for aspartic acid, 3.5 – 4.2 for threonine, 2.4 – 3.0 for serine, 2.7 – 3.2 for glutamic acid, 1.7 – 2.2 for for proline, 2.7 – 3.2 for glycine, 1.6 – 2.2 for valine, 0.8 – 1.2 for 2-aminosubaric acid, 4.5 – 5.2 for leucine, 0.7 – 1.2 for tyrosine, 1.7 – 2.2 for lysine, 0.8 – 1.2 for histidine and 0.7 – 1.2 for arginine.

**Operating conditions**

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated styrene-divinylbenzene copolymer (3 \( \mu \)m in particle diameter).

Column temperature: Varied between 50°C and 65°C.

Chemical reaction vessel temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Buffer solutions A, B, C and D, with sodium ion concentrations of 0.10 mol/L, 0.135 mol/L, 1.26 mol/L and 0.20 mol/L, respectively. The ion concentration of the mobile phase is changed stepwise from 0.10 mol/L to 1.26 mol/L by using these buffer solutions.

<table>
<thead>
<tr>
<th>Components of buffer solutions</th>
<th>Buffer solution:</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid monohydrate</td>
<td>8.85 g</td>
<td>7.72 g</td>
<td>6.10 g</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Trisodium citrate dihydrate</td>
<td>3.87 g</td>
<td>10.05 g</td>
<td>26.67 g</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>—</td>
<td>—</td>
<td>2.50 g</td>
<td>8.00 g</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.54 g</td>
<td>1.87 g</td>
<td>54.35 g</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Ethanol (95)</td>
<td>60.0 mL</td>
<td>—</td>
<td>—</td>
<td>60.0 mL</td>
<td></td>
</tr>
<tr>
<td>Thioglycol</td>
<td>5.0 mL</td>
<td>5.0 mL</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Purified water</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td></td>
</tr>
<tr>
<td>Total amount</td>
<td>1000 mL</td>
<td>1000 mL</td>
<td>1000 mL</td>
<td>1000 mL</td>
<td></td>
</tr>
</tbody>
</table>

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-
propanol, add water to make 2000 mL, stir for about 20 minutes while passing Nitrogen, and use this solution as solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for about 20 minutes while passing Nitrogen, and use this solution as solution B. Mix solution A and solution B before use.

Flow rate of mobile phase: Adjust the flow rate so that the retention time of arginine is about 75 minutes.

Flow rate of reaction reagent: About 0.2 mL per minute.

Selection of column: Proceed with 10 µL of the standard solution under the above operating conditions. Use a column from which aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, 2-aminosuberic acid, leucine, tyrosine, lysine, histidine and arginine are eluted in this order, with complete separation of each peak.

**Purity (1)** Acetic acid—Weigh accurately 3 – 6 mg of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, add exactly 1 mL of the internal standard solution to dissolve it, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of acetic acid (100), and add the internal standard solution to make exactly 100 mL. Pipet 5 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_t and Q_s, of the peak area of acetic acid to that of the internal standard: the amount of acetic acid is not more than 7.0%.

\[
\text{Amount} \,(\%) \, \text{of acetic acid} \,(\text{CH}_2\text{COOH}) = \frac{M_{st}}{M_{sa}} \times \frac{Q_t}{Q_s} \times 50
\]

*M_st:* Amount (g) of acetic acid (100)

*M_sa:* Amount (mg) of sample

**Internal standard solution—** A solution of citric acid monohydrate (1 in 4000).

**Operating conditions—**

Detecting instrument: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 13.2 g of diammonium hydrogen phosphate in 900 mL of water, add phosphoric acid to adjust the pH to 2.5, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 4 minutes.

Selection of column: Proceed with 20 µL of the standard solution under the above operating conditions. Use a column from which acetic acid and citric acid are eluted in this order with the resolution between their peaks being not less than 2.0.

(2) Related substances—Dissolve 1.0 mg of Elcatonin in 1 mL of a mixture of trifluoroacetic acid TS and acetonitrile (2:1), and use this solution as the sample solution. Take exactly 0.3 mL of the sample solution, add a mixture of trifluoroacetic acid TS and acetonitrile (2:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than the peak of elcatonin from the sample solution is not larger than 1/3 times the peak area of elcatonin from the standard solution, and the total of the peak areas other than the peak of elcatonin is not larger than the peak area of elcatonin from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of trifluoroacetic acid TS and acetonitrile (change the ratio linearly from 85:15 to 55:45 in 30 minutes).

Flow rate: Adjust the flow rate so that the retention time of elcatonin is about 25 minutes.

Selection of column: Dissolve 2 mg of Elcatonin in 200 µL of trypsin TS for test of elcatonin, warm at 37°C for 1 hour, then add 1 drop of acetic acid (100), and heat at 95°C for 1 minute. To 10 µL of this solution add 50 µL of the sample solution, and mix. Proceed with 10 µL of this solution under the above operating conditions, and calculate the resolution. Use a column such that the resolution between the peak of elcatonin and the peak which appears immediately before the peak of elcatonin is not less than 2.0, and the retention time of elcatonin is about 25 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of elcatonin from 10 µL of the standard solution is between 50 mm and 200 mm.

Time span of measurement: Continue measurement until the regularly changing base-line of the chromatogram disappears, beginning after the solvent peak.

**Water** <2.48> Weigh accurately 1 – 3 mg of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, and perform the test as directed in 2. Coulometric titration: not more than 8.0%.

**Nitrogen content** Weigh accurately 0.015 – 0.02 g of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, and perform the test as directed under Nitrogen Determination <1.08>: it contains not less than 16.1% and not more than 18.7% of nitrogen (N: 14.01) in the peptide, calculated on the dehydrated and de-acetic acid basis.

**Assay (i)** Animals: Select healthy male Sprague-Dawley rats each weighing between 90 g and 110 g. Keep the rats for not less than 3 days before use, providing an appropriate uniform diet and water.

(ii) Diluent for elcatonin: Dissolve 2.72 g of sodium acetate in water to make 200 mL, add 0.2 g of bovine serum albumin, and adjust the pH to 6.0 with acetic acid (100). Prepare before use.

(iii) Standard solution: Dissolve Elcatonin RS in the diluent for elcatonin to make two standard solutions, one to contain exactly 0.075 Unit in each mL which is designated as
the high-dose standard solution, $S_H$, and the other to contain exactly 0.0375 Unit in each mL which is designated as the low-dose standard solution, $S_L$.

(iv) Sample solution: Weigh accurately 0.5 - 2.0 mg of Elcatonin quickly under conditions of $25 \pm 2^\circ C$ and 50 - 5% relative humidity, and dissolve in the diluent for elcatonin to make two sample solutions, $T_H$, which contains the Units per mL equivalent to $S_H$ and the low-dose sample solution, $T_L$, which contains the Units per mL equivalent to $S_L$.

(v) Deproteinizing solution for elcatonin: Dissolve 160 g of trichloroacetic acid and 30.6 g of strontium chloride in water to make 3600 mL.

(vi) Procedure: Divide the animals into 4 equal groups of not less than 10 animals each. Withhold all food, but not water, for 18 to 24 hours before the injections, and withhold water during the assay until the final blood sample is taken. Handle the animals with care in order to avoid undue excitement.

Inject exactly 0.2 mL of each of the standard solutions and the sample solutions into the tail vein of each animal as indicated in the following design:

- First group $S_H$  
- Second group $S_L$  
- Third group $T_H$  
- Fourth group $T_L$

At 1 hour after the injection, take a sufficient blood sample to perform the test from the carotid artery and vein of each animal under ether anesthesia, centrifuge the blood samples to separate serum, and determine the serum calcium according to the following (vii).

(vii) Serum calcium determination: Take exactly 0.3 mL of the serum, add the deproteinizing solution for elcatonin to make exactly 3 mL, mix well, centrifuge, and use the supernatant liquid as the sample solution for calcium determination. Separately, pipet 1 mL of Standard Calcium Solution for Atomic Absorption Spectrophotometry $<2.23>$, and add a solution of sodium chloride (17 in 2000) to make exactly 10 mL. Pipet 5 mL of this solution, add the deproteinizing solution for elcatonin to make exactly 50 mL, and use this solution as the standard solution for calcium determination. Determine the absorbances, $A_1$ and $A_2$, of the sample solution and the standard solution as directed under Atomic Absorption Spectrophotometry $<2.23>$ according to the following conditions. Determine the absorbance, $A_0$, of a solution obtained in the same manner used for preparation of the standard solution, but with 1 mL of water instead of the standard solution.

Amount (mg) of calcium (Ca) in 100 mL of the serum

\[ M = 0.01 \times (A_1 - A_0)/(A_S - A_0) \times 10 \times 100 \]

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: Calcium hollow-cathode lamp.

Wavelength: 422.7 nm.

(viii) Calculation: Amounts of calcium in 100 mL of the serum obtained with $S_H$, $S_L$, $T_H$ and $T_L$ in (vii) are symbolized as $y_1$, $y_2$, $y_3$, and $y_4$, respectively. Sum up individual $y_1$, $y_2$, $y_3$, and $y_4$ to obtain $Y_1$, $Y_2$, $Y_3$, and $Y_4$, respectively.

Units per mg of peptide, calculated on the dehydrated and de-acetic acid basis

\[ M = 0.3010 \times Y_5/Y_6 \]

\[ Y_5 = -Y_1 - Y_2 + Y_3 + Y_4 \]

\[ Y_6 = Y_1 - Y_2 + Y_3 - Y_4 \]

\[ a: \text{Amount (mg) of the sample} \]

\[ \times [100 - \text{water content (\%)} + \text{acetic acid content (\%)}]/100 \]

\[ b: \text{Total volume (mL) of the high-dose sample solution prepared by dissolving the sample with diluent for elcatonin} \]

$F'$ computed by the following equation should be smaller than $F$ shown in the table against $n$ with which $s^2$ is calculated. Calculate $L (P = 0.95)$ by use of the following equation: $L$ should be not more than 0.20. If $F'$ exceeds $F$, or if $L$ exceeds 0.20, repeat the test, increasing the number of animals or arranging the assay conditions so that $F'$ is not more than $F$ and $L$ is not more than 0.20.

\[ F' = (- Y_1 + Y_2 + Y_3 - Y_4)/4fs^2 \]

\[ n = 4 (f - 1) \]

\[ L = 2f(C - 1)(CM^2 + 0.09062) \]

\[ C = Y^2/(Y^2 - 4fs^2t^2) \]

$t^2$: Value shown in the following table against $n$ used to calculate $s^2$.

<table>
<thead>
<tr>
<th>$n$</th>
<th>$t^2$</th>
<th>$n$</th>
<th>$t^2$</th>
<th>$n$</th>
<th>$t^2$</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>161.45</td>
<td>13</td>
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<tr>
<td>2</td>
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<td>4.747</td>
<td>24</td>
<td>4.260</td>
<td>300</td>
<td>3.685</td>
</tr>
</tbody>
</table>

Containers and storage

Containers—Tight containers.

Storage—Not exceeding 8°C.
Emorfazone

エモルファゾン

C_{11}H_{17}N_{3}O_{3}: 239.27
4-Ethoxy-2-methyl-5-(morpholin-4-yl)pyridazin-3(2H)-one

Emorfazone, when dried, contains not less than 98.5% and not more than 101.0% of C_{11}H_{17}N_{3}O_{3}.

**Description**
Emorfazone occurs as colorless crystals or a white to light yellow crystalline powder.

It is very soluble in ethanol (99.5), and freely soluble in water and in acetic anhydride.

It dissolves in 1 mol/L hydrochloric acid TS.

It gradually turns yellow and decomposes on exposure to light.

**Identification**

(1) Dissolve 20 mg of Emorfazone in 2 mL of 1 mol/L hydrochloric acid TS, and add 5 drops of Reinecke’s TS: light red floating matters are formed.

(2) Determine the absorption spectrum of a solution of Emorfazone (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Emorfazone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.60> 89 – 92°C (after drying).

**Purity**

(1) Chloride <1.03> — Perform the test with 1.0 g of Emorfazone. Prepare the control solution with 0.50 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(2) Heavy metals <1.07> — Proceed with 2.0 g of Emorfazone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Arsenic** <1.11> — Prepare the test solution with 2.0 g of Emorfazone according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances — Conduct this procedure using light-resistant vessels. Dissolve 0.5 g of Emorfazone in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than emorfazone obtained from the sample solution is not larger than 1/10 times the peak area of emorfazone from the standard solution, and the total area of the peaks other than the peak of emorfazone is not larger than 1/2 times the peak area of emorfazone from the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (11:10).

Flow rate: Adjust the flow rate so that the retention time of emorfazone is about 5 minutes.

Time span of measurement: About 2.5 times as long as the retention time of emorfazone, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, add the mobile phase to make exactly 20 mL. Confirm that the peak area of emorfazone obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that with 20 μL of the standard solution.

System performance: Dissolve 16 mg of Emorfazone and 30 mg of 2,4-dinitrophenylhydrazine in 100 mL of methanol. When the procedure is run with 20 μL of this solution under the above operating conditions, emorfazone and 2,4-dinitrophenylhydrazine are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of emorfazone is not more than 1.0%.

**Loss on drying** <2.4I> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay**
Weigh accurately about 0.2 g of Emorfazone, previously dried, dissolve in 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 23.93 mg of C_{11}H_{17}N_{3}O_{3}

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

**Emorfazone Tablets**

Emorfazone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of emorfazone (C_{11}H_{17}N_{3}O_{3}: 239.27).

**Method of preparation** Prepare as directed under Tablets, with Emorfazone.

**Identification** To a quantity of powdered Emorfazone Tablets, equivalent to 0.1 g of Emorfazone according to the labeled amount, add 100 mL of water, shake well, and cen-
trifuge. Filter the supernatant liquid, and to 1 mL of the filtrate add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry. It exhibits maxima between 237 nm and 241 nm, and between 310 nm and 314 nm, and a shoulder between 288 nm and 298 nm.

**Uniformity of dosage units**

Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Emorfazone Tablets add methanol to make exactly 50 mL so that each mL contains about 4 mg of emorfazone (C11H17N3O3), and shake well to disintegrate. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of emorfazone (C}_{11}\text{H}_{17}\text{N}_{3}\text{O}_{3}) = M_S \times \frac{Q_t}{Q_s} \times V/5
\]

\[
M_S: \text{Amount (mg) of emorfazone for assay}
\]

**Internal standard solution** — A solution of 2,4-dinitrophenyl-hydrazine in methanol (3 in 2000). Prepare before use.

**Dissolution**

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Emorfazone Tablets is not less than 80%.

Start the test with 1 tablet of Emorfazone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 50 mL so that each mL contains about 11 μg of emorfazone (C11H17N3O3) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of emorfazone for assay, previously dried in vacuum conditions, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_t\) and \(A_o\), of the sample solution and standard solution at 239 nm as directed under Ultraviolet-visible Spectrophotometry.

\[
\text{Dissolution rate (cm)}^{2}\text{cm}^{-1}\text{min}^{-1}\]

\[
= M_S \times \frac{A_t}{A_o} \times \frac{V'}{V} \times \frac{1}{C} \times 36
\]

\[
M_S: \text{Amount (mg) of emorfazone for assay}
\]

**Assay**

To 10 tablets of Emorfazone Tablets add 200 mL of methanol, shake well to disintegrate, add methanol to make exactly 250 mL, and centrifuge. Pipet a volume of the supernatant liquid, equivalent to about 8 mg of emorfazone (C11H17N3O3), add exactly 10 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of emorfazone for assay, previously dried in vacuum conditions, and dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, \(Q_t\) and \(Q_s\), of the peak area of emorfazone to that of the internal standard.

\[
\text{Amount (mg) of emorfazone (C}_{11}\text{H}_{17}\text{N}_{3}\text{O}_{3}) = M_S \times \frac{Q_t}{Q_s} \times 2/5
\]

\[
M_S: \text{Amount (mg) of emorfazone for assay}
\]

**Internal standard solution** — A solution of 2,4-dinitrophenyl-hydrazine in methanol (3 in 2000). Prepare before use.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 313 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (11:10).

Flow rate: Adjust the flow rate so that the retention time of emorfazone is about 5 minutes.

**System suitability**

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, emorfazone and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of emorfazone to that of the internal standard is not more than 1.0%.

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant.

**Enalapril Maleate**

エナラプリルマレイン酸塩

\[
\text{C}_{20}\text{H}_{28}\text{N}_{2}\text{O}_{5}\cdot\text{C}_{4}\text{H}_{4}\text{O}_{4}: \quad 492.52
\]

\[
(2S)-1-\{(255)-2-\{(1S)-1\text{-Ethoxycarbonyl-3-phenylpropylamino\}propanoyl\}pyrrolidine-2-carboxylic acid monomaleate
\]

[76959-16-4]

Enalapril Maleate, when dried, contains not less than 98.0% and not more than 102.0% of C20H28N2O5·C4H4O4.

**Description**

Enalapril Maleate occurs as white crystals or a white crystalline powder.

It is freely soluble in methanol, sparingly soluble in water and in ethanol (99.5), and slightly soluble in acetonitrile.

Melting point: about 145°C (with decomposition).
Identification (1) Determine the infrared absorption spectra of Enalapril Maleate as directed in the potassium bromide disc method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Enalapril Maleate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 20 mg of Enalapril Maleate add 5 mL of 1 mol/L hydrochloric acid TS, shake, add 5 mL of diethyl ether, and shake for 5 minutes. Take 3 mL of the upper layer, distil off the diethyl ether on a water bath, add 5 mL of water to the residue with shaking, and add 1 drop of potassium permanaganate TS: the red color of the test solution immediately disappears.

Optical rotation <2.49> \(\left[\alpha\right]_D^20 = -41.0 - 43.5^\circ\) (after drying, 0.25 g, methanol, 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Enalapril Maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 30 mg of Enalapril Maleate in 100 mL of a mixture of sodium dihydrogen phosphate TS, pH 2.5 and acetonitrile (19:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of sodium dihydrogen phosphate TS, pH 2.5 and acetonitrile (19:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 \(\mu\)L of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than maleic acid and enalapril obtained from the sample solution is not larger than twice the peak area of enalapril from the standard solution. Furthermore, the total area of the peaks other than maleic acid and enalapril is not larger than twice the peak area of enalapril from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phases, mobile phase flow, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of enalapril, beginning after the peak of maleic acid.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of sodium dihydrogen phosphate TS, pH 2.5 and acetonitrile (19:1) to make exactly 10 mL. Confirm that the peak area of enalapril obtained from 50 \(\mu\)L of this solution is equivalent to 7 to 13% of that from 50 \(\mu\)L of the standard solution.

System performance: When the procedure is run with 50 \(\mu\)L of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of enalapril are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 \(\mu\)L of the standard solution under the above conditions, the relative standard deviation of the peak area of enalapril is not more than 2.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, 60°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Enalapril Maleate and Enalapril Maleate RS, both previously dried, and dissolve in a mixture of sodium dihydrogen phosphate TS, pH 2.5 and acetonitrile (19:1) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 50 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of enalapril, \(A_T\) and \(A_S\), of both solutions.

\[
\text{Amount (mg) of } C_9H_{13}N_2O_5 \cdot C_4H_4O_4 = M_S \times A_T / A_S
\]

Operating conditions—


Column: A stainless steel column 4.1 mm in inside diameter and 15 cm in length, packed with porous styrene-divinylbenzene copolymer for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 70°C.

Mobile phase A: Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 6.8 with a solution of sodium hydroxide (1 in 4), and add water to make 1000 mL. To 950 mL of this solution, add 50 mL of acetonitrile for liquid chromatography.

Mobile phase B: Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 6.8 with a solution of sodium hydroxide (1 in 4), and add water to make 1000 mL. To 340 mL of this solution, add 660 mL of acetonitrile for liquid chromatography.

Mobile phase flow: Control the concentration gradient by changing the ratio of the mobile phases A and B as follows.

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>Mobile phase A (%vol)</th>
<th>Mobile phase B (%vol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>0 – 20</td>
<td>95 (\rightarrow) 40</td>
<td>5 (\rightarrow) 60</td>
</tr>
<tr>
<td>20 – 25</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>

Flow rate: 1.4 mL per minute.

System suitability—

System performance: When the procedure is run with 50 \(\mu\)L of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of enalapril are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 \(\mu\)L of the standard solution under the above conditions, the relative standard deviation of the peak area of enalapril is not more than 1.0%.

Containers and storage Containers—Well-closed containers.
Enalapril Maleate Tablets

エナラプリルメレート酸塩錠

Enalapril Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of enalapril maleate (C$_{20}$H$_{28}$N$_{2}$O$_{5}$·C$_{4}$H$_{4}$O$_{4}$: 492.52).

Method of preparation Prepare as directed under Tablets, with Enalapril Maleate.

Identification To a quantity of powdered Enalapril Maleate Tablets equivalent to 50 mg of Enalapril Maleate according to the labeled amount, add 20 mL of methanol, shake, centrifuge, and then use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of enalapril maleate in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-Layer Chromatography.<2.01> , Spot 20 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of water, acetone, 1-butanol, acetic acid (100) and toluene (1:1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the Rf values of the 2 spots obtained from the sample solution and the 2 spots obtained from the standard solution are equivalent.

Purity Enalaprilat and enalapril diketopiperazine—Use the sample solution obtained in the Assay as the standard solution. Pipet 1 mL of the sample solution, add sodium dihydrogen phosphate TS, pH 2.2 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 µL each of the sample solution and standard solution as directed under Liquid Chromatography.<2.07> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the peak area of enalaprilat, having the relative retention time of about 0.5 with respect to enalapril obtained from the sample solution, is not larger than 2 times the peak area of enalapril from the standard solution. Also, the peak area of enalapril diketopiperazine, having the relative retention time of about 1.5 is not larger than the peak area of enalapril from the standard solution.

Operating conditions—
Proceed as directed in the operating conditions in the Assay.

System suitability—
Test for required detectability: Pipet 1 mL of the standard solution, and add sodium dihydrogen phosphate TS, pH 2.2 to make exactly 10 mL. Confirm that the peak area of enalapril obtained from 50 µL of this solution is equivalent to 7 to 13% of that from 50 µL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 50 µL of the standard solution under the above conditions, the relative standard deviation of the peak area of enalapril is not more than 2.0%.

Uniformity of dosage units.<6.02> Perform the test according to the following method: it meets the requirement of the

Content uniformity test.
Take 1 tablet of Enalapril Maleate Tablets, add V/2 mL of sodium dihydrogen phosphate TS, pH 2.2, treat with ultrasonic waves for 15 minutes, shake for 30 minutes, and add sodium dihydrogen phosphate TS, pH 2.2 to make exactly V mL so that 1 mL of the solution contains about 0.1 mg of enalapril maleate (C$_{20}$H$_{28}$N$_{2}$O$_{5}$·C$_{4}$H$_{4}$O$_{4}$). Treat this solution with ultrasonic waves for 15 minutes, filter through a membrane filter with a pore size not exceeding 0.45 µm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of enalapril maleate (C$_{20}$H$_{28}$N$_{2}$O$_{5}$·C$_{4}$H$_{4}$O$_{4}$) = M$_{5}$ × A$_{1}$/A$_{5}$ × V/200

M$_{5}$: Amount (mg) of Enalapril Maleate RS

Dissolution.<6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates are 15 minutes of a 2.5- and 5-mg tablet and in 30 minutes of a 10-mg tablet are not less than 85%, respectively.

Start the test with 1 tablet of Enalapril Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 2.8 µg of enalapril maleate (C$_{20}$H$_{28}$N$_{2}$O$_{5}$·C$_{4}$H$_{4}$O$_{4}$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 14 mg of Enalapril Maleate RS, previously dried in vacuum at 60°C for 2 hours, and dissolve in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 µL each of the sample solution and standard solution as directed under Liquid Chromatography.<2.07> according to the following conditions, and determine the enalapril peak areas, A$_{1}$ and A$_{2}$, of both solutions.

Dissolution rate (%) with respect to the labeled amount of enalapril maleate (C$_{20}$H$_{28}$N$_{2}$O$_{5}$·C$_{4}$H$_{4}$O$_{4}$) $= M_{5} × A_{1}/A_{5} × V/V × 1/C × 18$

M$_{5}$: Amount (mg) of Enalapril Maleate RS

C: Labeled amount (mg) of enalapril maleate (C$_{20}$H$_{28}$N$_{2}$O$_{5}$·C$_{4}$H$_{4}$O$_{4}$) in 1 tablet

Operating conditions—
Detector, column, column temperature, and flow rate: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 1.88 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 2.2 with phosphoric acid, and add water to make 1000 mL. To 750 mL of this solution add 250 mL of acetonitrile.

System suitability—
System performance: When the procedure is run with 50 µL of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of enalapril are not less than 300 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 µL of the standard solution under the above conditions, the relative standard deviation of the peak area of enalapril is not more than 2.0%.
**Enalapril Maleate**

**Assay**  Weigh accurately not less than 20 Enalapril Maleate Tablets, and powder. Weigh accurately a portion of the powder equivalent to about 10 mg of enalapril maleate ((C₂₀H₂₈N₂O₅.C₄H₄O₄), add 50 mL of sodium dihydrogen phosphate TS, pH 2.2, treat with ultrasonic waves for 15 minutes, shake for 30 minutes, and then add sodium dihydrogen phosphate TS, pH 2.2 to make exactly 100 mL. Treat this solution with ultrasonic waves for 15 minutes, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of Enalapril Maleate RS, previously dried in vacuum at 60°C for 2 hours, dissolve in sodium dihydrogen phosphate TS, pH 2.2 to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the enalapril peak areas, A₁ and A₅, of both solutions.

\[
M₅ = M₅ \times A₁/A₅ \times 1/2
\]

- **Operating conditions—**
  - Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
  - Column temperature: A constant temperature of about 50°C.
  - Mobile phase: A mixture of sodium dihydrogen phosphate TS, pH 2.2 and acetonitrile (3:1).
  - Flow rate: Adjust the flow rate so that the retention time of enalapril is about 5 minutes.
  - **System suitability—**
    - System performance: Heat to fusion about 20 mg of enalapril maleate. After cooling, add 50 mL of acetonitrile, and treat with ultrasonic waves to dissolve. To 1 mL of this solution, add the standard solution to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 50 μL of the solution for system suitability test under the above conditions, enalapril and enalapril diketopiperazine, which has a relative retention time of 1.5 to enalapril, are eluted in this order with the resolution between these peaks being not less than 2.0.
    - System repeatability: When the test is repeated 6 times with 50 μL of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of enalapril is not more than 1.0%.

**Containers and storage**  Containers—Well-closed containers.

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**Enflurane**

エンフルラン

C₇H₅ClF₂O: 184.49
(2RS)-2-Chloro-1-(difluoromethoxy)-1,1,2-trifluoroethane [13838-16-9]

**Description**  Enflurane is a clear, colorless liquid. It is slightly soluble in water. It is miscible with ethanol (95) and with diethyl ether. It is a volatile, and not an inflammable. It shows no optical rotation.

**Boiling point**  54 – 57°C

**Identification (1)**  Take 50 μL of Enflurane, and prepare the test solution as directed to the Oxygen Flask Combustion Method <1.06> using 40 mL of water as the absorbing liquid. The test solution responds to the Qualitative Tests <1.09> for chloride and fluoride.

(2)  Determine the infrared absorption spectrum of Enflurane as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Refractive index**  <2.45>  \(n^20\): 1.302 – 1.304

**Specific gravity**  <2.56>  d₂₀: 1.520 – 1.540

**Purity (1)**  Acidity or alkalinity—To 60 mL of Enflurane add 60 mL of freshly boiled and cooled water, shake for 3 minutes, separate the water later, and use the layer as the sample solution. To 20 mL of the sample solution add one drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is purple. To 20 mL of the sample solution add one drop of bromocresol purple TS and 0.06 mL of 0.01 mol/L hydrochloric acid VS: the color of the solution is yellow.

(2)  Chloride <1.09>—To 20 g of Enflurane add 20 mL of water, shake well, and separate the water layer. Take 10 mL of the water layer add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.001%).

(3)  Related substances—Proceed the test with 5 μL of Enflurane as directed under Gas chromatography <2.02> according to the following conditions. Determine each peak area other than the peak of air which appears soon after injection of the sample by the automatic integration method, and calculate the amount of each peak by the area percentage method: the amount of the substances other than enflurane is not more than 0.10%.

**Operating conditions—**

- Detector: A thermal conductivity detector.
- Column: A column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μm in particle diameter, coated with diethylene glycol succinate ester for gas chromatography in the ratio of 20%.
- Column temperature: A constant temperature of about 180 to 250°C.
Enoxacin Hydrate

**JP XVI**

80°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of enfurane is about 3 minutes.

Time span of measurement: About 3 times as long as the retention time of enfurane.

**System suitability**—

Test for required detection: To exactly 1 mL of enfurane add 2-propanol to make exactly 100 mL. To exactly 2 mL of this solution add 2-propanol to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add 2-propanol to make exactly 10 mL. Confirm that the peak area of enfurane obtained from 5 μL of this solution is equivalent to 7 to 13% of that from 5 μL of the solution for system suitability test.

System performance: Mix 5 mL of Enflurane and 5 mL of 2-propanol. When the procedure is run with 5 μL of this mixture under the above operating conditions, enfurane and 2-propanol are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of enfurane is about 3 minutes.

**2.24** Not more than 0.10% (10 g, volumetric titration, direct titration).

**Containers and storage**—Tight containers.

**Storage**—Not exceeding 30°C.

**Enoxacin Hydrate**

エノキサシン水和物

![Chemical Structure](image)

C₁₅H₁₇FN₄O₃ · 1½H₂O: 347.34

1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid sesquihydrate [84294-96-2]

Enoxacin Hydrate, when dried, contains not less than 98.5% of enoxacin (C₁₅H₁₇FN₄O₃: 320.32)

**Description**—Enoxacin Hydrate occurs as white to pale yellow-brown crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol, very slightly soluble in chloroform, and practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS.

It is gradually colored by light.

**Identification**—(1) Place 0.02 g of Enoxacin Hydrate and 0.05 g of sodium in a test tube, and heat gradually to ignition with precaution. After cooling, add 0.5 mL of methanol and then 5 mL of water, and heat to boiling. To this solution add 2 mL of dilute acetic acid, and filter: the filtrate responds to the Qualitative Tests <1.099 (2) for fluoride.

(2) Dissolve 0.05 g of Enoxacin Hydrate in dilute sodium hydroxide TS to make 100 mL. To 1 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Enoxacin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point**—225 – 229°C (after drying).

**Purity**—(1) Sulfate <1.14>—Dissolve 1.0 g of Enoxacin Hydrate in 50 mL of dilute sodium hydroxide TS, shake with 10 mL of dilute hydrochloric acid, and centrifuge. Filter the supernatant liquid, and to 30 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 25 mL of dilute sodium hydroxide TS, 5 mL of dilute hydrochloric acid TS and water to make 50 mL (not more than 0.048%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Enoxacin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Enoxacin Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Enoxacin Hydrate in 25 mL of a mixture of chloroform and methanol (7:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (7:3) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**—<2.41> 7.0 – 9.0% (1 g, 105°C, 3 hours).

**Residue on ignition**—<2.44> Not more than 0.1% (1 g, platinum crucible).

**Assay**—Weigh accurately about 0.3 g of Enoxacin Hydrate, previously dried, dissolve in 30 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.03 mg of C₁₅H₁₇FN₄O₃

**Containers and storage**—Tight containers.
Enviomycin Sulfate

エンビオマイシン硫酸塩

Tuberactinomycin N Sulfate

(C$_{25}$H$_{43}$N$_{13}$O$_{10}$)$_{2.3}$H$_2$SO$_4$: 1665.62

Tuberactinomycin O Sulfate

(C$_{25}$H$_{43}$N$_{13}$O$_{10}$)$_{2.3}$H$_2$SO$_4$: 1633.62

Tuberactinomycin N Sulfate

(3R,4R)-N-[(3S,9S,12S,15S)-9,12-Bis(hydroxymethyl)-3-[(4R)-2-iminohexahydropyrimidin-4-yl]-2,5,8,11,14-pentaaoxo-6(Z)-ureidomethylene-1,4,7,10,13-pentaazacyclohexadec-15-yl]-3,6-diamino-4-hydroxyhexanamide sesquisulfate

[33103-22-9, Tuberactinomycin N]

Tuberactinomycin O Sulfate

(3S)-N-[(3S,9S,12S,15S)-9,12-Bis(hydroxymethyl)-3-[(4R)-2-iminohexahydropyrimidin-4-yl]-2,5,8,11,14-pentaaoxo-6(Z)-ureidomethylene-1,4,7,10,13-pentaazacyclohexadec-15-yl]-3,6-diaminohexanamide sesquisulfate

[33137-73-4, Tuberactinomycin O]

Enviomycin Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of Streptomyces griseoverticilla var. tuberacticus.

It contains not less than 770 μg (potency) per mg, calculated on the dried basis. The potency of Enviomycin Sulfate is expressed as mass (potency) of tuberactinomycin N (C$_{25}$H$_{43}$N$_{13}$O$_{10}$) 685.69.

Description

Enviomycin Sulfate occurs as a white powder. It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification

(1) To 5 mL of a solution of Enviomycin Sulfate (1 in 200) add 1.5 mL of sodium hydroxide TS, and add 1 drop of a mixture of 0.01 mol/L citric acid TS and copper (II) sulfate TS (97:3) : a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Enviomycin Sulfate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) To 2 mL of a solution of Enviomycin Sulfate (1 in 20) add 1 drop of barium chloride TS: a white precipitate is produced.

Optical rotation

$\langle 2.4 \rangle$ $[\alpha]_D^{20} -16 \sim -22^\circ$ (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH

$\langle 2.5 \rangle$ The pH of a solution obtained by dissolving 2.0 g of Enviomycin Sulfate in 20 mL of water is between 5.5 and 7.5.

Content ratio of the active principle

Dissolve 0.1 g of Enviomycin Sulfate in water to make 100 mL, and use this solution as the sample solution. Perform the test with 3 μL of the sample solution as directed under Liquid Chromatography, according to the following conditions, and determine the peak areas, $A_{T1}$ and $A_{T2}$, of tuberactinomycin N and tuberactinomycin O, having the relative retention time, 1.4 ± 0.4, with respect to tuberactinomycin N, by the automatic integration method: $A_{T2}/(A_{T1} + A_{T2})$ is between 0.090 and 0.150.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of ammonium acetate TS, 1,4-dioxane, tetrahydrofuran, water and ammonia solution (28:100:75:50:23:2).

Flow rate: Adjust the flow rate so that the retention time of tuberactinomycin N is about 9 minutes.

System suitability

System performance: When the procedure is run with 3 μL of the sample solution under the above operating conditions, tuberactinomycin N and tuberactinomycin O are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 3 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of tuberactinomycin N is not more than 2.0%.

Purity

(1) Clarity and color of solution—Dissolve 1.0 g of Enviomycin Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals $\langle 1.0 \rangle$—Proceed with 2.0 g of Enviomycin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic $\langle 1.1 \rangle$—Prepare the test solution with 2.0 g of Enviomycin Sulfate according to Method 1, and perform the test (not more than 1 ppm).

Loss on drying $\langle 2.4 \rangle$ Not more than 4.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Assay

Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics, according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633

Purity (1) Heavy metals $<1.07$—Proceed with 1.0 g of Eperisone Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Piperidine hydrochloride—Dissolve 1.0 g of Eperisone Hydrochloride in 20 mL of water, add 2.0 mL of diluted hydrochloric acid (1 in 2), 2.0 mL of a solution of copper (II) sulfate pentahydrate (1 in 1000) add 18 mL of water, 2.0 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 1.5 mL of ammonia solution (28), and use this solution as the sample solution. Separately, to 2.0 mL of a solution of piperidine hydrochloride (1 in 1000) add 18 mL of water, 2.0 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 1.5 mL of ammonia solution (28), and use this solution as the standard solution. To each of the sample solution and standard solution add 10 mL of a mixture of isopropylether and carbon disulfide (3:1), shake for 30 seconds, allow them to stand for 2 minutes, and compare the color of the upper layer: the color obtained from the sample solution is not more darker than that from the standard solution.

(3) Related substances—Dissolve 0.1 g of Eperisone Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography (2.24), according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of eperisone from the sample solution is not larger than 1/5 times the peak area of eperisone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeccylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of methanol, 0.0375 mol/L sodium 1-decanesulfonate TS and perchloric acid (600:400:1).

Flow rate: Adjust the flow rate so that the retention time of eperisone is about 17 minutes.

Time span of measurement: About 2 times as long as the retention time of eperisone.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of eperisone obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of eperisone are not less than 4000 steps and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of eperisone is not more than 3.0%.

Water (2.48) Not more than 0.2% (0.1 g, coulometric
Ephedrine Hydrochloride / Official Monographs

**Residue on ignition**<sup>2.44</sup> Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.6 g of Ephedrine Hydrochloride, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 25.95 mg of C₁₀H₁₅NO.HCl

**Containers and storage** Containers—Well-closed containers.

**Ephedrine Hydrochloride**

エフェドリン塩酸塩

C₁₀H₁₅NO.HCl: 201.69

(1R,2S)-2-Methylamino-1-phenylpropan-1-ol monohydrochloride [50-98-6]

Ephedrine Hydrochloride, when dried, contains not less than 99.0% of C₁₀H₁₅NO.HCl.

**Description** Ephedrine Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, soluble in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in acetonitrile and in acetic anhydride.

**Identification (1)** Determine the absorption spectrum of a solution of Ephedrine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ephedrine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ephedrine Hydrochloride (1 in 15) responds to the Qualitative Tests <1.09> for chloride.

**Optical rotation**<sup>2.49</sup> [α]₂₀° = 33.0 – 36.0° (after drying, 1 g, water, 20 mL, 100 mm).

**pH**<sup>2.54</sup> Dissolve 1.0 g of Ephedrine Hydrochloride in 20 mL of water: the pH of this solution is between 4.5 and 6.5.

**Melting point**<sup>2.60</sup> 218 – 222°C

**Purity (1)** Clarity and color of solution—Dissolve 0.5 g of Ephedrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate—Dissolve 0.05 g of Ephedrine Hydrochloride in 40 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS, and allow to stand for 10 minutes: no turbidity is produced.

(3) Heavy metals<sup>1.07</sup>—Proceed with 1.0 g of Ephedrine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.05 g of Ephedrine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the areas of each peak by the automatic integration method: the total area of the peaks other than ephedrine from the sample solution is not larger than the peak area of ephedrine from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of a solution of sodium lauryl sulfate (1 in 128), acetonitrile and phosphoric acid (640:360:1).

Flow rate: Adjust the flow rate so that the retention time of ephedrine is about 14 minutes.

Time span of measurement: About 3 times as long as the retention time of ephedrine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of ephedrine obtained from 10 µL of this solution is equivalent to 4 to 6% of that from 10 µL of the standard solution.

System performance: Dissolve 1 mg of ephedrine hydrochloride for assay and 4 mg of atropine sulfate in 100 mL of diluted methanol (1 in 2). When the procedure is run with 10 µL of this solution under the above operating conditions, ephedrine and atropine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ephedrine is not more than 2.0%.

**Loss on drying**<sup>2.41</sup> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition**<sup>2.44</sup> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Ephedrine Hydrochloride, previously dried, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming. Cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 20.17 mg of C₁₀H₁₅NO.HCl
Containers and storage  Containers—Well-closed containers.

Ephedrine Hydrochloride Injection

Ephedrine Hydrochloride Injection is aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of ephedrine hydrochloride (C₁₀H₁₅NO.HCl: 201.69).

Method of preparation  Prepare as directed under Injections, with Ephedrine Hydrochloride.

Description  Ephedrine Hydrochloride Injection is a clear, colorless liquid. pH: 4.5 – 6.5

Identification  To a volume of Ephedrine Hydrochloride Injection, equivalent to 0.05 g of Ephedrine Hydrochloride according to the labeled amount, add water to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.242>: it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Bacterial endotoxins <4.01>  Less than 7.5 EU/mg.

Extractable volume <6.05>  It meets the requirement.

Foreign insoluble matter <6.06>  Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07>  It meets the requirement.

Sterility <4.06>  Perform the test according to the Membrane filtration method: it meets the requirement.

Assay  To an exact volume of Ephedrine Hydrochloride Injection, equivalent to 40 mg of ephedrine hydrochloride (C₁₀H₁₅NO.HCl), add exactly 10 mL of the internal standard solution and water to make 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution to dissolve, add water to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₅, of the peak area of ephedrine to that of the internal standard of each solution.

Amount (mg) of ephedrine hydrochloride (C₁₀H₁₅NO.HCl) = M₅ × Q₁/Q₅

M₅: Amount (mg) of ephedrine hydrochloride for assay

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

10% Ephedrine Hydrochloride Powder

Ephedrine Hydrochloride Powder

Ephedrine Hydrochloride Powder contains not less than 9.3% and not more than 10.7% of ephedrine hydrochloride (C₁₀H₁₅NO.HCl: 201.69).

Method of preparation  Prepare as directed under Granules or Powders, with the above ingredients.

Identification  To 0.5 g of 10% Ephedrine Hydrochloride Powder add 100 mL of water, shake for 20 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.242>: it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Assay  Weigh accurately about 0.4 g of 10% Ephedrine Hydrochloride Powder, add 150 mL of water, and extract with the aid of ultrasonicator for 10 minutes with occasional shaking. Shake more for 10 minutes, then add exactly 10 mL of the internal standard solution and water to make 200 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 40 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution to dissolve, add water to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₅, of the peak area of ephedrine to that of the internal standard of each solution.

Amount (mg) of ephedrine hydrochloride (C₁₀H₁₅NO.HCl) = M₅ × Q₁/Q₅

M₅: Amount (mg) of ephedrine hydrochloride for assay

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Hermetic containers. Storage—Light-resistant.
Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Perform as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Ephedrine Hydrochloride Tablets

エフェドリン塩酸塩錠

Ephedrine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of ephedrine hydrochloride (C₁₀H₁₅NO.HCl: 201.69).

Method of preparation Prepare as directed under Tablets, with Ephedrine Hydrochloride.

Identification To an amount of powdered Ephedrine Hydrochloride Tablets, equivalent to 0.05 g of Ephedrine Hydrochloride, add 100 mL of water, shake for 20 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Uniformity of dosage units (6.02) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ephedrine Hydrochloride Tablets add V mL of water so that each mL contains 0.25 mg of ephedrine hydrochloride (C₁₀H₁₅NO.HCl), then add exactly V/4 mL of the internal standard solution, disperse the tablet into small particles using ultrasonic waves, then stir for a further 10 minutes in the same way. Shake this solution for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution, disperse the tablet into small pieces using ultrasonic waves, then stir for a further 10 minutes in the same way. Shake this solution for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of ephedrine hydrochloride (C₁₀H₁₅NO.HCl) = Mₕ × Qₗ/Qₘ × V/100

Mₕ: Amount (mg) of ephedrine hydrochloride for assay

Internal standard solution—A solution of etilefrine hydrochloride (1 in 2000).

Dissolution (6.10) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Ephedrine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Ephedrine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, Aₗ and Aₘ, of ephedrine in each solution.

Dissolution rate (%) with respect to the labeled amount of ephedrine hydrochloride (C₁₀H₁₅NO.HCl)

\[ Mₕ \times Aₗ/ₘ \times 1/C \times 90 \]

Mₕ: Amount (mg) of ephedrine hydrochloride for assay

C: Labeled amount (mg) of ephedrine hydrochloride (C₁₀H₁₅NO.HCl) in 1 tablet

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ephedrine are not less than 10,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 2.0%.

Assay Weigh accurately not less than 20 tablets of Ephedrine Hydrochloride Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 40 mg of ephedrine hydrochloride (C₁₀H₁₅NO.HCl), add 150 mL of water, and extract with the aid of ultrasonicator for 10 minutes with occasional shaking. Shake more for 10 minutes, then add exactly 10 mL of the internal standard solution and water to make 200 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 40 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution to dissolve, add water to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, Qₗ and Qₘ, of the peak area of ephedrine to that of the internal standard of each solution.

Amount (mg) of ephedrine hydrochloride (C₁₀H₁₅NO.HCl) = Mₕ × Qₗ/Qₘ
Epirizole
Mepirizole

Epirizole occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in methanol and in acetic acid (100), freely soluble in ethanol (95), and sparingly soluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sulfuric acid.

The pH of a solution of Epirizole (1 in 100) is between 6.0 and 7.0.

Identification

1 To 0.1 g of Epirizole add 0.1 g of vanillin, 5 mL of water and 2 mL of sulfuric acid, and mix with shaking for a while: a yellow precipitate is formed.

2 Dissolve 0.1 g of Epirizole in 10 mL of water, and add 10 mL of 2,4,6-trinitrophenol TS: a yellow precipitate is produced. Collect the precipitate by filtration, wash with 50 mL of water, and dry at 105°C for 1 hour: it melts between 163°C and 169°C.

3 Determine the absorption spectrum of a solution of Epirizole in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point

Purity

Clarity and color of solution—Dissolve 0.20 g of Epirizole in 20 mL of water: the solution is clear and colorless.

Chloride

Add 0.5 g of Epirizole to a ground mixture of 0.7 g of potassium nitrate and 1.2 g of anhydrous sodium carbonate, mix well, transfer little by little to a platinum crucible, previously heated, and heat until the reaction is completed. After cooling, add 15 mL of dilute sulfuric acid and 5 mL of water to the residue, boil for 5 minutes, filter, wash the insoluble matter with 10 mL of water, and add 6 mL of dilute nitric acid and water to the combined filtrate and washings to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: proceed with the same quantities of the same reagents as directed for the preparation of the test solution, and add 0.25 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL (not more than 0.018%).

Heavy metals

Proceed with 2.0 g of Epirizole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Arsenic

Prepare the test solution with 1.0 g of Epirizole according to Method 3, and perform the test (not more than 2 ppm).

Related substances

Dissolve 1.0 g of Epirizole in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a mixture of isopropyl diethyl ether, ethanol (95) and water (23:10:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Place this plate in a chamber filled with iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Readily carbonizable substances

Perform the test with 0.10 g of Epirizole: the solution has no more color than Matching Fluid A.

Loss on drying

Not more than 0.5% (1 g, silica gel, 4 hours).

Residue on ignition

Not more than 0.1% (1 g).

Assay

Weigh accurately about 0.5 g of Epirizole, previously dried, dissolve in 40 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS) until the color of the solution changes from purple through blue-green to green.

Perform a blank determination, and make any necessary correction.
Each mL of 0.1 mol/L perchloric acid VS = 23.43 mg of C₁₁H₁₄N₄O₂

Containers and storage  Containers—Well-closed containers.

Epirubicin Hydrochloride  エピルビシン塩酸塩

C₂₇H₃₂N₄O₁₁·HCl: 579.98
(2S,4S)-4-(3-Amino-2,3,6-trideoxy-α-L-arabino-hexopyranosyloxy)-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione mono hydrochloride
[56590-09-1]

Epirubicin Hydrochloride is the hydrochloride of a derivative of daunorubicin.

It contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of the residual solvent. The potency of Epirubicin Hydrochloride is expressed as mass (potency) of epirubicin anhydrous basis and corrected by the amount of epirubicin hydrochloride (C₂₇H₃₂N₄O₁₁·HCl).

Description  Epirubicin Hydrochloride occurs as a pale yellowish red to brownish red powder.

It is soluble in water and in methanol, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

Identification (1)  Determine the absorption spectrum of a solution of Epirubicin Hydrochloride in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)  Determine the infrared absorption spectrum of Epirubicin Hydrochloride and Epirubicin Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation  <2.49>  [α]D²⁰  = +310° – +340° (10 mg calculated on the anhydrous basis and collected by the amount of the residual solvent, methanol, 20 mL, 100 mm).

pH  <2.54>  Dissolve 10 mg of Epirubicin Hydrochloride in 2 mL of water: the pH of the solution is between 4.0 and 5.5.

Purity (1)  Clarity and color of solution—Dissolve 50 mg of Epirubicin Hydrochloride in 5 mL of water: the solution is clear and dark red.

(2)  Heavy metals <1.07>—Proceed with 1.0 g of Epirubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3)  Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.07> according to the following conditions, determine each peak area by the automatic integration method, and calculate the sum amount of the peaks other than epirubicin and 2-naphthalenesulfonic acid by the area percentage method: not more than 5.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of epirubicin beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of epirubicin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

(4)  Residual solvents <2.46>—Weigh accurately about 0.3 g of Epirubicin Hydrochloride, add exactly 0.6 mL of the internal standard solution, add N,N-dimethylformamide to make 6 mL, and use this solution as the sample solution. Separately, pipet 1 mL of methanol, add N,N-dimethylformamide to make exactly 25 mL, and use this solution as methanol standard stock solution. Take exactly 125 μL of acetone, 30 μL of ethanol (99.5), 32 μL of 1-propanol and 17 μL of the methanol standard stock solution, add exactly 10 mL of the internal standard solution and N,N-dimethylformamide to make 100 mL, and use this solution as the sample solution. Perform the test with 1 μL of each of the sample solution and standard solution as directed under Gas Chromatography <2.07> according to the following conditions, and calculate the ratios of the peak areas of acetone, ethanol, 1-propanol and methanol to that of the internal standard, Q₁₀, Q₈₅, Q₁₇₈ and Q₂₈, Q₁₀ and Q₂₈, respectively. Calculate the amounts of acetone, ethanol, 1-propanol and methanol by the following equations: the amounts of acetone, ethanol, 1-propanol and methanol are not more than 1.5%, not more than 0.5%, not more than 0.5% and not more than 0.1%, respectively.

Amount (%) of acetone = 1/M₁ × Q₁₀/Q₈₅ × 593
Amount (%) of ethanol = 1/M₁ × Q₁₀/Q₂₈ × 142
Amount (%) of 1-propanol = 1/M₁ × Q₁₀/Q₈₅ × 154
Amount (%) of methanol = 1/M₁ × Q₁₀/Q₂₈ × 2.23
M₁: Amount (mg) of Epirubicin Hydrochloride

Internal standard solution—A solution of 1,4-dioxane in N,N-dimethylformamide (1 in 100).

Operating conditions—

Detector: Hydrogen flame-ionization detector.
Column: A fused silica column 0.53 mm in inside diameter
and 30 m in length, coated with polyethylene glycol for gas-chromatography 1 μm in thickness.

Column temperature: 40°C for 11 minutes after injection of the sample, then rise to 90°C at a rate of 10°C per minute. If necessary, rise to 130°C at a rate of 50°C per minute and maintain the temperature for 30 minutes.

Injection port temperature: A constant temperature of about 120°C.

Detector temperature: A constant temperature of about 150°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 8 minutes.

Split ratio: 1:15

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, the relative standard deviations of the ratios of the peak areas of acetone and the internal standard being not less than 30.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of acetone, methanol, ethanol and 1-propanol are not more than 4.0%, respectively.

Water <2.48> Not more than 8.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.5% (0.1 g).

Assay Weigh accurately an amount of Epirubicin Hydrochloride and Epirubicin Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in the internal standard chloride and Epirubicin Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in the internal standard.

\[ \text{Amount} = M_s \times \frac{Q_1}{Q_2} \times 1000 \]

where:
- \( M_s \): Amount [mg (potency)] of Epirubicin Hydrochloride RS
- \( Q_1 \): Amount [μg (potency)] of \( \text{C}_27\text{H}_29\text{NO}_{11}\text{HCl} \)
- \( Q_2 \): Amount [μg (potency)] of \( \text{C}_44\text{H}_{36}\text{O} \)


Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with trimethylsilanized silica gel for liquid chromatography (6 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2 g of sodium lauryl sulfate in a mixture of water, acetonitrile, methanol and phosphoric acid (540:290:170:1) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of epirubicin is about 9.5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and epirubicin are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of epirubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—At a temperature between 0°C and 5°C.

**Ergocalciferol**

**Calciferol**

**Vitamin D₂**

エルゴカルシフェロール

C₃₈H₄₆O: 396.65
(3S,5Z,7E,22E)-9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol

[50-14-6]

Ergocalciferol contains not less than 97.0% and not more than 103.0% of C₃₈H₄₆O.

Description Ergocalciferol occurs as white crystals. It is odorless, or has a faint, characteristic odor.

It is freely soluble in ethanol (95), in diethyl ether and in chloroform, sparingly soluble in isooctane, and practically insoluble in water.

It is affected by air and by light.

Melting point: 115 – 118°C Transfer Ergocalciferol to a capillary tube, and dry for 3 hours in a desiccator (in vacuum at a pressure not exceeding 2.67 kPa). Immediately fire-seal the capillary tube, put it in a bath fluid, previously heated to a temperature about 10°C below the expected melting point, and heat at a rate of rise of about 3°C per minute, and read the melting point.

Identification (1) Dissolve 0.5 mg of Ergocalciferol in 5 mL of chloroform, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake: a red color is produced, and rapidly changes through purple and blue to green.

(2) Determine the infrared absorption spectrum of Ergocalciferol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ergocalciferol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.
Ergometrine Maleate / Official Monographs

Absorbance
\[< 2.24 \times 10^{17} (265 \text{ nm}) \quad 445 - 485 \quad (10 \text{ mg}, \text{ ethanol (95), 100 mL}).\]

Optical rotation
\[< 2.49^\circ \quad [\alpha]_{D}^{20} + 102 - + 107^\circ (0.3 \text{ g}, \text{ ethanol (95), 20 mL, 100 nm}).\] Prepare the solution of Ergocalciferol within 30 minutes after the container has been opened, and determine the rotation within 30 minutes after the solution has been prepared.

Purity
Ergosterol—Dissolve 10 mg of Ergocalciferol in 2.0 mL of diluted ethanol (9 in 10), add a solution of 20 mg of digitonin in 2.0 mL of diluted ethanol (9 in 10), and allow the mixture to stand for 18 hours: no precipitate is formed.

Assay
Weigh accurately about 30 mg each of Ergocalciferol and Ergocalciferol RS, and dissolve each in isooctane to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 3 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 to 20 mg of digitonin in 2.0 mL of diluted ethanol (9 in 10), and allow the mixture to stand for 18 hours: no precipitate is formed.

Chromatography
Stationary phase—A silica gel for liquid chromatography (250 mm particle diameter).
Flow rate: Adjust the flow rate so that the retention time of Ergocalciferol is about 25 minutes.
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with a silica gel for liquid chromatography (10 μm particle diameter).
Mobile phase: A mixture of hexane and tert-amylalcohol (97:3).
Column temperature: A constant temperature of about 20°C.
Flow rate: Adjust the flow rate so that the retention time of ergocalciferol is about 25 minutes.
System suitability—
System performance: Dissolve 15 mg of Ergocalciferol RS in 25 mL of isooctane. Transfer this solution to a flask, heat in an oil bath under a reflux condenser for 2 hours, and cool immediately to room temperature. Transfer the solution to a quartz test tube, and irradiate with a short-wave lamp (main wavelength: 254 nm) and a long-wave lamp (main wavelength: 365 nm) for 3 hours. To 10 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the ratios of the retention time of previtamin D₂, trans-vitamin D₂ and tachysterol₂ to that of ergocalciferol are about 0.5, about 0.6 and about 1.1, respectively, and the resolution between previtamin D₂ and trans-vitamin D₂ is not less than 0.7, and that between ergocalciferol and tachysterol₂ is not less than 1.0.
System retestability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ergocalciferol to that of the internal standard is not more than 1.0%.

Containers and storage
Containers—Hermetic containers.
Storage—Light-resistant, under Nitrogen atmosphere, and in a cold place.

Ergometrine Maleate
エルゴメトリンマレイン酸塩

C₁₉H₂₃N₃O₂.C₄H₄O₄: 441.48
(85)-N-[[(1S)-2-Hydroxy-1-methylethyl]-6-methyl-9,10-dihydroergoline-8-carboxamide monomaleate

Ergometrine Maleate, when dried, contains not less than 98.0% of C₁₉H₂₃N₃O₂.C₄H₄O₄.

Description
Ergometrine Maleate occurs as a white to pale yellow, crystalline powder. It is odorless.
It is sparingly soluble in water, slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.
Melting point: About 185°C (with decomposition).
It gradually changes to yellow in color on exposure to light.

Identification
(1) Prepare a solution of Ergometrine Maleate (1 in 50); the solution shows a blue fluorescence.
(2) Dissolve 1 mg of Ergometrine Maleate in 5 mL of water. To 1 mL of this solution add 2 mL of 4-dimethylaminobenzaldehyde-ferric chloride TS, shake, and allow to stand for 5 to 10 minutes: a deep blue color develops.
(3) To 5 mL of a solution of Ergometrine Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the solution disappears immediately.

Optical rotation
\[< 2.49^\circ \quad [\alpha]_{D}^{20} + 102 - + 107^\circ (0.3 \text{ g}, \text{ ethanol (95), 20 mL, 100 mm}).\]

pH
\[< 2.54^\circ \quad \text{Dissolve 0.10 g of Ergometrine Maleate in 10 mL of water. The pH of the solution is between 3.0 and 5.0.}\]

Purity
(1) Clarity and color of solution—Dissolve 0.10 g of Ergometrine Maleate in 10 mL of water: the solution is clear and colorless to light yellow.
(2) Ergotamine and ergotoxine—To 0.02 g of Ergometrine Maleate add 2 mL of a solution of sodium hydroxide (1 in 10), and heat to boiling: the gas evolved does not change moistened red litmus paper to blue.
(3) Related substances—Dissolve 5.0 mg each of Ergometrine Maleate and Ergometrine Maleate RS in 1.0 mL of methanol, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography \[< 2.07^\circ \]. Spot 10 μL each of the sample solution and standard solution on a plate, prepared with silica gel for
thin-layer chromatography and dilute sodium hydroxide TS. Develop the plate with a mixture of chloroform and methanol (4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde-iron (III) chloride TS on the plate: the spots obtained from the sample solution and the standard solution show a red-purple color and the same RF value, and any spot from the sample solution other than that corresponding to the spot from the standard solution does not appear.

Loss on drying $<2.4\%$ Not more than 2.0% (0.2 g, silica gel, 4 hours).

Assay Weigh accurately about 10 mg each of Ergometrine Maleate and Ergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, to separate glass-stoppered test tubes, and proceed as directed in the Assay under Ultraviolet-visible Spectrophotometry $<2.27>$, using a solution, prepared with 2 mL of water in the same manner, as the blank. Determine the absorbances, $A_T$ and $A_S$, of the subsequent solutions of the sample solution and the standard solution at 550 nm, respectively.

\[
M_S = A_T / A_S
\]

$M_S$: Amount (mg) of Ergometrine Maleate RS

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ergometrine Maleate Injection

エルゴメトリンメレイン酸塩注射液

Ergometrine Maleate Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of ergometrine maleate ($\text{C}_{19}\text{H}_{23}\text{N}_3\text{O}_2\cdot\text{C}_4\text{H}_4\text{O}_4$; 441.48).

Method of preparation Prepare as directed under Injections, with Ergometrine Maleate.

Description Ergometrine Maleate Injection is a clear, colorless to pale yellow liquid.

pH: 2.7 – 3.5

Identification (1) Measure a volume of Ergometrine Maleate Injection, equivalent to 3 mg of Ergometrine Maleate according to the labeled amount, if necessary, dilute with water or evaporate on a water bath to make 15 mL, and use this solution as the sample solution. The sample solution shows a blue fluorescence.

(2) To 1 mL of the sample solution obtained in (1) add 1 mL of ammonia TS, and extract with 20 mL of diethyl ether. To the diethyl ether extract add 1 mL of dilute sulfuric acid, shake, and warm to remove diethyl ether in a water bath.

Cool, to the residue obtained add 2 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and allow to stand for 5 to 10 minutes: a deep blue color develops.

(3) To 5 mL of the sample solution obtained in (1) add 1 drop of potassium permanganate TS: a red color disappears immediately.

Bacterial endotoxins $<4.01>$ Less than 1500 EU/mg.

Extractable volume $<6.05>$ It meets the requirement.

Foreign insoluble matter $<6.06>$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter $<6.07>$ It meets the requirement.

Sterility $<4.06>$ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Transfer an exactly measured volume of Ergometrine Maleate Injection, equivalent to about 2 mg of ergometrine maleate ($\text{C}_{19}\text{H}_{23}\text{N}_3\text{O}_2\cdot\text{C}_4\text{H}_4\text{O}_4$), and add sodium chloride in a ratio of 0.3 g to 1 mL of the solution. To this mixture add 20 mL of diethyl ether and 2 mL of ammonia TS, shake, and extract. Further, extract with three 15-mL portions of diethyl ether, combine all the extracts, add 5 g of anhydrous sodium sulfate, filter through a pledget of absorbent cotton, and wash with three 5-mL portions of diethyl ether. Add the washings to the filtrate, shake with 5 mL of dilute sulfuric acid, evaporate the diethyl ether by warming in a current of nitrogen, to the remaining solution add water to make exactly 50 mL, and use this solution as the sample solution.

Separately, weigh accurately about 2 mg of Ergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, add water to make exactly 50 mL, and use this solution as the standard solution. Transfer 2 mL each of the sample solution and standard solution, accurately measured, to separate glass-stoppered test tubes, and proceed as directed in the Assay under Ergometrine Maleate.

Amount (mg) of ergometrine maleate ($\text{C}_{19}\text{H}_{23}\text{N}_3\text{O}_2\cdot\text{C}_4\text{H}_4\text{O}_4$) $M_S = A_T / A_S$

$M_S$: Amount (mg) of Ergometrine Maleate RS

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant, and in a cold place.

Ergometrine Maleate Tablets

エルゴメトリンメレイン酸塩錠

Ergometrine Maleate Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of ergometrine maleate ($\text{C}_{19}\text{H}_{23}\text{N}_3\text{O}_2\cdot\text{C}_4\text{H}_4\text{O}_4$; 441.48).

Method of preparation Prepare as directed under Tablets, with Ergometrine Maleate.

Identification To a quantity of powdered Ergometrine Maleate Tablets, equivalent to 3 mg of Ergometrine Maleate according to the labeled amount, add 15 mL of warm water, shake, and filter: the filtrate shows a blue fluorescence. Proceed with this solution as directed in the Identification (2)
and (3) under Ergometrine Maleate.

**Uniformity of dosage units** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Ergometrine Maleate Tablets to a brown glass-stoppered centrifuge tube, and add a solution of L-tartaric acid (1 in 100) to make exactly $V \text{ mL}$ so that each mL contains about 40 μg of ergometrine maleate ($C_{19}H_{23}N_{3}O_{2} \cdot C_{4}H_{4}O_{4}$). Stopper the tube, shake for 30 minutes vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 4 mg of Ergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution into separate brown glass-stoppered test tubes, add exactly 8 mL of a solution of 4-dimethylaminobenzaldehyde-iron (III) chloride TS while cooling in an ice bath, after shaking, and allow to stand for 1 hour at ordinary temperature. Perform the test with these solutions as directed under Ultraviolet-Visible Spectrophotometry <2.24>, using a solution, prepared with 4 mL of water in the same manner, as the blank. Determine the absorbances, $A_1$ and $A_5$, of the subsequent solutions of the sample solution and the standard solution at 550 nm, respectively.

Amount (mg) of ergometrine maleate ($C_{19}H_{23}N_{3}O_{2} \cdot C_{4}H_{4}O_{4}$)  

$$M_S \times A_1 / A_5 \times V / 100$$

$M_S$: Amount (mg) of Ergometrine Maleate RS

**Assay** Weigh accurately, and powder not less the 20 Ergometrine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 2 mg of ergometrine maleate ($C_{19}H_{23}N_{3}O_{2} \cdot C_{4}H_{4}O_{4}$), transfer to a glass filter (G4), add 10 mL of a solution of L-tartaric acid (1 in 100), and filter with thorough shaking. Repeat the procedures 3 times, combine the filtrates, add a solution of L-tartaric acid (1 in 100) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 2 mg of Ergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, dissolve in a solution of L-tartaric acid (1 in 100) to make exactly 50 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and standard solution, and proceed as directed in the Assay under Ergometrine Maleate.

Amount (mg) of ergometrine maleate ($C_{19}H_{23}N_{3}O_{2} \cdot C_{4}H_{4}O_{4}$)  

$$M_S \times A_1 / A_5$$

$M_S$: Amount (mg) of Ergometrine Maleate RS

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

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**Ergotamine Tartrate**

エルゴタミン酒石酸塩

$$(C_{19}H_{23}N_{3}O_{2} \cdot C_{4}H_{4}O_{4}); C_{19}H_{23}N_{3}O_{3} \cdot C_{4}H_{4}O_{4}; \text{ 1313.41}

(5'S)-5'-Benzyl-12'-hydroxy-2'-methylergotaman-3',6',18-trione hemitartrate

[C17H35N6O16] 

Ergotamine Tartrate contains not less than 98.0% of ($C_{33}H_{35}N_{5}O_{5}$)2:C4H6O6, calculated on the dried basis.

**Description** Ergotamine Tartrate occurs as colorless crystals, or a white to pale yellowish white or grayish white, crystalline powder.

It is slightly soluble in water and in ethanol (95). Melting point: about 180°C (with decomposition).

**Identification** (1) Dissolve 1 mg of Ergotamine Tartrate in 10 mL of a mixture of acetic acid (100) and ethyl acetate (1:1). To 0.5 mL of this solution add slowly 0.5 mL of sulfuric acid, with shaking in cold water, and allow to stand: a purple color develops. To this solution add 0.1 mL of diluted iron (III) chloride TS (1 in 12): the color of the solution changes to blue to blue-purple.

(2) Dissolve 1 mg of Ergotamine Tartrate in 5 mL of a solution of L-tartaric acid (1 in 100). To 1 mL of this solution add 2 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and shake: a blue color develops.

**Optical rotation** <2.49> Ergotamine base [α]$\text{D}^\circ$ = −155 to −165°. Dissolve 0.35 g of Ergotamine Tartrate in 25 mL of a solution of L-tartaric acid (1 in 100), add 0.5 g of sodium hydrogen carbonate, shake gently and sufficiently, and extract with four 10-mL portions of ethanol-free chloroform. Filter the extracts successively through a small filter paper, moistened with ethanol-free chloroform, into a 50-mL volumetric flask. Allow the flask to stand in a water bath at 20°C for 10 minutes, and determine the optical rotation in a 100-mm cell. Separately, pipet 25 mL of this solution, evaporate to dryness under reduced pressure at a temperature not higher than 45°C, dissolve the residue in 25 mL of acetic acid (100), and titrate <2.50> with 0.05 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS). Perform a blank determination, and make any necessary correction. Calculate the specific rotation of the ergotamine base from the consumed volume of 0.05 mol/L perchloric acid VS and the optical rotation.

Each mL of 0.05 mol/L perchloric acid VS  

$$= 29.08 \text{ mg of } C_{33}H_{35}N_{5}O_{5}$$

**Purity** Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. To 40 mg of Ergotamine Tartrate add 10 mL of a solution of L-
tartaric acid in diluted methanol (1 in 2) (1 in 1000), dissolve with thorough shaking, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of L-tartaric acid in diluted methanol (1 in 2) (1 in 1000) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.03 > \). Spot 10 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \( <2.4 > \) Not more than 5.0% (0.1 g, in vacuum, 60°C, 4 hours).

**Assay** Weigh accurately about 0.2 g of Ergotamine Tartrate, dissolve in 15 mL of a mixture of acetic acid (100) and acetic anhydride (50:3), and titrate \( <2.50 > \) with 0.05 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 32.84 mg of \((C_{17}H_{22}N_{2}O_{5})\cdot C_{4}H_{6}O_{6}\)

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under nitrogen atmosphere, and not exceeding 5°C.

**Erythromycin**

エリスロマイシン

\[
\text{C}_{37}\text{H}_{67}\text{NO}_{13}; 733.93
\]

\((2R,3S,4S,5R,6R,8R,10R,11R,12S,13R)-5-(3,4,6-Trideoxy-3-dimethylamino-\beta-D-xylo-hexopyranosoyloxy)-3-(2,6-dideoxy-3-C-methyl-3-O-methyl-\alpha-L-ribo-hexopyranosoyloxy)-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide}

[114-07-8]

Erythromycin is a macrolide substance having antibacterial activity produced by the growth of *Saccharopolyspora erythraea*.

It contains not less than 930 \( \mu g \) (potency) and not more than 1020 \( \mu g \) (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin is expressed as mass (potency) of erythromycin \((C_{37}H_{67}NO_{13})\).

**Description** Erythromycin occurs as a white to light yellowish white powder.

It is very soluble in \(N,N\)-dimethylformamide, freely soluble in methanol and in ethanol (95), and very slightly soluble in water.

**Identification** (1) Determine the infrared absorption spectrum of Erythromycin as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.2S > \), and compare the spectrum with the Reference Spectrum or the spectrum of Erythromycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 10 mg each of Erythromycin and Erythromycin RS in 1 mL of methanol, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.03 > \). Spot 5 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (50:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 100°C for 15 minutes: the principal spot from the sample solution and the spot from the standard solution are dark purple in color, and their \( R_f \) values are the same.

**Optical rotation** \( <2.49 > \) [\( \alpha \)]\(_D\)\(^{20} = -71 \sim -78^\circ\) (1 g calculated on the anhydrous basis, ethanol (95), 50 mL, 100 mm).

**Purity** (1) Heavy metals \( <1.07 > \)—Proceed with 1.0 g of Erythromycin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \( <1.11 > \)—Prepare the test solution with 1.0 g of Erythromycin according to Method 5 using hydrochloric acid instead of diluted hydrochloric acid (1 in 2), and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 40 mg of Erythromycin in 2 mL of methanol, add a mixture of phosphate buffer solution, \( \text{pH} 7.0 \) and methanol (15:1) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 16 mg of Erythromycin RS in 2 mL of methanol, add a mixture of phosphate buffer solution, \( \text{pH} 7.0 \) and methanol (15:1) to make exactly 10 mL, and use this solution as the standard stock solution. Dissolve 5 mg each of erythromycin B and erythromycin C in 2 mL of methanol, add exactly 2 mL of the standard stock solution, add a mixture of phosphate buffer solution, \( \text{pH} 7.0 \) and methanol (15:1) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 100 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 > \) according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of erythromycin B and erythromycin C from the sample solution are not larger than those of erythromycin B and erythromycin C from the standard solution, respectively, and each area of the peaks other than erythromycin, erythromycin B and erythromycin C is not larger than the area of the peak of erythromycin from the standard solution.

**Operating conditions**—


Column: A stainless steel column 4.6 mm in inside diame-
Erythromycin Enteric-Coated Tablets

Erythromycin Enteric-Coated Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of erythromycin (C_{37}H_{67}NO_{13}: 733.93).

**Method of preparation** Prepare as directed under Tablets, with Erythromycin.

**Identification** To a quantity of powdered Erythromycin Enteric-Coated Tablets, equivalent to 10 mg (potency) of Erythromycin according to the labeled amount, add 1 mL of methanol, shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of Erythromycin RS in 1 mL of methanol, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Erythromycin.

**Loss on drying** Not more than 10.0% (0.2 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

**Uniformity of dosage units** It meets the requirement of the Mass variation test.

**Disintegration** It meets the requirement. For the test with 2nd fluid for disintegration test, use the disk.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (i) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Erythromycin RS, equivalent to about 25 mg (potency), dissolve in 25 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 4°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration sample solution, respectively.

containers and storage Containers—Well-closed containers.
C_{37}H_{67}NO_{13}: 733.93.

Erythromycin Ethylsuccinate is a derivative of erythromycin.

It contains not less than 780 μg (potency) and not more than 900 μg (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin Ethylsuccinate is expressed as mass (potency) of erythromycin on the anhydrous basis.

**Description**

Erythromycin Ethylsuccinate occurs as a white powder.

It is freely soluble in methanol and in acetone, soluble in ethanol (95), and practically insoluble in water.

**Identification (1)**

Dissolve 3 mg of Erythromycin Ethylsuccinate in 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color develops and is immediately changed to deep purple.

(2) Determine the infrared absorption spectrum of Erythromycin Ethylsuccinate, previously dried in a desiccator at reduced pressure, silica gel for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Water**

Not more than 5.0% (0.5 g, volumetric titration, direct titration).

**Assay**

Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics according to the following conditions.

(i) Test organism—Staphylococcus aureus ATCC 6538

(ii) Culture medium—Use the medium in (i) for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Erythromycin RS equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Erythromycin Ethylsuccinate equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and Storage**

Containers—Tight containers.

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**Erythromycin Lactobionate**

C_{37}H_{67}NO_{13}.C_{12}H_{22}O_{12}: 1092.22

Erythromycin Lactobionate is the lactobionate of erythromycin.

It contains not less than 560 μg (potency) and not more than 700 μg (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin Lactobionate is expressed as mass (potency) of erythromycin on the anhydrous basis.

**Description**

Erythromycin Lactobionate occurs as a white powder.

It is freely soluble in water, in methanol and in ethanol (99.5), and very slightly soluble in acetone.

**Identification (1)**

To 3 mg of Erythromycin Lactobionate add 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color is produced, and it changes immediately to red to deep purple.

(2) Transfer about 0.3 g of Erythromycin Lactobionate to a separator, add 15 mL of ammonia TS and 15 mL of chloroform, shake, and take the separated aqueous layer.

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Wash the aqueous layer with three 15-mL portions of chloroform, and evaporate the aqueous liquid on a water bath to dryness. Dissolve the residue in 10 mL of a mixture of methanol and water (3:2), and use this solution as the sample solution. Separately, dissolve 0.10 g of lactobionic acid in 10 mL of a mixture of methanol and water (3:2), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography _<2.03>_. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the upper layer obtained from a mixture of water, 1-butanol and acetic acid (100) (3:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid, and heat at 105°C for 20 minutes: the principal spot obtained from the sample solution shows a deep brown and the RF value which are the same as those of the principal spot from the standard solution.

\[
\text{pH < 2.5} \quad \text{The pH of a solution obtained by dissolving 0.5 g of Erythromycin Lactobionate in 10 mL of water is between 5.0 and 7.5.}
\]

**Water < 2.48** Not more than 5.0% (0.5 g, volumetric titration, direct titration).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics _<4.02>_ according to the following conditions.

(i) **Test organism**— _Staphylococcus aureus_ ATCC 6538 P

(ii) **Culture medium**—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) **Standard solutions**—Weigh an amount of Erythromycin RS, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C between 5.0 and 7.5.

Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C between 5.0 and 7.5.

Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C between 5.0 and 7.5.

(iv) **Sample solutions**—Weigh an amount of Erythromycin Lactobionate, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

**Erythromycin Stearate**

エリスロマイシンステアリン酸塩

C₁₇H₂₂NO₁₅.C₁₈H₃₆O₂·10H₂O: 1018.40
(C₂₉₅₃₅₄S₅₆₇₈O₉₁₀₁₁₁₂₁₃₁₄₁₅₁₆₁₇₁₈₁₉₂₀): 733.93

- Not less than 600 μg (potency) and not more than 720 μg (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin Stearate is expressed as mass (potency) of erythromycin (C₃₇H₆₇NO₁₃: 733.93).

**Description** Erythromycin Stearate occurs as a white powder.

It is freely soluble in ethanol (95) and in acetone, soluble in methanol, and practically insoluble in water.

**Identification** (1) Dissolve 3 mg of Erythromycin Stearate in 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color develops and is immediately changed to red to deep purple.

(2) Determine the infrared absorption spectrum of Erythromycin Stearate, previously dried in a desiccator (reduced pressure, silica gel) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry _<2.25>_, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Water < 2.48** Not more than 5.0% (0.5 g, volumetric titration, direct titration).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics _<4.02>_ according to the following conditions.

(i) **Test organism**— _Staphylococcus aureus_ ATCC 6538 P

(ii) **Culture medium**—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) **Standard solutions**—Weigh accurately an amount of Erythromycin RS equivalent to about 50 mg (potency), dis-
solve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Erythromycin Stearate equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of the sample, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Estazolam

エスタゾラム

C₁₆H₁₁ClN₄: 294.74
8-Chloro-6-phenyl-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine [29975-16-4]

Estazolam, when dried, contains not less than 98.5% of C₁₆H₁₁ClN₄.

Description Estazolam occurs as white to pale yellowish white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is soluble in methanol and in acetic anhydride, sparingly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification (1) Dissolve 0.01 g of Estazolam in 3 mL of sulfuric acid: the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Estazolam in 1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Estazolam as directed under Flame Coloration Test (2) <1.04>: a green color appears.

Melting point <2.60> 229 – 233°C

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Estazolam in 10 mL of ethanol (95): the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 1.0 g of Estazolam in 10 mL of ethanol (95) by heating, add 40 mL of water, cool with shaking in ice water, allow to stand to attain ordinary temperature, and filter. To 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS and 6 mL of ethanol (95) (not more than 0.015%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Estazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Estazolam according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Estazolam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, chloroform and methanol (5:3:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the principal spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (2 g).

Assay Weigh accurately about 0.25 g of Estazolam, previously dried, dissolve in 100 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration), until the solution changes to the second equivalence point. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 14.74 mg of C₁₆H₁₁ClN₄

Containers and storage Containers—Well-closed containers.
**Estradiol Benzoate**

エストラジオール安息香酸エステル

![Chemical Structure](image)

C₂₅H₂₈O₃: 376.49  
Estra-1,3,5(10)-triene-3,17β-diol 3-benzoate  
[50-50-0]

Estradiol Benzoate, when dried, contains not less than 97.0% of C₂₅H₂₈O₃.

**Description**  
Estradiol Benzoate occurs as a white, crystalline powder. It is odorless. It is sparingly soluble in acetone, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

**Identification (1)**  
To 2 mg of Estradiol Benzoate add 2 mL of sulfuric acid: a yellowish green color with a blue fluorescence is produced, and the color of the solution changes to light orange on the careful addition of 2 mL of water.

(2) Determine the infrared absorption spectrum of Estradiol Benzoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry< 2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Estradiol Benzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation**< 2.49> [α]D<sub>20</sub>: +54 - +58° (after drying, 0.1 g, acetone, 10 mL, 100 mm).

**Melting point**< 2.60> 191 – 198°C

**Purity (1)**  
17β-Estradiol—Dissolve 5.0 mg each of Estradiol Benzoate and Estradiol Benzoate RS in acetone to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Place exactly 2 mL each of the sample solution and standard solution in separate glass-stoppered test tube, add boiling stones, exactly 2 mL each of the sample solution and standard solution on a water bath, shake in a water bath for several seconds, and heat for 2 minutes. Cool the solutions in ice for 2 minutes, then add methanol to make 20 mL, and use these solutions as the sample solution and standard solution as directed under Liquid Chromatography< 2.01> according to the following conditions, and calculate the ratios, Q<sub>T</sub> and Q<sub>S</sub>, of the peak area of estradiol benzoate to that of the internal standard.

Amount (mg) of C₂₅H₂₈O₃: = M<sub>T</sub> × Q<sub>T</sub>/Q<sub>S</sub>

M<sub>S</sub>: Amount (mg) of Estradiol Benzoate RS

**Internal standard solution**—A solution of progesterone in methanol (13 in 80,000).

**Operating conditions**—  
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of estradiol benzoate is about 10 minutes.

**System suitability**—  
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and estradiol benzoate are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of estradiol benzoate to that of the internal standard is not more than 1.0%.

**Containers and storage**  
Containers—Tight containers.

Storage—Light-resistant.

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**Estradiol Benzoate Injection**

エストラジオール安息香酸エステル注射液

Estradiol Benzoate Injection is an oily solution for injection. It contains not less than 90.0% and not more than 110.0% of the labeled amount of estradiol benzoate.
Estradiol Benzoate Injection (Aqueous Suspension)

エストラジオール安息香酸エステル水性懸濁注射液

Estradiol Benzoate Injection (Aqueous Suspension) is an aqueous suspension for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of estradiol benzoate (C_{25}H_{28}O_{3}: 376.49).

Method of preparation Prepare as directed under Injection, with Estradiol Benzoate.

Description Estradiol Benzoate Injection (Aqueous Suspension) produces a white turbidity on shaking.

Identification Extract a volume of Estradiol Benzoate Injection (Aqueous Suspension), equivalent to 1 mg of Estradiol Benzoate according to the labeled amount, with 5 mL of chloroform, and use this solution as the standard solution. Separately, dissolve 1 mg of Estradiol Benzoate RS in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (99:1) to a distance of about 15 cm, and air-dry the plate.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: easily detectable foreign matters are not observed.

Sterility <4.06> Perform the test according to the Direct inoculation method: it meets the requirement.

Assay Measure exactly a volume of well-mixed Estradiol Benzoate Injection (Aqueous Suspension), equivalent to about 2 mg of estradiol benzoate (C_{25}H_{28}O_{3}), dissolve the crystals with an appropriate quantity of methanol, and add methanol to make exactly 20 mL. Pipet 10 mL of this solution, add methanol to make exactly 30 mL, and use this solution as the standard solution. Transfer 2 mL each of the sample solution and standard solution, exactly measured, to light-resistant 20-mL volumetric flasks, and evaporate to dryness on a water bath with the aid of a current of air. Dissolve the residue in 1 mL of methanol, add 10 mL of boric acid-methanol buffer solution, shake, and boil under a reflux condenser for 30 minutes. Cool, add 5 mL of boric acid-methanol buffer solution, shake, and cool with ice. To each solution add 2 mL of ice-cold diazo TS quickly, shake vigorously, add 2 mL of sodium hydroxide TS, then add water to make 20 mL, and filter after shaking. Discard the first 3 mL of the filtrate, and perform the test with the subsequent filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution, prepared with 2 mL of methanol in the same manner, as the blank. Determine the absorbances, \( A_7 \) and \( A_{38} \), of the subsequent solutions obtained from the sample solution and standard solution in a 4-cm cell at 490 nm, respectively.

\[
\text{Amount (mg) of estradiol benzoate (C}_{25}\text{H}_{28}\text{O}_{3}) = M_3 \times \frac{A_7}{A_{38}} \times \frac{2}{5} \\
M_3: \text{Amount (mg) of Estradiol Benzoate RS}
\]

Containers and storage Containers—Hermetic containers.
methanol (13 in 100,000).

Containers and storage Containers—Hermetic containers.

**Estriol**

![Image](image.png)

C₁₈H₂₄O₃: 288.38  
Estra-1,3,5(10)-triene-3,16α,17β-triol  
[50-27-7]

Estriol, when dried, contains not less than 97.0% and not more than 102.0% of C₁₈H₂₄O₃.

Description Estriol occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol, slightly soluble in ethanol (95) and in 1,4-dioxane, and practically insoluble in water and in diethyl ether.

Identification (1) Dissolve 0.01 g of Estriol in 100 mL of ethanol (95) by warming, and use this solution as the sample solution. Evaporate 1 mL of the sample solution on a water bath to dryness, add 5 mL of a solution of sodium p-phenolsulfonate in diluted phosphoric acid (1 in 50), heat at 150°C for 10 minutes, and cool: a red-purple color develops.

(2) Determine the absorption spectrum of the sample obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Estriol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Estriol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Estriol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D: +54° to +62° (after drying, 40 mg, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 281 – 286°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Estriol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 40 mg of Estriol in 10 mL of ethanol (95) by warming, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and acetic acid (100:18:1:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, and heat at 105°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 25 mg each of Estriol and Estriol RS, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of estriol to that of the internal standard, respectively.

\[
\text{Amount (mg) of } C_{18}H_{24}O_3 = M_s \times \frac{Q_1}{Q_2}
\]

Mₛ: Amount (mg) of Estriol RS

Internal standard solution—A solution of methyl benzoate for estriol limit test in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (51:49).

Flow rate: Adjust the flow rate so that the retention time of estriol is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, estriol and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of estriol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

**Estriol Injection (Aqueous Suspension)**

エストリオール水性懸濁注射液

Estriol Injection (Aqueous Suspension) is an aqueous suspension for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of estriol (C₁₈H₂₄O₃: 288.38).
Method of preparation  Prepare as directed under Injections, with Estriol.

Description  Shake Estriol Injection (Aqueous Suspension): a white turbidity is produced.

Identification  (1) Shake well, take a volume of Estriol Injection (Aqueous Suspension), equivalent to 2 mg of Estriol according to the labeled amount, add ethanol (95) to make 20 mL, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Estriol.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 279 nm and 283 nm.

Extractable volume  It meets the requirement.

Foreign insoluble matter  Perform the test according to Method 1: easily detectable foreign matters are not observed.

Sterility  Perform the test according to the Direct inoculation method: it meets the requirement.

Assay  Shake well, pipet a volume of Estriol Injection (Aqueous Suspension), equivalent to about 5 mg of estriol (C\(_{18}\)H\(_{24}\)O\(_3\)), and dissolve in methanol to make exactly 20 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Estriol RS, previously dried at 105 °C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Estriol.

\[
\text{Amount (mg) of estriol (C}_{18}\text{H}_{24}\text{O}_3) = M_S \times \frac{Q_T}{Q_S} \times \frac{1}{5}
\]

\(M_S\): Amount (mg) of Estriol RS

Internal standard solution—A solution of methyl benzoate for estriol limit test in ethanol (95) (1 in 5000).

Containers and storage  Containers—Hermetic containers.

Estriol Tablets

エストリオール錠

Estriol Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of estriol (C\(_{18}\)H\(_{24}\)O\(_3\)) 288.38).

Method of preparation  Prepare as directed under Tablets, with Estriol.

Identification  (1) Weigh a portion of powdered Estriol Tablets, equivalent to 2 mg of Estriol according to the labeled amount, add ethanol (95) to make 20 mL, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Estriol.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 279 nm and 283 nm.

Uniformity of dosage units  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Estriol Tablets add exactly 5 mL of water, disperse the fine particles with ultrasonic wave, add exactly 15 mL of methanol, and shake for 15 minutes. Centrifuge this solution for 10 minutes, pipet a definite amount of the supernatant liquid, and add methanol to make exactly a definite amount of solution so that each mL of the solution contains about 5 µg of estriol (C\(_{18}\)H\(_{24}\)O\(_3\)). Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Proceed with 20 µL of the sample solution as directed in the Assay under Estriol. Use a solution of methyl benzoate in methanol (1 in 40,000) as the internal standard solution. Calculate the mean value from each ratio of peak areas of 10 samples: the samples conform to the requirements if the deviation (%) of the mean value and each ratio of peak areas is within 15%. If the deviation (%) exceeds 15%, and 1 sample shows deviation within 25%, repeat the test with 20 samples. Calculate the deviation (%) of the mean value from each ratio of peak areas of the 30 samples used in the 2 tests and each ratio of peak areas: the samples conform to the requirements if the deviation exceeds 15%, not more than 1 sample shows deviation within 25%, and no sample shows deviation exceeding 25%.

Dissolution  When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Estriol Tablets is not less than 80%.

Start the test with 1 tablet of Estriol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 µm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 10 mL so that each mL contains about 0.1 µg of estriol (C\(_{18}\)H\(_{24}\)O\(_3\)) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Estriol RS, previously dried at 105 °C for 3 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 µL each of the sample solution and standard solution as directed under Liquid chromatography: it following the according to the following conditions, and determine the peak areas, \(A_T\) and \(A_S\), of estriol.

\[
\text{Dissolution rate} \times \% = \frac{M_S \times A_T}{A_S} \times \frac{V}{V_{\text{in}}} \times \frac{C}{1000} \times \frac{C}{1000}
\]

\(M_S\): Amount (mg) of Estriol RS

\(C\): Labeled amount (mg) of estriol (C\(_{18}\)H\(_{24}\)O\(_3\)) in 1 tablet

Operating conditions— Proceed as directed in the operating conditions in the Assay under Estriol.
**System suitability**

Proceed as directed in the system suitability in the Assay under Estriol.

**Assay**

Weigh accurately and powder not less than 20 Estriol Tablets. Weigh accurately a portion of the powder, equivalent to about 1 mg of estriol \((C_{18}H_{24}O_3)\), add exactly 5 mL of water, disperse the fine particles with ultrasonic wave, shake with 25 mL of methanol for 10 minutes, centrifuge, and take the supernatant liquid. Add 25 mL of methanol, repeat the above procedure twice, combine the supernatant liquids, and make up to 100 mL with methanol. Add exactly 5 mL of the internal standard solution, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Estriol RS, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the standard solution. Proceed with 20 μL each of the sample solution and standard solution as directed in the Assay under Estriol.

\[
\text{Amount (mg) of estriol (C}_{18}\text{H}_{24}\text{O}_{3}) = M_s \times \frac{Q_T}{Q_b} \times \frac{1}{25}
\]

\(M_s\): Amount (mg) of Estriol RS

**Internal standard solution**—A solution of methyl benzoate for estriol limit test in methanol (1 in 5000).

**Containers and storage** Containers—Tight containers.

---

**Etacrynic Acid**

エタクリン酸

\[\text{C}_{13}\text{H}_{12}\text{Cl}_{2}\text{O}_{4}: 303.14\]

\([2,3\text{-Dichloro-4-(2-ethylacryloyl)phenoxy]acetic acid [58-54-8]}

Etacrynic Acid, when dried, contains not less than 98.0% of \(\text{C}_{13}\text{H}_{12}\text{Cl}_{2}\text{O}_{4}\).

**Description**

Etacrynic Acid occurs as a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in methanol, freely soluble in ethanol (95), in acetic acid (100) and in diethyl ether, and very slightly soluble in water.

**Identification**

(1) Dissolve 0.2 g of Etacrynic Acid in 10 mL of acetic acid (100), and to 5 mL of this solution add 0.1 mL of bromine TS: the color of the test solution disappears. To the remaining 5 mL of the solution add 0.1 mL of potassium permanganate TS: the color of the test solution changes to light orange immediately.

(2) To 0.01 g of Etacrynic Acid add 1 mL of sodium hydroxide TS, and heat in a water bath for 3 minutes. After cooling, add 1 mL of chlorotrope acid TS, and heat in a water bath for 10 minutes: a deep purple color develops.

(3) Determine the absorption spectrum of a solution of Etacrynic Acid in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Etacrynic Acid as directed under Flame Coloration Test (2) \(<1.04\>\): a green color appears.

**Melting point** \(<2.60\>\quad 121 - 125°C\)

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of Etacrynic Acid in 10 mL of methanol: the solution is clear and colorless.

(2) Heavy metals \(<1.07\>—\)Proceed with 1.0 g of Etacrynic Acid according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic \(<1.11\>—\)Prepare the test solution with 1.0 g of Etacrynic Acid according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Etacrynic Acid in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.05\>\). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethyl acetate and acetic acid (100) (6:5:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.41\>\quad \text{Not more than 0.25% (1 g, in vacuum, 60°C, 2 hours)}.

**Residue on ignition** \(<2.44\>\quad \text{Not more than 0.1% (1 g)}.

**Assay**

Weigh accurately about 0.1 g of Etacrynic Acid, previously dried, place in an iodine bottle, dissolve in 20 mL of acetic acid (100), and add exactly 20 mL of 0.05 mol/L bromine VS. To this solution add 3 mL of hydrochloric acid, stopper tightly at once, shake, and allow to stand in a dark place for 60 minutes. Add carefully 50 mL of water and 15 mL of potassium iodide TS, stopper tightly at once, shake well, and titrate \(<2.50\>\) the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS

\[
= 15.16 \text{ mg of C}_{13}\text{H}_{12}\text{Cl}_{2}\text{O}_{4}
\]

**Containers and storage** Containers—Well-closed containers.
Etacrynic Acid Tablets

エタクリン酸誘

Etacrynic Acid Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of etacrynic acid (C_{13}H_{12}Cl_{2}O_{4}: 303.14).

Method of preparation  Prepare as directed under Tablets, with Etacrynic Acid.

Identification (1) Weigh a quantity of powdered Etacrynic Acid Tablets, equivalent to 0.3 g of Etacrynic Acid according to the labeled amount, add 25 mL of 0.1 mol/L hydrochloric acid TS, and extract with 50 mL of Dichloromethane. Filter the dichloromethane extract, and evaporate the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1), (2) and (4) under Etacrynic Acid.

(2) Prepare a solution of the residue obtained in (1), equivalent to a solution of Etacrynic Acid in methanol (1 in 20,000), and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 268 nm and 272 nm.

Dissolution 6.10  When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Etacrynic Acid Tablets is not less than 70%.

Start the test with 1 tablet of Etacrynic Acid Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 28 μg of etacrynic acid (C_{13}H_{12}Cl_{2}O_{4}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 55 mg of etacrynic acid for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_M and A_s, at 277 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of etacrynic acid (C_{13}H_{12}Cl_{2}O_{4})

\[ M_s = M \times (V/V) \times (1/C) \times 45 \]

where

\[ M \] is the weight of the tablet
\[ V \] is the volume of water
\[ C \] is the concentration of the solution

M_s: Amount (mg) of etacrynic acid for assay
C: Labeled amount (mg) of etacrynic acid (C_{13}H_{12}Cl_{2}O_{4}) in 1 tablet

Assay  Weigh accurately and powder not less than 20 Etacrynic Acid Tablets. Weigh accurately a portion of the powder, equivalent to 0.1 g of etacrynic acid (C_{13}H_{12}Cl_{2}O_{4}), add 25 mL of 0.1 mol/L hydrochloric acid TS, and extract with three 30-mL portions of dichloromethane. Filter the dichloromethane extracts through a pledget of absorbent cotton into an iodine bottle. Wash the pledget of absorbent cotton with a small amount of dichloromethane, and combine the washing with the extracts. Evaporate this solution on a water bath to dryness in a current of air, to the residue add 20 mL of acetic acid (100), and proceed as directed in the Assay under Etacrynic Acid.

Each mL of 0.05 mol/L bromine VS
\[ = 15.16 \text{ mg of C}_{13}H_{12}Cl_{2}O_{4} \]

Containers and storage  Containers—Well-closed containers.

Ethambutol Hydrochloride

エタンブトール塩酸塩

Ethambutol Hydrochloride, when dried, contains not less than 98.5% of C_{10}H_{24}N_{2}O_{2}.2HCl.

Description  Ethambutol Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

The pH of a solution of Ethambutol Hydrochloride (1 in 20) is between 3.4 and 4.0.

Identification (1) To 10 mL of a solution of Ethambutol Hydrochloride (1 in 100) add 0.5 mL of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a deep blue color is produced.

(2) Dissolve 0.1 g of Ethambutol Hydrochloride in 40 mL of water, add 20 mL of 2,4,6-trinitrophenol TS, and allow to stand for 1 hour. Collect the precipitate, wash with 50 mL of water, and dry at 105°C for 2 hours: the precipitate melts <2.60> between 193°C and 197°C.

(3) A solution of Ethambutol Hydrochloride (1 in 30) responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> [α]_D: +5.5° – +6.1° (after drying, 5 g, water, 50 mL, 200 mm).

Melting point <2.60> 200 – 204°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ethambutol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g Ethambutol Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.17>—Prepare the test solution with 1.0 g of Ethambutol Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) 2-Aminobutanol—Dissolve 5.0 g of Ethambutol Hydrochloride in methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve...
0.05 g of 2-amino-1-butanol in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{<2.02>} \). Spot 2 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100), hydrochloric acid and water (11:7:1:1) to a distance of about 10 cm, air-dry the plate, and heat at 105°C for 5 minutes. Cool, spray evenly ninhydrin-L-ascorbic acid TS upon the plate, air-dry the plate, and heat at 105°C for 5 minutes: the resulting liquid is clear. Control solution: water.

**Purity (1)** Clarity and color of solution—Ethanol is clear and colorless. To 1.0 mL of Ethanol add water to make 20 mL, and allow to stand for 5 minutes: the resulting liquid is clear. Control solution: water.

**Acidity or alkalinity**—To 20 mL of Ethanol add 20 mL of freshly boiled and cooled water and 0.1 mL of a solution prepared by addition of 7.0 mL of ethanol (95) and 2.0 mL of water to 1.0 mL of phenolphthalein TS: no color develops. Add 1.0 mL of 0.01 mol/L sodium hydroxide VS to this solution: a light red color develops.

**Identification** pipet 500 mL of Ethanol, add 150 \( \mu L \) of 4-methylpentan-2-ol, and use this solution as the sample solution. Separately, to 100 \( \mu L \) of anhydrous methanol add Ethanol to make exactly 50 mL. Pipet 5 mL of this solution, add Ethanol to make exactly 50 mL, and use this solution as the standard solution (1). Separately, to exactly 50 \( \mu L \) each of anhydrous methanol and acetaldehyde add Ethanol to make exactly 50 mL. To exactly 100 \( \mu L \) of this solution add Ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to exactly 150 \( \mu L \) of acetal add Ethanol to make exactly 50 mL. To exactly 100 \( \mu L \) of this solution add Ethanol to make exactly 10 mL, and use this solution as the standard solution (3). Separately, to exactly 100 \( \mu L \) of benzene add Ethanol to make exactly 100 mL. To exactly 100 \( \mu L \) of this solution add Ethanol to make exactly 50 mL, and use this solution as the standard solution (4). Perform the test with exactly 1 \( \mu L \) each of Ethanol, the sample solution and standard solutions (1), (2), (3) and (4) as directed under Gas Chromatography \( \text{<2.02>} \). According to the following conditions, and determine the area of acetaldehyde, \( A_{E} \), benzene, \( B_{E} \) and acetal, \( C_{E} \) obtained with Ethanol, and the peak area of methanol with the standard solution (1), the peak area of acetaldehyde, \( A_{C} \) with the standard solution (2), the peak area of acetal, \( C_{C} \) with the standard solution (3) and the peak area of benzene, \( B_{C} \): the peak area of methanol is not larger than 1/2 times the peak area of methanol with the standard solution (1). When calculate the amounts of the volatile impurities by the following equation, the total amount of acetaldehyde and acetal is not more than 10 vol ppm as acetaldehyde, and the amount of benzene is not more than 2 vol ppm. The total area of the peaks other than the peak mentioned above and the peak having the area not more than 3% that of 4-methylpentan-2-ol is not larger than the peak area of 4-methylpentan-2-ol.

\[
\text{Total amount (vol ppm) of acetaldehyde and acetal} = (10 \times A_{E})/(A_{C} - A_{E}) + (30 \times C_{E})/(C_{C} - C_{E})
\]

Amount (vol ppm) of benzene = \( 2B_{E}/(B_{C} - B_{E}) \)

If necessary, identify the peak of benzene by using a different stationary liquid phase and suitable chromatographic conditions.

**Operating conditions**—

**Detector:** A hydrogen flame-ionization detector.

**Column:** A fused silica tube 0.32 mm in inside diameter and 30 m in length, coated with 6% cyanopropyl phenyl-94% dimethyl silicone polymer for gas chromatography in
1.8 µm thickness.

Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 12 minutes, then rise up to 240°C at the rate of 10°C per minute, and maintain at a constant temperature of about 240°C for 10 minutes.

Carrier gas: Helium.

Flow rate: 35 cm per minute.


System suitability—

System performance: When the procedure is run with 1 µL of the standard solution (2) under the above operating conditions, acetaldehyde and methanol are eluted in this order with the resolution between these peaks being not less than 1.5.

(4) Other impurities (absorbance)—Perform the test with Ethanol as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbances at 240 nm, between 250 nm and 260 nm and between 270 nm and 340 nm are not more than 0.40, 0.30, and 0.10, respectively. The absorption spectrum determined in a 5-cm cell using water as a blank shows a flat absorption curve between 235 nm and 340 nm.

(5) Residue on evaporation—Evaporate 100 mL of Ethanol, exactly measured, in a tared dish on a water bath, and dry for 1 hour at 105°C: the mass of the residue does not exceed 2.5 mg.

Containers and storage  • Containers—Tight containers.

Storage—Without exposure to light.

Anhydrous Ethanol

Dehydrated Alcohol

無水エタノール

C₂H₆O: 46.07
Ethanol

64-17-5

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols ( • •).

Anhydrous Ethanol contains not less than 99.5 vol% (by specific gravity) of C₂H₆O at 15°C.

• Description Anhydrous Ethanol is a clear, colorless liquid.

It is miscible with water.

It is flammable and burns with a light blue flame on ignition.

It is volatile.

Boiling point: 78 – 79°C •

Identification Determine the infrared absorption spectrum of Anhydrous Ethanol as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <2.56> \( d_\text{15}^0 \): 0.794 – 0.797

Purity (1) Clarity and color of solution—Anhydrous Ethanol is clear and colorless. To 1.0 mL of Anhydrous Ethanol add water to make 20 mL, and allow to stand for 5 minutes: the resulting liquid is clear. Control solution: water.

(2) Acidity or alkalinity—To 20 mL of Anhydrous Ethanol add 20 mL of freshly boiled and cooled water and 0.1 mL of a solution obtained by addition of 7.0 mL of ethanol (95) and 2.0 mL of water to 1.0 mL of phenolphthalein TS: no color develops. Add 1.0 mL of 0.01 mol/L sodium hydroxide VS to this solution: a light red color develops.

(3) Volatile impurities—Pipet 500 mL of Anhydrous Ethanol, add 150 µL of 4-methylpentan-2-ol, and use this solution as the sample solution. Separately, to 100 µL of anhydrous methanol add Anhydrous Ethanol to make exactly 50 mL. Pipet 5 mL of this solution, add Anhydrous Ethanol to make exactly 50 mL, and use this solution as the standard solution (1). Separately, to exactly 50 µL each of anhydrous methanol and acetaldehyde add Anhydrous Ethanol to make exactly 50 mL. To exactly 100 µL of this solution add Anhydrous Ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to exactly 150 µL of acetal add Anhydrous Ethanol to make exactly 50 mL. To exactly 100 µL of this solution add Anhydrous Ethanol to make exactly 10 mL, and use this solution as the standard solution (3). Separately, to exactly 100 µL of benzene add Anhydrous Ethanol to make exactly 100 mL. To exactly 100 µL of this solution add Anhydrous Ethanol to make exactly 50 mL, and use this solution as the standard solution (4). Perform the test with exactly 1 µL each of Anhydrous Ethanol, the sample solution and standard solutions (1), (2), (3) and (4) as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas of acetaldehyde, \( A_\text{T} \), benzene, \( B_\text{T} \) and acetal, \( C_\text{T} \), obtained with Anhydrous Ethanol, and the peak area of methanol with the standard solution (1), the peak area of acetaldehyde, \( A_\text{E} \), benzene, \( B_\text{E} \) and acetal, \( C_\text{E} \), obtained with Anhydrous Ethanol, and the peak area of methanol with the standard solution (2), the peak area of acetaldehyde, \( A_\text{T} \), with the standard solution (2), the peak area of acetal, \( C_\text{E} \) with the standard solution (3) and the peak area of benzene, \( B_\text{T} \): the peak area of methanol is not larger than 1/2 times the peak area of methanol with the standard solution (1). When calculate the amounts of the volatile impurities by the following equation, the total amount of acetaldehyde and acetal is not more than 10 vol ppm as acetaldehyde, and the amount of benzene is not more than 2 vol ppm. The total area of the peaks other than the peak mentioned above and the peak having the area not more than 3% that of 4-methylpentan-2-ol is not larger than the peak area of 4-methylpentan-2-ol.

Total amount (vol ppm) of acetaldehyde and acetal

\[
= (10 \times A_\text{T})/(A_\text{T} - A_\text{E}) + (30 \times C_\text{E})/(C_\text{E} - C_\text{T})
\]

Amount (vol ppm) of benzene

\[
= 2B_\text{T}/(B_\text{T} - B_\text{E})
\]

If necessary, identify the peak of benzene by using a different stationary liquid phase and suitable chromatographic conditions.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica tube 0.32 mm in inside diameter and 30 m in length, coated with 6% cyanopropyl phenyl-94%dimethyl silicone polymer for gas chromatography in 1.8 µm thickness.

Column temperature: Inject at a constant temperature of
about 40°C, maintain the temperature for 12 minutes, then rise up to 240°C at the rate of 10°C per minute, and maintain at a constant temperature of about 240°C for 10 minutes.

Carrier gas: Helium.
Flow rate: 35 cm per minute.
System suitability—
System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, acetaldehyde and methanol are eluted in this order with the resolution between these peaks being not less than 1.5.

(4) Other impurities (absorbance)—Perform the test with Ethanol as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbances at 240 nm, between 250 nm and 260 nm and between 270 nm and 340 nm are not more than 0.40, 0.30, and 0.10, respectively. The absorption spectrum determined in a 5-cm cell using water as a blank shows a flat absorption curve between 235 nm and 340 nm.

(5) Residue on evaporation—Evaporate 100 mL of Anhydrous Ethanol, exactly measured, in a tared dish on a water bath, and dry for 1 hour at 105°C: the mass of the residue does not exceed 2.5 mg.

Containers and storage—Containers—Tight containers.
Storage—Without exposure to light.

Ethanol for Disinfection
消毒用エタノール

Ethanol for Disinfection contains not less than 76.9 vol% and not more than 81.4 vol% (by specific gravity) of ethanol (C\textsubscript{2}H\textsubscript{6}O: 46.07) at 15°C.

Method of preparation

Prepare by mixing the above ingredients.

Description
Ethanol for Disinfection is a colorless, clear liquid.

It is miscible with water.

It burns with a light blue flame on ignition.

It is volatile.

Identification (1) To 1 mL of Ethanol for Disinfection add 2 mL of iodine TS and 1 mL of sodium hydroxide TS, and mix: light yellow precipitates appear.

(2) To 1 mL of Ethanol for Disinfection add 1 mL of acetic acid (100) and 3 drops of sulfuric acid, and heat: the odor of ethyl acetate is produced.

Specific gravity <2.56> d\textsubscript{15}: 0.860 – 0.873

Purity Proceed as directed in the Purity under Ethanol.

Containers and storage—Containers—Tight containers.

Storage—Light-resistant.

Ethenzamide
エテンザミド

C\textsubscript{9}H\textsubscript{11}NO\textsubscript{2}: 165.19
2-Ethoxybenzamide [938-73-8]

Ethenzamide, when dried, contains not less than 98.0% of C\textsubscript{9}H\textsubscript{11}NO\textsubscript{2}.

Description
Ethenzamide occurs as white crystals or crystalline powder.

It is soluble in methanol, in ethanol (95), and in acetone, and practically insoluble in water.

It begins to sublime slightly at about 105°C.

Identification (1) Determine the absorption spectrum of a solution of Ethenzamide in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ethenzamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethenzamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ethenzamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 131 – 134°C

Purity (1) Chloride <1.07>—Dissolve 0.5 g of Ethenzamide in 30 mL of acetone, add 6 mL of dilute nitric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.7 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone and 6 mL of dilute nitric acid, and dilute with water to make 50 mL (not more than 0.050%).

(2) Sulfate <1.14>—Dissolve 0.5 g of Ethenzamide in 30 mL of acetone, add 1 mL of dilute hydrochloric acid, and dilute with water to 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 30 mL of acetone and 1 mL of dilute hydrochloric acid, and dilute with water to 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Ethenzamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—To 0.40 g of Ethenzamide add 0.3 g of potassium nitrate and 0.5 g of anhydrous sodium carbonate, mix thoroughly, ignite the mixture gradually, and cool. Dissolve the residue in 10 mL of dilute sulfuric acid, and
heat the solution until white fumes begin to evolve. After cooling, add water carefully to make 5 mL, use this solution as the test solution, and perform the test (not more than 5 ppm).

(5) Salicylamide—Dissolve 0.20 g of Ethenzamide in 15 mL of diluted ethanol (95) (2 in 3), and add 2 to 3 drops of dilute iron (III) chloride TS: no purple color develops.

Loss on drying <2.4d> Not more than 1.0% (1 g, silica gel, 3 hours).

Residue on ignition <2.4d> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Ethenzamide and Ethenzamide RS, previously dried, and dissolve each in 70 mL of ethanol (95) by warming, and after cooling, add ethanol (95) to make exactly 50 mL. Pipet 5 mL each of these solutions, add ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, \( A_t \) and \( A_s \), of the sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry <2.2d>, using ethanol (95) as the blank.

\[
\text{Amount (mg) of C}_9\text{H}_11\text{NO}_2 = M_s \times A_t/A_s
\]

\( M_s \): Amount (mg) of Ethenzamide RS

Containers and storage Containers—Well-closed containers.

Ether エーテル

\[ \text{C}_2\text{H}_6\text{O}: 74.12 \]

Diethyl ether [60-29-7]

Ether contains not less than 96% and not more than 98% (by specific gravity) of \( \text{C}_2\text{H}_6\text{O} \).

It contains a small quantity of ethanol and water. It cannot be used for anesthesia.

Description Ether is a colorless, clear, mobile liquid, having a characteristic odor.

It is miscible with ethanol (95).

It is soluble in water.

It is highly volatile and flammable.

It is slowly oxidized by the action of air and light, with the formation of peroxides.

Its vapor, when mixed with air and ignited, may explode violently.

Boiling point: 35 – 37°C

Specific gravity <2.5d> \( d_{20}^5 \): 0.718 – 0.721

Purity (1) Foreign odor—Place 10 mL of Ether in an evaporating dish, and allow it to evaporate spontaneously to a volume of about 1 mL: no foreign odor is perceptible. Drop this residue onto a piece of clean, odorless filter paper to evaporate the ether: no foreign odor is perceptible.

(2) Acidity—Place 10 mL of diluted ethanol (95) (4 in 5) and 0.5 mL of phenolphthalein TS in a 50-mL glass-stopped flask, and add 0.2 mol/L sodium hydroxide drop-wise to produce a red color which persists after shaking for 30 seconds. Add 25 mL of Ether, stopper the flask, shake gently, and add 0.40 mL of 0.02 mol/L sodium hydroxide VS with shaking: a red color develops.

(3) Aldehyde—Place 10 mL of Ether in a Nessler tube, add 1 mL of potassium hydroxide TS, and allow the mixture to stand for 2 hours, protecting from light, with occasional shaking: no color is produced in the ether layer and the aqueous layer.

(4) Peroxide—Place 10 mL of Ether in a Nessler tube, add 1 mL of a freshly prepared solution of potassium iodide (1 in 10), shake for 1 minute, then add 1 mL of starch TS, and shake well: no color is produced in the ether layer and in the aqueous layer.

(5) Residue on evaporation—Evaporate 140 mL of Ether, and dry the residue at 105°C for 1 hour: the mass of the residue does not more than 1.0 mg.

Containers and storage Containers—Tight containers.

Storage—Without fill up, light-resistant, remote from fire, and not exceeding 25°C.

Anesthetic Ether 麻酔用エーテル

\[ \begin{array}{c}
\text{H}_3\text{C} \quad \text{O} \quad \text{CH}_3 \\
\end{array} \]

\( \text{C}_2\text{H}_6\text{O}: 74.12 \)

Diethyl ether [60-29-7]

Anesthetic Ether contains not less than 96% and not more than 98% (by specific gravity) of \( \text{C}_2\text{H}_6\text{O} \).

It contains small quantities of ethanol and water. Suitable stabilizers may be added.

It is not to be used for anesthesia if it has been removed from the original container for more than 24 hours.

Description Anesthetic Ether occurs as a colorless, clear, mobile liquid, having a characteristic odor.

It is miscible with ethanol (95).

It is soluble in water.

It is highly volatile and flammable.

It is slowly oxidized by the action of air and light, with the formation of peroxides.

Its vapor, when mixed with air and ignited, may explode violently.

Boiling point: 35 – 37°C

Specific gravity <2.5d> \( d_{20}^5 \): 0.718 – 0.721

Purity (1) Foreign odor—Place 10 mL of Anesthetic Ether in an evaporating dish, and allow it to evaporate spontaneously to a volume of about 1 mL: no foreign odor is perceptible. Drop this residue onto a piece of clean, odorless filter paper to evaporate the ether: no foreign odor is perceptible.

(2) Acidity—Place 10 mL of diluted ethanol (95) (4 in 5) and 0.5 mL of phenolphthalein TS in a 50-mL glass-stopped flask, and add 0.2 mol/L sodium hydroxide drop-wise to produce a red color which persists after shaking for
30 seconds. Add 25 mL of Anesthetic Ether, stopper the flask, shake gently, and add 0.40 mL of 0.02 mol/L sodium hydroxide VS with shaking: a red color develops.

(3) Aldehyde—To 100 mL of water in a 200-mL glass-stoppered flask add 10 mL of Anesthetic Ether and 1 mL of a solution of sodium hydrogen sulfite (1 in 1000), stopper tightly, shake vigorously for 10 seconds, and allow the mixture to stand in a cool place for 30 minutes, protected from light. Add 2 mL of starch TS, and add dropwise 0.01 mol/L iodine VS until a pale blue color develops. Shake with about 2 g of sodium hydrogen carbonate to decolorize the solution, and add 1 mL of diluted 0.01 mol/L iodine VS (9 in 40): a blue color develops. Keep the temperature of the solution below 18°C during the procedure.

(4) Peroxide—Place 10 mL of Anesthetic Ether in a Nessler tube, add 1 mL of a freshly prepared solution of potassium iodide (1 in 10), shake occasionally for 1 hour, prepare in a test tube, dissolve in 10 mL of a solution of silver nitrate (1 in 20), and titrate with 0.01 mol/L sodium hydroxide VS with shaking: a red color develops.

Residue on evaporation—Evaporate 50 mL of Anesthetic Ether, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Containers and storage
Containers—Tight containers.
Storage—Without fill up, light-resistant, remote from fire, and not exceeding 25°C.

**Ethinylestradiol Tablets**

Ethinylestradiol Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of ethinylestradiol (C₉H₂₄O₂: 296.40).

**Method of preparation** Prepare as directed under Tablets, with Ethinylestradiol.

**Identification (1)** Evaporate to dryness 5 mL of the sample solution obtained in Assay, and add 2 mL of a mixture of sulfuric acid and ethanol (95) (2:1) to the residue: a light red color with a yellow fluorescence develops. To the solution add carefully 4 mL of water: the color of the solution changes to red-purple.

(2) Evaporate to dryness 10 mL of the sample solution obtained in Assay, add 0.2 mL of acetic acid (31) and 2 mL of phosphoric acid to the residue, and heat on a water bath for 5 minutes: a red color with a yellow-green fluorescence develops.

**Uniformity of dosage units** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Ethinylestradiol Tablets in a separator, add 10 mL of 2nd fluid for disintegration test, and shake until the tablet is disintegrated. Add 10 mL of dilute sulfuric acid and 20 mL of chloroform, shake vigorously for 5 minutes, and filter the chloroform layer into a conical flask through filter paper on which 5 g of anhydrous sodium sulfate is placed. Extract the aqueous layer with two 20-mL portions of chloroform, proceed with the extracts in the same manner as before, and combine the filtrates with the
previous one. Evaporate gently the combined filtrate on a water bath with the aid of a current of nitrogen, dissolve the residue in exactly 100 mL of methanol, and centrifuge, if necessary. Pipet x mL of the supernatant liquid, add methanol to make exactly V mL of a solution containing about 0.04 µg of ethinylestradiol (C_{18}H_{20}O_{2}) per mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ethinylestradiol RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in methanol, dilute to a volume containing about 0.04 µg of ethinylestradiol (C_{18}H_{20}O_{2}) per mL, and use this solution as the standard solution. Pipet 4 mL each of sulfuric acid-methanol TS into three glass-stoppered test tubes, T, S and B, cool in ice, to each tube add exactly 1 mL each of the sample solution, the standard solution and methanol, shake immediately, and allow to stand in a water bath at 30°C for 40 minutes, then allow to stand in a water bath at 20°C for 5 minutes. Perform the test with these solutions as directed under Fluorometry <2.22>. Determine the fluorescence intensities, F_T, F_S and F_B, of these solutions using the fluorophotometer, at about 460 nm of the excitation and at about 493 nm of the fluorescence.

\[
\text{Amount (mg) of ethinylestradiol (C}_{18}\text{H}_{20}\text{O}_{2}) = M_T \times \left(\frac{F_T - F_B}{F_S - F_B}\right) \times \frac{1}{2500} \times \frac{1}{x}
\]

\[
M_S: \text{Amount (mg) of Ethinylestradiol RS}
\]

**Dissolution** (6.10) Being specified separately.

**Assay** (i) Chromatographic tube: Pack a pledget of glass wool in the bottom of a tube 25 mm in inside diameter and 300 mm in length, and place 5 g of anhydrous sodium sulfate on the glass wool.

(ii) Chromatographic column: Place 5 g of siliceous earth for chromatography in a 200-mL beaker, soak well in 4 mL of 1 mol/L hydrochloric acid TS, and mix uniformly. Put the siliceous earth into the chromatographic tube in small portions to make 60 to 80 mm in height in proper hardness with a tamping rod.

(iii) Standard solution: Weigh accurately about 10 mg of Ethinylestradiol RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in chloroform to make exactly 100 mL. Pipet 5 mL of this solution, and add chloroform to make exactly 100 mL.

(iv) Sample: Weigh accurately not less than 20 Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.5 mg of ethinylestradiol (C_{18}H_{20}O_{2}), place in a 50-mL beaker, add 2 mL of water, shake well, add 3 mL of chloroform, and shake well again. Add 4 g of siliceous earth for chromatography, mix well until the contents do not stick to the inner wall of the beaker, and use the substance as the sample.

(v) Procedure: To the chromatographic column add the sample with a funnel, and pack in proper hardness. Mix well the sample sticking to the beaker with 0.5 g of siliceous earth for chromatography, and place in the chromatographic tube. Wipe off the sample solution sticking to the beaker and the tamping rod with glass wool, and place it in the chromatographic tube. Push down the sample, and press lightly on the chromatographic column to make the height of the column 110 mm to 130 mm. Take 70 mL of chloroform, rinse the inner wall of the chromatographic tube with a portion of the chloroform, and transfer the remaining portion to the chromatographic tube. Collect the effluent solution at a flow rate not more than 0.8 mL per minute. After completing the elution, rinse the lower end of the chromatographic tube with a small quantity of chloroform, add chloroform to make exactly 100 mL, and use this solution as the sample solution. Transfer 6 mL each of the sample solution and standard solution to each separator, and add 20 mL each of isoctane. Add exactly 10 mL of a mixture of sulfuric acid and methanol (7:3), shake vigorously for 5 minutes, allow to stand in a dark place for 15 minutes, and centrifuge. Perform the test with the resulting color solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 6 mL of chloroform in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions obtained from the sample solution and standard solution at 540 nm, respectively.

\[
\text{Amount (mg) of ethinylestradiol (C}_{18}\text{H}_{20}\text{O}_{2}) = M_S \times \frac{A_T}{A_S} \times \frac{1}{20}
\]

**Ethionamide**

**Containers and storage** Coniners—Well-closed containers.

**C_{8}H_{10}N_{2}S**: 166.24
2-Ethylpyridine-4-carbothioamide [536-33-4]

Ethionamide, when dried, contains not less than 98.5% and not more than 101.0% of C_{8}H_{10}N_{2}S.

**Description** Ethionamide occurs as yellow crystals or crystalline powder, having a characteristic odor.

It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5) and in acetone, and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Ethionamide in methanol (3 in 160,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethionamide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.60> 161 – 165°C

**Purity** (1) Acidity—Dissolve 3.0 g of Ethionamide in 30 mL of methanol by warming, add 90 mL of water, allow to stand in ice water for 1 hour, and filter. To 80 mL of the filtrate add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.
Ethosuximide / Official Monographs

(2) Heavy metals \(<1.07\>—Proceed with 1.0 g of Ethionamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic \(<1.11\>—Prepare the test solution with 1.0 g of Ethionamide according to Method 3. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.20 g of Ethionamide in 10 mL of acetic acid (100), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution (1). Separately, pipet exactly 0.2 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.07\>.

Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, hexane and methanol (6:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution (1), and number of the spot other than the principal spot obtained with the sample solution which is more intense than the spot with the standard solution (2) is not more than one.

Loss on drying \(<2.41\>—Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition \(<2.44\>—Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Ethionamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate \(<2.50\> with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-red to dark orange-brown (indicator: 2 mL of \(p\)-naphtholbenzein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 16.62 mg of \(C_7H_{11}NO_2\)

Containers and storage Containers—Well-closed containers.

Ethosuximide

エトスクシミド

\[
\begin{array}{c}
O \\
\text{C}_7\text{H}_{11}\text{NO}_2: 141.17 \\
(2RS)-2-Ethyl-2-methylsuccinimide \\
[77-67-9]
\end{array}
\]

Ethosuximide contains not less than 98.5% of \(C_7H_{11}NO_2\) calculated on the anhydrous basis.

Description Ethosuximide occurs as a white, paraffin-like solid or powder. It is odorless or has a slight, characteristic odor.

It is very soluble in methanol, in ethanol (95), in diethyl ether, and in \(N,N\)-dimethylformamide, and freely soluble in water.

Melting point: about 48°C

Identification (1) To 0.2 g of Ethosuximide add 10 mL of sodium hydroxide TS, and boil: the gas evolved turns a moistened red litmus paper blue.

(2) Dissolve 0.05 g of Ethosuximide in 1 mL of ethanol (95), add 3 drops of a solution of copper (II) acetate monohydrate (1 in 100), warm slightly, and add 1 to 2 drops of sodium hydroxide TS: a purple color is produced.

(3) Determine the absorption spectrum of a solution of Ethosuximide in ethanol (95) (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ethosuximide in 10 mL of water: the solution is clear and colorless.

(2) Chloride \(<1.03\>—With 1.0 g of Ethosuximide, perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Heavy metals \(<1.07\>—Proceed with 1.0 g of Ethosuximide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic \(<1.11\>—Prepare the test solution with 1.0 g of Ethosuximide, according to Method 1, and perform the test (not more than 2 ppm).

(5) Acid anhydride—Dissolve 0.50 g of Ethosuximide in 1 mL of ethanol (95), add 1 mL of hydroxylammonium chloride-iron (III) chloride TS, and allow to stand for 5 minutes. Add 3 mL of water, mix, and allow to stand for 5 minutes: the red to red-purple color of this solution is not more intense than that of the following control solution.

Control solution: Dissolve 70 mg of succinic anhydride in ethanol (95) to make exactly 100 mL. To 1.0 mL of this solution add 1 mL of hydroxylammonium chloride-iron (III) chloride TS, and proceed in the same manner.

(6) Cyanide—Dissolve 1.0 g of Ethosuximide in 10 mL of ethanol (95), and add 3 drops of iron (II) sulfate TS, 1 mL of sodium hydroxide TS and 2 to 3 drops of iron (III) chloride TS. Warm gently, and acidify with dilute sulfuric acid: not a blue precipitate and a blue color are produced within 15 minutes.

Water \(<2.48\>—Not more than 0.5% (2 g, volumetric titration, direct titration).

Residue on ignition \(<2.44\>—Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ethosuximide, dissolve in 20 mL of \(N,N\)-dimethylformamide, and titrate \(<2.50\> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 14.12 mg of \(C_7H_{11}NO_2\)
Ethyl Aminobenzoate

Anestamine

Benzocaine

Ethyl 4-aminobenzoate

C₉H₁₁NO₂: 165.19
Ethyl 4-aminobenzoate
[94-09-7]

Ethyl Aminobenzoate, when dried, contains not less than 99.0% of C₉H₁₁NO₂.

Description Ethyl Aminobenzoate occurs as white crystals or crystalline powder. It is odorless. It has a slightly bitter taste, numbing the tongue.

It is freely soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It dissolves in dilute hydrochloric acid.

Identification (1) Dissolve 0.01 g of Ethyl Aminobenzoate in 1 mL of dilute hydrochloric acid and 4 mL of water. This solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Dissolve 0.1 g of Ethyl Aminobenzoate in 5 mL of water with the aid of dilute hydrochloric acid added dropwise, and add iodine TS dropwise: a brown precipitate is produced.

(3) Warm 0.05 g of Ethyl Aminobenzoate with 2 drops of acetic acid (31) and 5 drops of sulfuric acid: the odor of ethyl acetate is perceptible.

Melting point <2.60> 89 – 91°C

Purity (1) Acidity—Dissolve 1.0 g of Ethyl Aminobenzoate in 10 mL of neutralized ethanol, and add 10 mL of water, 2 drops of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color is produced.

(2) Chloride—Dissolve 0.20 g of Ethyl Aminobenzoate in 5 mL of ethanol (95), add 2 to 3 drops each of dilute nitric acid and of silver nitrate TS: no change occurs immediately.

(3) Heavy metals <1.07>—Dissolve 2.0 g of Ethyl Aminobenzoate in 20 mL of ethanol (95), add 2 mL of dilute acetic acid and ethanol (95) to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and sufficient ethanol (95) to make 50 mL (not more than 10 ppm).

(4) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Ethyl Aminobenzoate: the solution has no more color than Matching Fluid A.

Loss on drying <2.47> Not more than 1.0% (1 g, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Ethyl Aminobenzoate, previously dried, dissolve in 10 mL of hydrochloric acid and 70 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), and cool to a temperature below 15°C. Then titrate <2.50> with 0.1 mol/L sodium nitrite VS by the potentiometric titration or the amperometric titration.

Each mL of 0.1 mol/L sodium nitrite VS
= 16.52 mg of C₉H₁₁NO₂

Containers and storage Containers—Tight containers.

Ethyl L-Cysteine Hydrochloride

Ethyl Cysteine Hydrochloride

Ethyl (2R)-2-amino-3-sulfanylpropanoate monohydrochloride

C₅H₁₁NO₂S.HCl: 185.67
Ethyl (2R)-2-amino-3-sulfanylpropanoate monohydrochloride
[868-59-7]

Ethyl L-Cysteine Hydrochloride, when dried, contains not less than 98.5% of C₅H₁₁NO₂S.HCl.

Description Ethyl L-Cysteine Hydrochloride occurs as white crystals or crystalline powder. It has a characteristic odor, and has a bitter taste at first with a burning aftertaste. It is very soluble in water, and freely soluble in ethanol (95).

Melting point: about 126°C (with decomposition).

Identification (1) Determine the infrared absorption spectrum of Ethyl L-Cysteine Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Ethyl L-Cysteine Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> (1) for chloride.

Optical rotation <2.49> [α]D: −10.0 – −13.0° (after drying, 2.0 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity (1) Sulfate <1.14>—Perform the test with 0.6 g of Ethyl L-Cysteine Hydrochloride. Prepare the the control solution with 0.35 mL of 0.005 mol/L sulfuric acid (not more than 0.028%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ethyl L-Cysteine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure rapidly. Dissolve 0.05 g each of Ethyl L-Cysteine Hydrochloride and N-ethylmaleimide in 5 mL of mobile phase, allow to stand for 30 minutes, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sam-
Ethyl Icosapentate

**Containers and storage**
Containers—Tight containers.

**Ethyl Icosapentate**

**Description**
Ethyl Icosapentate is a colorless or pale yellow, clear liquid. It has a faint, characteristic odor. It is miscible with ethanol (99.5), with acetic acid (100) and with hexane. It is practically insoluble in water and in ethylene glycol.

**Identification**
(1) To 20 mg of Ethyl Icosapentate add 3 mL of a solution of potassium hydroxide in ethylene glycol (21 in 100), stopper tightly while passing a current of nitrogen, and heat at 180°C for 15 minutes. After cooling, add methanol to make 100 mL. To 4 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared in the same manner as the sample solution with 3 mL of the solution of potassium hydroxide in ethylene glycol (21 in 100), as a control, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ethyl Icosapentate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethyl Icosapentate as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ethyl Icosapentate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Refractive index**
<2.45> $n^D_{20}$: 1.481 - 1.491

**Specific gravity**
<2.56> $d^D_{20}$: 0.905 - 0.915

**Acid value**
<1.13> Not more than 0.5.

**Saponification value**
<1.13> 165 - 175

**Iodine value**
<1.13> 365 - 395 Perform the test with 20 mg of Ethyl Icosapentate.

**Purity**
(1) Heavy metals <1.07>—Mix 1.0 g of Ethyl Icosapentate with ethanol (99.5), and add 2 mL of dilute acetic acid and ethanol (99.5) to make 50 mL. Perform the test with this solution as the test solution. Control solution: To 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and ethanol (99.5) to make 50 mL (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g
of Ethyl Icosapentate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—To 0.40 g of Ethyl Icosapentate add hexane to make 50 mL, and use this solution as the sample solution. Perform the test with 1.5 μL of the sample solution as directed under Gas Chromatography (2.02) according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of these peaks by the area percentage method: the area of the peak, having the relative retention time of about 0.53 with respect to ethyl icosapentate, is not more than 0.5%, the area of the two peaks, having the relative retention time of about 0.80 and 0.93, is not more than 1.0%, the area of the peak other than the principal peak and other than the peak mentioned above is not more than 1.0%, and the total amount of these peaks other than the principal peak is not more than 3.5%.

Operating conditions—

Detector, column, column temperature, carrier gas, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of ethyl icosapentate, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the sample solution add hexane to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add hexane to make exactly 10 mL. Confirm that the peak area of ethyl icosapentate obtained from 1.5 μL of this solution is equivalent to 7 to 13% of that from 1.5 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 1.5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethyl icosapentate to that of the internal standard is about 2.0%.

(4) Peroxide—Weigh accurately about 1 g of Ethyl Icosapentate, put in a 200-mL glass-stoppered conical flask, add 25 mL of a mixture of acetic acid (100) and chloroform (3:2), and dissolve by gentle shaking. Add 1 mL of saturated potassium iodide solution TS, immediately stopper tightly, shake gently, and allow to stand in a dark place for 10 minutes. Then add 30 mL of water, shake vigorously for 5 to 10 seconds, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears after addition of 1 mL of starch TS. Calculate the amount of peroxide by the following equation: not more than 2 mEq/kg.

Amount (mEq/kg) of peroxide = \( V \times \frac{M}{Z} \times 10 \)

\( V \): Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed

\( M \): Amount (g) of the sample

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Ethyl Icosapentate, and add hexane to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of Ethyl Icosapentate RS, and add hexane to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 3 μL each of the sample solution and standard solution as directed under Gas Chromatography (2.02) according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of ethyl icosapentate to that of the internal standard.

\[ M_S = \text{Amount (mg) of Ethyl Icosapentate RS} \]

Internal standard solution—A solution of methyl docosanate in hexane (1 in 125).

Operating conditions—

Detector: A hydrogen-flame ionization detector.

Column: A glass column in 4 mm inside diameter and 1.8 m in length, packed with siliceous earth for gas chromatography (175 to 246 μm in particle diameter), coated with diethylene glycol succinate polyester for gas chromatography in the ratio of 25%.

Column temperature: A constant temperature of about 190°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of ethyl icosapentate is about 30 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the internal standard and ethyl icosapentate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethyl icosapentate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Being fully filled, or replacing the air with Nitrogen.

Ethyl Parahydroxybenzoate

パラオキシ安息香酸エチル

(1) 

\( \text{C}_8\text{H}_8\text{O}_3 \): 166.17

Ethyl 4-hydroxybenzoate

[120-47-8]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (*).
Ethylenediamine / Official Monographs

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ethyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add water to make 1000 mL.

(2) Acidity—Dissolve 0.20 g of Ethyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Ethyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).

(4) Related substances—Dissolve 0.10 g of Ethyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.60>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot is not more intense than the spot obtained with the standard solution.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1.0 g of Ethyl Parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point. (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS = 166.2 mg of C₉H₁₀O₃

Ethylparahydroxybenzoic acid, melting point between 115°C and 118°C.

*Containers and storage Containers—Well-closed containers.

Identification (1) The melting point <2.60> of Ethyl Parahydroxybenzoate is between 115°C and 118°C.

(2) Determine the infrared absorption spectrum of Ethyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Ethylenediamine contains not less than 97.0% of C₉H₁₂N₂.

Description Ethylenediamine is a clear, colorless to pale yellow liquid. It has an ammonia-like odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

It has a caustic nature and an irritating property.

It is gradually affected by air.

Specific gravity \( d_{20}^{20} \approx 0.898 \)

Identification (1) A solution of Ethylenediamine (1 in 500) is alkaline.

(2) To 2 mL of copper (II) sulfate TS add 2 drops of Ethylenediamine: a blue-purple color develops.

(3) To 0.04 g of Ethylenediamine add 6 drops of benzoyl chloride and 2 mL of a solution of sodium hydroxide (1 in 10), warm for 2 to 3 minutes with occasional shaking, collect the white precipitate formed, and wash with water. Dissolve the precipitate in 8 mL of ethanol (95) by warming, promptly add 8 mL of water, cool, filter the crystals, wash with water, and dry at 105°C for 1 hour: it melts <2.60> between 247°C and 251°C.

Purity (1) Heavy metals <1.07>—Place 1.0 g of Ethylenediamine in a porcelain crucible, evaporate to dryness on a water bath, cover loosely, ignite at a low temperature until charred, proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Residue on evaporation—Pipet 5 mL of Ethylenediamine, heat on a water bath to dryness, and dry to constant mass at 105°C: the mass of the residue does not exceed 3.0 mg.

Distilling range <2.57> 114 – 119°C, not less than 95 vol%.

Assay Weigh accurately about 0.7 g of Ethylenediamine in a glass-stoppered conical flask, add 50 mL of water, and titrate <2.50> with 1 mol/L hydrochloric acid VS (indicator: 3 drops of bromophenol blue TS).

Each mL of 1 mol/L hydrochloric acid VS = 30.05 mg of C₉H₁₂N₂

Containers and storage Containers—Tight containers.

Storge—Light-resistant, and almost well-filled.
Ethylmorphine Hydrochloride Hydrate

Dionin

Description

Ethylmorphine Hydrochloride Hydrate occurs as white to pale yellow crystals or crystalline powder. It is very soluble in methanol and in acetic acid (100), freely soluble in water, soluble in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether. It is affected by light. Melting point: about 123°C (with decomposition).

Identification

(1) Determine the absorption spectrum of a solution of Ethylmorphine Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethylmorphine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ethylmorphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests (1.09) (2) for chloride.

Optical rotation

$\langle 2.45 \rangle \quad [\alpha]_{D}^{20} = -103 - -106^\circ$ (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH

$\langle 2.54 \rangle \quad$ Dissolve 0.10 g of Ethylmorphine Hydrochloride Hydrate in 10 mL of water: the pH of this solution is between 4.0 and 6.0.

Purity

Related substances—Dissolve 0.20 g of Ethylmorphine Hydrochloride Hydrate in 10 mL of diluted ethanol (1 in 2), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add diluted ethanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water

$\langle 2.48 \rangle \quad 8.0 - 10.0\% \quad (0.25\, g, \text{ volumetric titration}, \text{ direct titration}).$

Residue on ignition

Not more than 0.1% (0.5 g).

Assay

Weigh accurately about 0.5 g of Ethylmorphine Hydrochloride Hydrate, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS $= 34.99$ mg of C$_{19}$H$_{23}$NO$_3$.HCl.

Containers and storage

Containers—Tight containers. Storage—Light-resistant.

Etidronate Disodium

Description

Etidronate Disodium occurs as a white powder. It is freely soluble in water, and practically insoluble in ethanol (99.5). The pH of a solution prepared by dissolving 0.10 g of Etidronate Disodium in 10 mL of water is between 4.4 and 5.4.

It is hygroscopic.

Identification

(1) To 5 mL of a solution of Etidronate Disodium (1 in 100) add 1 mL of copper (II) sulfate TS, and mix for 10 minutes: a blue precipitate is formed.

(2) Determine the infrared absorption spectrum of Etidronate Disodium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Etidronate Disodium (1 in 50) responds to the Qualitative Tests (1.09) for sodium salt.

Purity

Heavy metals $\langle 1.07 \rangle$—Proceed with 1.0 g of Etidronate Disodium according to Method 4, and perform the test using the supernatant liquid obtained by centrifuging
after addition of 2 mL of dilute acetic acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \(<1.1\) Prepare the test solution with 1.0 g of Etidronate Disodium according to Method 1, and perform the test (not more than 2 ppm).

(3) Phosphite—Weigh accurately about 3.5 g of Etidronate Disodium, dissolve in 100 mL of 0.1 mol/L sodium dihydrogen phosphate TS adjusted the pH to 8.0 with sodium hydroxide TS, add exactly 20 mL of 0.05 mol/L iodine VS, and immediately stopper tightly. Allow to stand in a dark place for 30 minutes, add 1 mL of acetic acid (100), and titrate \(2.50\) the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction. The amount of phosphite (NaH₂PO₃) is not more than 1.0%.

Each mL of 0.05 mol/L iodine VS = 5.199 mg of NaH₂PO₃

(4) Methanol—Weigh accurately about 0.5 g of Etidronate Disodium, dissolve in water to make exactly 5 mL, and use this solution as the sample solution. Separately, pipet 1 mL of methanol, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 mL each of the sample solution and standard solution as directed under Gas Chromatography \(<2.02\) according to the following conditions, and determine the peak areas of methanol, \(A_1\) and \(A_2\), and determine the amount of methanol (CH₄O) by the following equation: not more than 0.1%.

\[
\text{Amount (\%)} = \frac{M}{1/M \times A_1/A_2} \times \frac{1}{20} \times 0.79
\]

\[M: \text{Amount (g) of sample}
\]

\[0.79: \text{Density (g/mL) of methanol}
\]

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 2 m in length, packed with porous copolymer beads for gas chromatography (180 – 250 \(\mu\)m in particle diameter).
Column temperature: A constant temperature of about 130°C.
Carrier gas: Nitrogen.
Flow rate: Adjust the flow rate so that the retention time of methanol is about 2 minutes.

System suitability—
System performance: To 1 mL of methanol and 1 mL of ethanol (99.5) add water to make 100 mL. To 1 mL of this solution add water to make 100 mL. When the procedure is run with 1 \(\mu\)L of this solution under the above operating conditions, methanol and ethanol are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 1 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methanol is not more than 5.0%.

Loss on drying \(<2.41\) Not more than 5.0% (0.5 g, 210°C, 2 hours).

Assay Weigh accurately about 0.5 g of Etidronate Disodium, previously dried, and dissolve in water to make exactly 50 mL. Transfer exactly 15 mL of this solution to a chromatographic column of 10 mm in internal diameter containing 5 mL of strongly acidic ion exchange resin for column chromatography (H type), allow to flow at a flow rate of about 1.5 mL per minute, and wash the column with two 25-mL portions of water. Combine the eluate and the washings, and titrate \(<2.50\) with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 12.50 mg of C₂H₆Na₂O₇P₂

Containers and storage Containers—Tight containers.

Etidronate Disodium Tablets

エチドロン酸二ナトリウム錠

Etidronate Disodium Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of etidronate disodium (C₂H₆Na₂O₇P₂: 249.99).

Method of preparation Prepare as directed under Tablets, with Etidronate Disodium.

Identification (1) Shake an amount of pulverized Etidronate Disodium Tablets, equivalent to 0.2 g of Etidronate Disodium according to the labeled amount, with 20 mL of water, and filter. Proceed with the filtrate as directed in the Identification (1) under Etidronate Disodium.

(2) Shake an amount of pulverized Etidronate Disodium Tablets, equivalent to 0.4 g of Etidronate Disodium according to the labeled amount, with 10 mL of water, and filter. Evaporate total amount of the filtrate to dryness under reduced pressure, shake the residue with 15 mL of ethanol (99.5), centrifuge, and dry the precipitate at 150°C for 4 hours. Determine the infrared absorption spectrum of the precipitate as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\): it exhibits absorption at the wave numbers of about 1170 cm\(^{-1}\), 1056 cm\(^{-1}\), 916 cm\(^{-1}\) and 811 cm\(^{-1}\).

Uniformity of dosage unit \(<6.02\) It meets the requirement of the Mass variation test.

Dissolution \(<6.10\) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Etidronate Disodium Tablets is not less than 85%.

Start the test with 1 tablet of Etidronate Disodium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \(\mu\)m. Discard the first 10 mL of the filtrate, take exactly \(V\) mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains about 0.22 mg of etidronate disodium (C₂H₆Na₂O₇P₂) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of etidronate disodium for assay, previously dried at 210°C for 2 hours, and dissolve in water to
make exactly 100 mL. Dilute exactly a suitable amount of this solution with water to make solutions so that each mL contains about 0.12 mg, about 0.21 mg and about 0.24 mg of etidronate disodium ([C2H6Na2O7P2]), and use these solutions as the standard solutions. Pipet 2 mL each of the sample solution and standard solutions, add exactly 2 mL of a solution of copper (II) sulfate (7 in 10,000) with water to make exactly 10 mL. Determine the absorbances of these solutions at 233 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by diluting exactly 2 mL of the solution of copper (II) sulfate (7 in 10,000) with water to make exactly 10 mL as the control. From the calibration curve obtained with the standard solutions calculate the concentration of etidronate disodium, C1, in the sample solution.

Dissolution rate (%) with respect to the labeled amount of etidronate disodium ([C2H6Na2O7P2])

\[ C_1 \times \frac{V}{V} \times \frac{1}{C} \times 90 \]

C1: Concentration (µg/mL) of etidronate disodium ([C2H6Na2O7P2]) in the sample solution
C: Labeled amount (mg) of etidronate disodium ([C2H6Na2O7P2]) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Etidronate Disodium Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of etidronate disodium ([C2H6Na2O7P2]), add 30 mL of water, shake vigorously for 10 minutes, add water to make exactly 50 mL, and filter. Proceed with exactly 15 mL of the filtrate as directed in the Assay under Etidronate Disodium.

Containers and storage Containers—Tight containers.

Etilefrine Hydrochloride

エチレフリン塩酸塩

C10H15NO2.HCl: 217.69

\((1R5)-2\text{-Ethylamino-1-(3-hydroxyphenyl)ethanol monohydrochloride} [943-17-9]\)

Etilefrine Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of C10H15NO2.HCl.

Description Etilefrine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in water, freely soluble in ethanol (99.5), and sparingly soluble in acetic acid (100).

It is gradually colored to yellow-brown by light.

A solution of Etilefrine Hydrochloride (1 in 20) shows no optical rotation.

Identification (1) Dissolve 5 mg of Etilefrine Hydrochloride in 100 mL of diluted hydrochloric acid (1 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etilefrine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Etilefrine Hydrochloride (1 in 1000) responds to the Qualitative Tests <1.07> (2) for chloride.

Melting point <2.6D> 118 – 122°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Etilefrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—To 10 mL of a solution of Etilefrine Hydrochloride (1 in 50) add 0.1 mL of methyl red TS for acid or alkali test and 0.2 mL of 0.01 mol/L sodium hydroxide VS: a yellow color develops, and the necessary volume of 0.01 mol/L hydrochloric acid VS to change the color to red is not more than 0.4 mL.

(3) Sulfate <1.14>—Perform the test with 0.85 g of Etilefrine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.020%).

(4) Heavy metals <1.07>—Dissolve 1.0 g of Etilefrine Hydrochloride in 30 mL of water and 2 mL of acetic acid (100), adjust with sodium hydroxide TS to a pH of 3.3, add water to make 50 mL, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.4J> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.4D> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Etilefrine Hydrochloride, previously dried, dissolve in 20 mL of acetic acid (100), adjust with sodium hydroxide TS to a pH of 3.3, add water to make 50 mL, and perform the test. Prepare the control solution with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

\[ = \frac{21.77 \text{ mg of } \text{C}_10\text{H}_15\text{N}_2\text{O}_2\text{HCl}}{2.50} \]

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Etilefrine Hydrochloride Tablets

エチレフリン塩酸塩錠

Etilefrine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of etilefrine hydrochloride ([C10H15NO2.HCl]: 217.69).

Method of preparation Prepare as directed under Tablets, with Etilefrine Hydrochloride.

Identification To a quantity of powdered Etilefrine Hydrochloride Tablets, equivalent to 5 mg of Etilefrine Hydrochlo-
Etilefrine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of etilefrine hydrochloride (C_{10}H_{15}NO_{2}.HCl), add 60 mL of diluted hydrochloric acid (1 in 1000), shake for 10 minutes, add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of etilefrine hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in diluted hydrochloric acid (1 in 1000) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as direct under Liquid Chromatography according to the following conditions, and determine the peak areas, A_{1} and A_{3}, of etilefrine.

\[
M_{5} = \frac{A_{1}}{A_{3}} \times \frac{V}{V' \times 1/C \times 18}
\]

Operating conditions—Proceed as directed in the operating conditions in the Assay.

Containers and storage—Containers—Light-resistant.

Storage—Light-resistant.
Etizolam

エチゾラム

\[
\text{C}_{17}\text{H}_{15}\text{ClN}_{4}\text{S}: 342.85 \\
4-(2-\text{Chlorophenyl})-2-\text{ethyl}-9-\text{methyl}-6\text{-thieno}[3,2-f][1,2,4]\text{triazolo}[4,3-d][1,4]\text{diazepine} \\
\text{(40054-69-1)}
\]

Etizolam, when dried, contains not less than 98.5% and not more than 101.0% of etizolam (C\text{17\text{H}_{15}\text{ClN}_{4}\text{S}}).

**Description** Etizolam occurs as a white to pale yellowish white crystalline powder.

It is soluble in ethanol (99.5), sparingly soluble in acetonitrile and in acetic anhydride, and practically insoluble in water.

**Identification (1)** Determine the absorption spectrum of a solution of Etizolam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etizolam as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.60> 146 - 149°C

**Purity (1)** Heavy metals <1.07>—Proceed with 2.0 g of Etizolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Etizolam in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 20 mL. Pipet 1 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than etizolam obtained from the sample solution is not larger than the peak area of etizolam from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of etizolam is about 6 minutes.

Time span of measurement: About 5 times as long as the retention time of etizolam beginning after the solvent peak.

**System suitability**—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of etizolam obtained from 10 μL of this solution is equivalent to 8 to 12% of that from 10 μL of the standard solution.

System performance: Dissolve 0.02 g each of Etizolam and ethyl parahydroxybenzoate in the mobile phase to make 50 mL. To 1 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ethyl parahydroxybenzoate and etizolam are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etizolam is not more than 2%.

**Loss on drying** <2.44> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Etizolam, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). The end point is the second equivalent point. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 17.14 mg of C\text{17\text{H}_{15}\text{ClN}_{4}\text{S}}

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

Etizolam Fine Granules

エチゾラム細粒

Etizolam Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of etizolam (C\text{17\text{H}_{15}\text{ClN}_{4}\text{S}}: 342.85).

**Method of preparation** Prepare as directed under Granules, with Etizolam.

**Identification (1)** To a quantity of powdered Etizolam Fine Granules, equivalent to 5 mg of Etizolam according to the labeled amount, add 10 mL of methanol, shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Evaporate the filtrate to dryness on a water bath, cool, and then dissolve the residue in 2 mL of sulfuric acid. The solution gives off a light yellow-green fluorescent when exposed to ultraviolet light (main wavelength: 365 nm).

(2) To a quantity of powdered Etizolam Fine Granules,
equivalent to 1 mg of Etizolam according to the labeled amount, add 80 mL of 0.1 mol/L hydrochloric acid TS, shake, and then filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry 2.20; it exhibits absorption maxima between 249 nm and 253 nm, and between 292 nm and 296 nm, when perform the measurement within 10 minutes.

**Uniformity of dosage units** §6.02 The Granules in single-unit containers meet the requirement of the Mass variation test.

**Dissolution** §6.10 When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Etizolam Fine Granules is not less than 75%.

Start the test with an accurately weighed amount of Etizolam Fine Granules, equivalent to about 1 mg of etizolam (C₁₇H₁₅ClN₄S) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of filtrate, pipet 2 mL of the subsequent filtrate, add exactly 2 mL of acetonitrile, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of etizolam for assay, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 4 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of acetonitrile, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography §2.01 according to the following conditions, and determine the peak areas, A₁ and A₅, of etizolam.

Dissolution rate (%) with respect to the labeled amount of etizolam (C₁₇H₁₅ClN₄S) = \( \frac{M₅}{M₈} \times \frac{A₅}{A₈} \times \frac{1}{C} \times \frac{1}{18} \times \frac{5}{5} \)

\( M₅ \): Amount (mg) of etizolam for assay
\( M₈ \): Amount (g) of sample
\( C \): Labeled amount (mg) of etizolam (C₁₇H₁₅ClN₄S) in 1 g

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of etizolam is about 7 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of etizolam are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etizolam is not more than 2.0%.

**Particle size** §6.03 It meets the requirements of Fine granules.

**Assay** Weigh accurately an amount of Etizolam Fine Granules, equivalent to about 4 mg of etizolam (C₁₇H₁₅ClN₄S), add 30 mL of water, and stir. Add 60 mL of methanol, stir for 20 minutes, add methanol to make exactly 100 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add dilute methanol (7 in 10) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of etizolam for assay, previously dried at 105°C for 3 hours, and dissolve in dilute methanol (7 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, and add dilute methanol (7 in 10) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add dilute methanol (7 in 10) to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography §2.01 according to the following conditions, and calculate the ratios, Q₇ and Q₉, of the peak area of etizolam to that of the internal standard.

\[ \text{Amount (mg) of etizolam (C₁₇H₁₅ClN₄S)} = M₅ \times \frac{Q₇}{Q₉} \times \frac{1}{25} \]

\( M₅ \): Amount (mg) of etizolam for assay

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in diluted methanol (7 in 10) (1 in 50,000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of etizolam is about 6 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and etizolam are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of etizolam to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.
Etizolam Tablets

エチゾラム錠

Etizolam Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of etizolam (C₁₇H₁₅ClN₄S: 342.85).  

Method of preparation  Prepare as directed under Tablets, with Etizolam.

Identification (1)  To a quantity of powdered Etizolam Tablets, equivalent to 5 mg of Etizolam according to the labeled amount, add 10 mL of methanol, shake, and filter. Evaporate the filtrate to dryness on a water bath, cool, and then dissolve the residue in 2 mL of sulfuric acid. The solution gives off a light yellow-green fluorescence when exposed to ultraviolet light (main wavelength: 365 nm).

(2)  To a quantity of powdered Etizolam Tablets, equivalent to 2 mg of Etizolam according to the labeled amount, add 80 mL of 0.1 mol/L hydrochloric acid TS, shake, and then filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of this filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits absorption maxima between 249 nm and 253 nm, and between 292 nm and 296 nm when performed under 10 minutes.

Uniformity of dosage units <6.02>  Perform the test according to the following method: to meet the requirement of the Content uniformity test.

Take 1 tablet of Etizolam Tablets, add 2.5 mL of water, and stir until it disintegrates. Add 20 mL of methanol, stir for 20 minutes, add methanol to make exactly 25 mL, and centrifuge. Pipet V mL of the supernatant liquid, add exactly 20 mL of the internal standard solution, add diluted methanol (9 in 10) to make exactly 25 mL, and use this solution as the sample solution. Pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of acetonitrile, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas of etizolam, AT and AS, of both solutions.

Dissolution rate (%) with respect to the labeled amount of etizolam (C₁₇H₁₅ClN₄S)

\[
M_S \times 100 \times V/V \times 1/C \times 9/5
\]

M₅: Amount (mg) of etizolam for assay
C: Labeled amount (mg) of etizolam (C₁₇H₁₅ClN₄S) in 1 tablet

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 243 nm)
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: A mixture of water and acetonitrile (1:1).
Flow rate: Adjust the flow rate so that the retention time of etizolam is about 7 minutes.

System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of etizolam are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etizolam is not more than 2.0%.

Assay  To 20 Etizolam Tablets add 50 mL of water, and stir until they disintegrate. Add 400 mL of methanol, stir for 20 minutes, add methanol to make exactly 500 mL, and centrifuge. Pipet an amount of the supernatant liquid, add exactly 10 mL of the internal standard solution, add diluted methanol (9 in 10) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 100 mg of etizolam for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (9 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, add diluted methanol (9 in 10) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add diluted methanol (9 in 10) to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q₂ and Q₃, of the peak area of etizolam to that of the internal standard.

Amount (mg) of etizolam (C₁₇H₁₅ClN₄S)

\[
M_S \times V/V \times 1/C \times 9/5
\]

M₅: Amount (mg) of etizolam for assay
Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (9 in 10) (1 in 50,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of etizolam is about 6 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and etizolam are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of etizolam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Etodolac

エトドラク

C_{17}H_{21}NO_{3}: 287.35
2-[(1RS)-1,8-Diethyl-1,3,4,9-tetrahydropyranoyl[3,4-b]indol-1-yl]acetic acid
[41340-25-4]

Etodolac, when dried, contains not less than 98.5% and not more than 101.0% of C_{17}H_{21}NO_{3}.

Description Etodolac occurs as white to pale yellow, crystals or crystalline powder.
It is freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.
A solution of Etodolac in methanol (1 in 50) shows no optical rotation.
Melting point: about 147°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Etodolac in ethanol (99.5) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.29>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave lengths.
(2) Determine the infrared absorption spectrum of Etodolac as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Etodolac according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
(2) Related substances—Dissolve 0.5 g of Etodolac in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution (1). Pipet 4 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Previously develop a plate of silica gel with fluorescent indicator for thin-layer chromatography in a developing container containing 2 cm depth of a solution of L-ascorbic acid in a mixture of methanol and water (4:1) (1 in 200 mL) to the distance of 3 cm, and air-dry for 30 minutes. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on the plate 2.5 cm away from the bottom of the plate, then immediately develop with a mixture of toluene, ethanol (95) and acetic acid (100) (140:60:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution (1), and the number of spots which are more intense than the spot with the standard solution (2) is not more than 2.

Loss on drying <2.4f> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition <2.4f> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Etodolac, previously dried, dissolve in 50 mL of ethanol (99.5), and titrate <2.5g> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 28.74 mg of C_{17}H_{21}NO_{3}

Containers and storage Containers—Tight containers.
Storage—Light-resistant.
Etoposide

エトポシド

\[
C_{29}H_{32}O_{13}: 588.56 \\
(5R,5aR,8aR,9S)-9\{[4,6-O-(1R)-Ethylidene-\beta-D-glucopyranosyloxy]5-(4-hydroxy-3,5-dimethoxyphenyl)\}-5,8a,9-tetrahydrofuro[3,4:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one \]

[33419-42-0]

Etoposide contains not less than 98.0% and not more than 102.0% of \( C_{29}H_{32}O_{13} \), calculated on the anhydrous basis.

**Description** Etoposide occurs as white crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Melting point: about 260°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Etoposide in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.44>\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Etoposide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etoposide as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum or the spectrum of Etoposide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \(<2.44>\ \ \left[\alpha\right]_{D}^0: -100 - 105° (0.1 g calculated on the anhydrous basis, methanol, 20 mL, 100 nm).

**Purity** (1) Heavy metals \(<1.07>\)—Proceed with 2.0 g of Etoposide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Etoposide in 10 mL of methanol, add the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and determine each peak area by the automatic integration method:

- the area of the peak other than etoposide with the sample solution is not larger than 1/5 times the peak area of etoposide with the standard solution, and the total area of the peaks other than the peak of etoposide is not larger than 1/2 times the peak area of etoposide with the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of etoposide beginning after the solvent peak.

**System suitability**

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of etoposide obtained with 50 \( \mu \)L of this solution is equivalent to 7 to 13% of that with 50 \( \mu \)L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 50 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etoposide is not more than 2.0%.

**Water** \(<2.44>\) Not more than 4.0% (0.5 g, volumetric titration, direct titration).

**Residue on ignition** \(<2.44>\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 25 mg each of Etoposide and Etoposide RS (previously determined the water \(<2.44>\) in the same manner as Etoposide) dissolve separately in methanol to make exactly 25 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 50 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of etoposide to that of the internal standard.

Amount (mg) of \( C_{29}H_{32}O_{13} = M_S \times Q_T/Q_S \)

**Residue on ignition**

**Internal standard solution**—A solution of 2,6-dichlorophenol in methanol (3 in 2500).

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 290 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with phenylsilanized silica gel for liquid chromatography (10 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 6.44 g of sodium sulfate dehydrate in dilute acetic acid (100) (1 in 100) to make 1000 mL, and add 250 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of etoposide is about 20 minutes.

System performance: Dissolve 10 mg of Etoposide in 2 mL of methanol, add 8 mL of the mobile phase, and mix well. Add 0.1 mL of diluted acetic acid (100) (1 in 25) and 0.1 mL
Eucalyptus Oil

Oleum Eucalypti

Eucalyptus Oil is the essential oil distilled with steam from the leaves of Eucalyptus globulus Labillardièrè or allied plants (Myrtaceae).

It contains not less than 70.0% of cineol (C_{10}H_{18}O: 154.25).

Description Eucalyptus Oil is a clear, colorless or pale yellow liquid. It has a characteristic, aromatic odor and a pungent taste.

It is neutral.

Identification Shake 1 mL of Eucalyptus Oil vigorously with 1 mL of phosphoric acid, and allow to stand: the solution congeals within 30 minutes.

Refractive index <2.45> n_{D}^{20}: 1.458 – 1.470

Specific gravity <1.13> d_{20}^{20}: 0.907 – 0.927

Purity (1) Clarity of solution—Mix 1.0 mL of Eucalyptus Oil with 5 mL of diluted ethanol (7 in 10): the solution is clear.

(2) Heavy metals <1.07>—Proceed with 1.0 mL of Eucalyptus Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Assay Weigh accurately about 0.1 g of Eucalyptus Oil, and dissolve in hexane to make exactly 25 mL. Pipet 5 mL of this solution, then add hexane to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of cineol for assay, proceed as directed in the sample solution, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Calculate the ratios, Q_{1} and Q_{2}, of the peak area of cineol to that of the internal standard of each solutions, respectively.

\[ M_{5} = \frac{M_{6} \times Q_{1}}{Q_{2}} \]

\[ M_{6} \]: Amount (mg) of cineol for assay

Internal standard solution—A solution of anisol in hexane (1 in 250).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 5 m in length, having alkylene glycol phthalate ester for gas chromatography coated at the ratio of 10% on silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 120°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of cineol is about 11 minutes.

Selection of column: Dissolve 0.1 g each of cineol and limonene in 25 mL of hexane. To 1 mL of this solution add hexane to make 20 mL. Proceed with about 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of limonene and cineol in this order with the resolution between these peaks being not less than 1.5.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Famotidine

ファモチジン

C_{11}H_{15}N_{7}O_{2}S_{3}: 337.45

N-Aminosulfonyl-3-[2-(diaminomethyleneamino)-1,3-thiazol-4-yl]methylsulfanyl]propanimidamide

Famotidine, when dried, contains not less than 98.5% of C_{11}H_{15}N_{7}O_{2}S_{3}.

Description Famotidine occurs as white to yellowish white crystals.

It is freely soluble in acetic acid (100), slightly soluble in ethanol (95), and very slightly soluble in water.

It dissolves in 0.5 mol/L hydrochloric acid TS.

It is gradually colored by light.

Melting point: about 164°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Famotidine in 0.05 mol/L potassium dihydrogenophosphate TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Famotidine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
**Purity (1)** Clarity and color of solution—Dissolve 0.5 g of Famotidine in 10 mL of 0.5 mol/L hydrochloric acid TS: the solution is clear and colorless to pale yellow.

(2) Heavy metals \(<0.07\) Proced with 2.0 g of Famotidine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.20 g of Famotidine in 10 mL of acetic acid (100), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetic acid (100) to make exactly 100 mL. Pipet 1 mL, 2 mL and 3 mL of this solution, add acetic acid (100) to make exactly 10 mL, respectively, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography. Under Thin-layer Chromatography, and dry in a stream of nitrogen.

Sample solution is not more than 0.5 ppm.

**Residue on ignition** Under Thin-layer Chromatography, and dry in a stream of nitrogen.

**Related substances**—Take a number of Famotidine (2) related substances—Dissolve 0.20 g of Famotidine in 10 mL of acetic acid (100), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetic acid (100) to make exactly 100 mL. Pipet 1 mL, 2 mL and 3 mL of this solution, add acetic acid (100) to make exactly 10 mL, respectively, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\) Spot 5 µL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel (5 to 7 µm) with fluorescent indicator for thin-layer chromatography, and dry in a stream of nitrogen. Develop the plate with a mixture of ethyl acetate, methanol, toluene and ammonium solution (28) (40:25:20:2) to a distance of about 8 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution (3). Total intensity of the spots other than the principal spot and the spot of the starting point from the sample solution is not more than 0.5% calculated on the basis of intensities of the spots from the standard solution (1) and the standard solution (2).

**Loss on drying** Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 4 hours).

**Residue on ignition** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Famotidine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate \(<2.50\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 16.87 mg of C$_8$H$_{15}$N$_7$O$_2$S$_3$

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Famotidine for Injection**

注射用ファモチジン

Famotidine for Injection is a preparation for injection which is dissolved before use. It contains not less than 94.0% and not more than 106.0% of the labeled amount of famotidine (C$_8$H$_{15}$N$_7$O$_2$S$_3$): 337.45.

**Method of preparation** Prepare as directed under Injec- tion, with Famotidine.

**Description** Famotidine for Injection occurs as white porous masses or powder.

**Identification** Dissolve an amount of Famotidine for Injec- tion, equivalent to 0.01 g of Famotidine according to the labeled amount, in 50 mL of 0.05 mol/L potassium dihydrogenphosphate TS. To 5 mL of this solution add 0.05 mol/L potassium dihydrogenphosphate TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\) it exhibits a maximum between 263 nm and 267 nm.

**pH** Dissolve an amount of Famotidine for Injection, equivalent to 0.02 g of Famotidine according to the labeled amount, in 1 mL of water: the pH of this solution is between 4.9 and 5.5.

**Purity (1)** Clarity and color of solution—Dissolve an amount of Famotidine for Injection, equivalent to 0.02 g of Famotidine according to the labeled amount, in 1 mL of water: the solution is clear and colorless.

(2) Related substances—Take a number of Famotidine for Injection, equivalent to about 0.1 g of famotidine (C$_8$H$_{15}$N$_7$O$_2$S$_3$), dissolve each content in water, wash the inside of the container with water, combine the solutions of the contents with the washings, add water to the combined solution to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than peak of famotidine from the sample solution is not larger than peak area of famotidine from the standard solution.

**System suitability**—Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of famotidine beginning after the solvent peak.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of famotidine is not more than 2.0%.

**Water** Not more than 1.5% (0.1 g, coulometric titration).

**Bacterial endotoxins** Not more than 15 EU/mg.

**Uniformity of dosage units** It meets the requirement of the Mass variation test.

**Foreign insoluble matter** Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** It meets the requirement.
Famotidine Injection

ファモチジン注射液

Famotidine Injection is an aqueous solution for injection.

It contains not less than 92.0% and not more than 108.0% of the labeled amount of famotidine (C₈H₁₅N₇O₂S₃: 337.45).

Method of preparation Prepare as directed under Injections, with Famotidine.

Description Famotidine Injection is a colorless or light yellow, clear liquid.

Identification To an amount of Famotidine Injection, equivalent to 10 mg of Famotidine according to the labeled amount, add water to make 100 mL. Run 1 mL of this solution on a column prepared by filling about 1 cm inside diameter chromatography tube with about 0.4 g of 55 - 105 μm octadecylsilanized silica gel for pretreatment. Wash the column with 15 mL of water, followed by elution with 5 mL of methanol. To the eluate add methanol to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 285 nm and 289 nm.

Osmotic pressure ratio Being specified separately.

pH Being specified separately.

Purity Related substances—To an exact amount of Famotidine Injection, equivalent to 25 mg of Famotidine according to the labeled amount, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 5 μL of each of the standard solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q₂ and Q₃, of the peak area of famotidine to that of the internal standard.

Amount (mg) of famotidine for assay

\[ M_5 = M_s \times \frac{Q_2}{Q_3} \]

\[ M_s: \text{Amount (mg) of famotidine for assay} \]

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust to pH 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 240 mL of acetonitrile and 40 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of famotidine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Sterility<4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take a number of Famotidine for Injection, equivalent to about 0.1 g of famotidine (C₈H₁₅N₇O₂S₃), dissolve each content in water, wash the inside of each container with water, combine the solutions of the contents with the washings, add water to the combined solution to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 5 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q₂ and Q₃, of the peak area of famotidine to that of the internal standard.

Amount (mg) of famotidine for assay

\[ M_5 = M_s \times \frac{Q_2}{Q_3} \times 2 \]

\[ M_s: \text{Amount (mg) of famotidine for assay} \]

Purity Related substances—To an amount of Famotidine Injection, equivalent to 25 mg of Famotidine according to the labeled amount, add water to make 100 mL. Run 1 mL of this solution on a column prepared by filling about 1 cm inside diameter chromatography tube with about 0.4 g of 55 - 105 μm octadecylsilanized silica gel for pretreatment. Wash the column with 15 mL of water, followed by elution with 5 mL of methanol. To the eluate add methanol to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 285 nm and 289 nm.

Osmotic pressure ratio Being specified separately.

pH Being specified separately.

Purity Related substances—To an exact amount of Famotidine Injection, equivalent to 25 mg of Famotidine according to the labeled amount, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of famotidine for assay, dissolve in methanol, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine the peak area of each solution by the automatic integration method, and calculate the amounts of the related substances by the following equation: the amounts of related substances, having the relative retention time about 1.3 and about 1.5 to famotidine are not more than 3.0% respectively, and the amount of other related substances except the above substances is not more than 0.5%, and the total amount of the related substances is not more than 5.0%.

Amount (%) of related substance = \[ M_5 \times A_T/A_S \times 1/10 \]

Total amount (%) of related substances = \[ M_5 \times \Sigma A_T/A_S \times 1/10 \]

\[ M_5: \text{Amount (mg) of famotidine for assay} \]

\[ A_T: \text{Peak area of famotidine in the standard solution} \]

\[ A_S: \text{Peak area of related substances in the sample solution} \]

\[ \Sigma A_T: \text{Total peak area of the related substances in the sample solution} \]

Operating conditions—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay.
Mobile phase: Dissolve 1.74 g of sodium 1-pentane sulfonate in 900 mL of water, adjust to pH 4.0 with diluted acetic acid (100) (1 in 10), and add water to make 1000 mL. To 840 mL of this solution add 80 mL of methanol and 40 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of famotidine is about 17 minutes.

Time span of measurement: About 4 times as long as the retention time of famotidine, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 50 mL. Confirm that the peak area of famotidine obtained with 20 μL of this solution is equivalent to 8 to 12% of that with 20 μL of the standard solution.

System performance: To 20 mg of famotidine for assay add 2 mL of a solution of methyl parahydroxybenzoate in acetonitrile (1 in 500), and add methanol to make 20 mL. To 5 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, famotidine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 19.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of famotidine is not more than 2.0%.

Bacterial endotoxins <4.01> Less than 15 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method I: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Famotidine Injection, equivalent to about 25 mg of famotidine (C8H15N7O2S3) according to the labeled amount, add exactly 2.5 mL of the internal standard solution, and add the mobile phase to make 50 mL. Pipet 10 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol, add exactly 5 mL of the internal standard solution, and add methanol to make 50 mL. Pipet 5 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.24> according to the following conditions. Calculate the ratios, Q1 and Q2 of the peak area of famotidine to that of the internal standard.

\[
\text{Amount (mg) of famotidine (C}_{8}\text{H}_{15}\text{N}_{7}\text{O}_{2}\text{S}_{3}) = M_5 \times \frac{Q_1}{Q_2} \times \frac{1}{2}
\]

M5: Amount (mg) of famotidine for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in acetonitrile (1 in 500).

Operating conditions—
Detector: An ultraviolet absorption photometer (Wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.74 g of sodium 1-pentane sulfonate in 900 mL of water, adjust to pH 4.0 with diluted acetic acid (100) (1 in 10), and add water to make 1000 mL. To 750 mL of this solution add 200 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of famotidine is about 4 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 26.
System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Famotidine Powder

ファモチジン散

Famotidine Powder contains not less than 94.0% and not more than 106.0% of the labeled amount of famotidine (C8H15N7O2S3; 337.45).

Method of preparation Prepare as directed under Granules or Powders, with Famotidine.

Identification Weigh a portion of Famotidine Powder, equivalent to 0.01 g of Famotidine according to the labeled amount, add 50 mL of 0.05 mol/L potassium dihydrogenphosphate TS, shake well, and centrifuge. To 5 mL of the supernatant liquid add 0.05 mol/L potassium dihydrogenphosphate TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 263 nm and 267 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: Famotidine Powder in single-unit containers meets the requirement of the Content uniformity test.

Take out the total amount of the content of 1 container of Famotidine Powder, add 10 mL of water per 10 mg of famotidine (C8H15N7O2S3), shake well, add 10 mL of methanol, shake well, add methanol to make exactly V mL so that each mL contains about 0.4 mg of famotidine (C8H15N7O2S3), and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Proceed as directed in the Assay.
Assay
Weigh accurately a portion of Famotidine Powder, phosphorus (V) oxide at 80 mL of the filtrate, and use the subsequent filtrate as the internal standard. Separately, weigh accurately about 0.1 g of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide. Pipet 5 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and determine the absorbance as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution <6.10>
When the test is performed at 50 revolutions per minute according to the Paddle method, use 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 as the dissolution medium, the dissolution rates in 15 minutes of a 20-mg/g powder and a 100-mg/g powder are not less than 80% and not less than 85%, respectively.

Start the test with an accurately weighed amount of Famotidine Powder, equivalent to about 20 mg of famotidine (C₈H₁₅N₇O₂S₃) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of famotidine for assay, previously dried in vacuum on phosphorus (V) oxide at 80°C for 4 hours, dissolve in the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₂, of the sample solution and standard solution at 266 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of famotidine (C₈H₁₅N₇O₂S₃)

\[ M_s = M_c \times \frac{Q_s}{Q_c} \times 1/C \times 45 \]

Mₕ: Amount (mg) of famotidine for assay
Mₖ: Amount (g) of the sample
C: Labeled amount (mg) of famotidine (C₈H₁₅N₇O₂S₃) in 1 g

Assay
Weigh accurately a portion of Famotidine Powder, equivalent to about 20 mg of famotidine (C₈H₁₅N₇O₂S₃) add 20 mL of water, and shake well. Add 20 mL of methanol, then shake well, add methanol to make exactly 50 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of famotidine for assay, previously dried in vacuum on phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of famotidine to that of the internal standard.

Amount (mg) of famotidine (C₈H₁₅N₇O₂S₃)

\[ M_s = M_c \times \frac{Q_s}{Q_c} \times 1/5 \]

Mₕ: Amount (mg) of famotidine for assay

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust to pH 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 240 mL of acetonitrile and 40 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of famotidine is about 6 minutes.

System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

### Famotidine Tablets

ファモチジン錠

Famotidine Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of famotidine (C₈H₁₅N₇O₂S₃: 337.45).

**Method of preparation** Prepare as directed under Tablets, with Famotidine.

**Identification** Weigh a portion of powdered Famotidine Tablets, equivalent to 0.01 g of Famotidine according to the labeled amount, add 50 mL of 0.05 mol/L potassium dihydrogenphosphate TS, shake well, and centrifuge. To 5 mL of the supernatant liquid add 0.05 mol/L potassium dihydrogenphosphate TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 263 nm and 267 nm.

**Uniformity of dosage units</6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Famotidine Tablets add 2 mL of water, shake to disintegrate, then add a suitable amount of methanol, and shake well. Add methanol to make exactly 10 mL of a solution containing about 0.2 mg of famotidine (C₈H₁₅N₇O₂S₃) per mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of famotidine for assay, previously dried in
vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, and add methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution, and add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the operating conditions described in the Assay, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of famotidine to that of the internal standard.

\[
M_S = \frac{M_S \times Q_T / Q_S \times V}{500}
\]

\( M_S \): Amount (mg) of famotidine for assay

Internal standard solution—To 5 mL of a solution of methyl paraphydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Dissolution—Being specified separately.

Assay—Take a number of Famotidine Tablets, equivalent to 0.2 g of famotidine (\( \text{C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3 \)), add 50 mL of water, and disintegrate by shaking well. Add 100 mL of methanol, then shake well, add methanol to make exactly 200 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of famotidine to that of the internal standard.

\[
M_S = \frac{M_S \times Q_T / Q_S \times 2}{V}
\]

\( M_S \): Amount (mg) of famotidine for assay

Internal standard solution—To 5 mL of a solution of methyl paraphydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylishanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust to pH 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 240 mL of acetonitrile and 40 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of famotidine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Faropenem Sodium Hydrate

ファロペネマナトリウム水和物

\( \text{C}_{12}\text{H}_{14}\text{O}_{5}\text{S} \) 2H₂O: 352.34

Monosodium (5R,6S)-6-[(1R)-1-hydroxyethyl]-7-oxo-3-[(2R)-tetrahydrofuran-2-yl]-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate hemipentahydrate [122547-49-3, anhydride]

Faropenem Sodium Hydrate contains not less than 870 μg (potency) and not more than 943 μg (potency) per mg, calculated on the anhydrous basis. The potency of Faropenem Sodium Hydrate is expressed as mass (potency) of faropenem (\( \text{C}_{12}\text{H}_{15}\text{NO}_{5}\text{S} \): 285.32).

Description—Faropenem Sodium Hydrate occurs as white to light yellow, crystals or crystalline powder. It is freely soluble in water and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification—(1) Dissolve 5 mg of Faropenem Sodium Hydrate in 1 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown to brown color develops.

(2) Determine the absorption spectra of solutions of Faropenem Sodium Hydrate and Faropenem Sodium RS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectra of Faropenem Sodium Hydrate and Faropenem Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D: +145° to +150° (0.5 g calculated as the anhydrous basis, water, 50 mL, 100 mm).

Purity—(1) Heavy metals <1.07>—Proceed with 2.0 g of Faropenem Sodium Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve a quantity of Faropenem Sodium Hydrate equivalent to 0.10 g (potency) in 200
mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine each peak area by the automatic integration method: the peak area of the epimer, having the relative retention time of about 1.1 with respect to faropenem, obtained from the sample solution is not larger than 3/10 times the peak area of faropenem from the standard solution, and the total area of the peaks other than the peak of faropenem from the sample solution is not larger than 1/2 times the peak area of faropenem from the standard solution.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Time span of measurement: About 6 times as long as the retention time of faropenem, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of faropenem obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution obtained in the Assay under the above operating conditions, the internal standard and faropenem are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of faropenem is not more than 2.0%.

Water <2.487> Not less than 12.6% and not more than 13.1% (20 mg, coulometric titration).

Assay Weigh accurately an amount of Faropenem Sodium Hydrate and Faropenem Sodium RS, equivalent to about 25 mg (potency), each of these, add exactly 10 mL each of the internal standard solution, add water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of faropenem to that of the internal standard.

\[
\text{Amount} \ [\mu g \text{ (potency)}] \text{ of faropenem} \ (C_{12}H_{15}NO_{5}S) = M_S \times \frac{Q_T}{Q_S} \times 1000
\]

\( M_S \): amount [mg (potency)] of Faropenem Sodium RS

Internal standard solution—Dissolve 0.5 g of \( m \)-hydroxyacetophenone in 20 mL of acetonitrile, and add water to make 200 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 305 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecytrimethylsilane silica gel for liquid chromatography (5 μm in particle diameter).

Temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.8 g of potassium dihydrogenphosphate, 5.4 g of disodium hydrogen phosphate dodecahydrate and 1.0 g of tetra n-butyl ammonium bromide in water to make 1000 mL. To 870 mL of this solution add 130 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of faropenem is about 11 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and faropenem are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of faropenem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Faropenem Sodium for Syrup シロップ用ファロペンナトリウム

Faropenem Sodium for Syrup is a preparation for syrup, which is dissolved before use.

It contains not less than 93.0% and not more than 106.0% of the labeled amount of faropenem (C_{12}H_{15}NO_{5}S: 285.32).

Method of preparation Prepare as directed under Preparations for Syrups, with Faropenem Sodium Hydrate.

Identification Dissolve an amount of pulverized Faropenem Sodium for Syrup, equivalent to 25 mg (potency) of Faropenem Sodium Hydrate according to the labeled amount, in 50 mL of water. To 5 mL of this solution add water to make 50 mL, filter, if necessary, and determine the absorption spectrum of the solution so obtained as directed under Ultraviolet-visible Spectrophotometry <2.247>: it exhibits maxima between 254 nm and 258 nm, and between 304 nm and 308 nm.

Purity Related substances—Powder Faropenem Sodium for Syrup, if necessary. To a part of the powder, equivalent to about 25 mg (potency) of Faropenem Sodium Hydrate according to the labeled amount, add about 10 mL of water, shake well, then add water to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak of cleaved derivative, having...
the relative retention time of about 0.71 with respect to faropenem, obtained from the sample solution is not larger than 1.5 times the peak area of faropenem from the standard solution, and the total area of the peaks other than the peak of faropenem from the sample solution is not larger than 2 times the peak area of faropenem from the standard solution. For these calculations use the area of the peak of cleaved derivative, having the relative retention time of about 0.71, after multiplying by the relative response factor 0.37.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase A: Dissolve 6.12 g of potassium dihydrogen phosphate, 1.79 g of disodium hydrogen phosphate dodecahydrate and 1.61 g of tetra n-butylammonium bromide in water to make 1000 mL.
Mobile phase B: A mixture of the mobile phase A and acetonitrile (1:1).
Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 54</td>
<td>84 → 30</td>
<td>16 → 70</td>
</tr>
</tbody>
</table>

Flow rate: 1.5 mL per minute.
Time span of measurement: 2.5 times as long as the retention time of faropenem, beginning after the solvent peak.
System suitability—
Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of faropenem obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.
System performance: When the procedure is run with 20 μL of the standard solution obtained in the Assay under the above operating conditions, the internal standard and faropenem are eluted in this order with the resolution between these peaks being not less than 11.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of faropenem is not more than 3.0%.

Water <2.48> Not less than 1.5% and not more than 2.1% (80 mg, coulometric titration).

Uniformity of dosage units <6.02> Faropenem Sodium for Syrup in single-unit containers meet the requirement of the Mass variation test.

Assay Powder, if necessary, and weigh accurately an amount of Faropenem Sodium for Syrup, equivalent to about 25 mg (potency) of faropenem (C_{12}H_{15}NO_{5}S), add exactly 10 mL of the internal standard solution and a suitable amount of water, shake well, and add water to make 50 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg (potency) of Faropenem Sodium RS, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Precede as directed in the Assay under Faropenem Sodium Hydrate.

Amount [mg (potency)] of faropenem (C_{12}H_{15}NO_{5}S) = M_{s} \times Q_{4}/Q_{3}
M_{s}: Amount [mg (potency)] of Faropenem Sodium RS

Internal standard solution—Dissolve 0.5 g of m-hydroxyacetophenone in 20 mL of acetonitrile, and add water to make 200 mL.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Faropenem Sodium Tablets
ファロペネムナトリウム錠

Faropenem Sodium Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of faropenem (C_{12}H_{15}NO_{5}S: 285.32).

Method of preparation Prepare as directed under Tablets, with Faropenem Sodium Hydrate.

Identification To pulverized Faropenem Sodium Tablets, equivalent to 70 mg (potency) of Faropenem Sodium Hydrate according to the labeled amount, add water to make 100 mL. To 5 mL of this solution add water to make 100 mL, filter, if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> : it exhibits maxima between 254 nm and 258 nm and between 304 nm and 308 nm.

Purity Related substances—Powder not less than 5 Faropenem Sodium Tablets. To a part of the powder, equivalent to about 25 mg (potency) of Faropenem Sodium Hydrate according to the labeled amount, add about 10 mL of water, shake well, then add water to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak of cleaved derivative, having the relative retention time of about 0.71 with respect to faropenem, obtained from the sample solution is not larger than 1.5 times the peak area of faropenem from the standard solution, and the total area of the peaks other than the peak of faropenem from the sample solution is not larger than 2.5 times the peak area of faropenem from the standard solution. For these calculation, use the area of the peak of cleaved derivative, having the relative retention time of about 0.71, after multiplying by the relative response factor 0.37.

Operating conditions—
Detector: An ultraviolet absorption photometer (wave-
length: 240 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 6.12 g of potassium dihydrogen phosphate, 1.79 g of disodium hydrogen phosphate dodecahydrate and 1.61 g of tetra n-butylammonium bromide in water to make 1000 mL.

Mobile phase B: A mixture of the mobile phases A and acetonitrile (1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

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</thead>
<tbody>
<tr>
<td>0 - 54</td>
<td>84 → 30</td>
<td>16 → 70</td>
</tr>
</tbody>
</table>

Flow rate: 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of faropenem, beginning after the solvent peak.

System suitability—
Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of faropenem obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution obtained in the Assay under the above operating conditions, the internal standard and faropenem are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of faropenem is not more than 3.0%.

Uniformity of dosage units e6.027 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Faropenem Sodium Tablets add 130 mL of water, shake vigorously until the tablets are disintegrated, and add water to make exactly V mL so that each mL contains about 1 mg (potency) of Faropenem Sodium Hydrate. Pipet 5 mL of this solution, add water to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg (potency) of Faropenem Sodium RS, equivalent to about 18 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry c2.24>, and determine the absorbances, A1 and A5, at 306 nm.

Dissolution c6.10D When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Faropenem Sodium Tablets is not less than 85%.

Start the test with 1 tablet of Faropenem Sodium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V’ mL so that each mL contains about 56 μg (potency) of Faropenem Sodium Hydrate according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Faropenem Sodium RS, equivalent to about 18 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Faropenem Sodium Hydrate.

Amount [mg (potency)] of faropenem (C12H15NO5S) = M5 × A1/AS × V2/25

M5: Amount [mg (potency)] of Faropenem Sodium RS

Dissolution rate (%) with respect to the labeled amount of faropenem (C12H15NO5S) = M5 × A1/AS × V/V’/V × 1/C × 225

M5: Amount [mg (potency)] of Faropenem Sodium RS

C: Labeled amount [mg (potency)] of faropenem (C12H15NO5S) in 1 tablet

Assay Weigh accurately the mass of not less than 5 Faropenem Sodium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg (potency) of faropenem (C12H15NO5S), add exactly 10 mL of the internal standard solution, shake well, and add water to make exactly 50 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg (potency) of Faropenem Sodium RS, add exactly 10 mL of the internal standard solution and water to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Faropenem Sodium Hydrate.

Amount [mg (potency)] of faropenem (C12H15NO5S) = M5 × Q/Q5

M5: Amount [mg (potency)] of Faropenem Sodium RS

Internal standard solution—Dissolve 0.5 g of m-hydroxyacetophenone in 20 mL of acetonitrile, and add water to make 200 mL.

Containers and storage Containers—Tight containers.
Felbinac

フェルビナク

Felbinac occurs as white to pale yellowish white crystals or crystalline powder.

It is soluble in methanol and in acetone, sparingly soluble in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Felbinac in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Felbinac as directed in the potassium bromide disc method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 163 – 166°C

Purity (1) Chloride <1.07>—Dissolve 1.0 g of Felbinac in 40 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution by combining 0.30 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.011%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Felbinac according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Felbinac in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add acetone to make exactly 100 mL. Pipet 5 mL of the sample solution, add acetone to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of heptane, acetone, and acetic acid (100:30:1) to a distance of about 12 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): spots other than the principal spot from the sample solution are not more in- tenser than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.3% (1 g, 105°C, 3 hours).

Fenbufen

フェンブフェン

Fenbufen occurs as a white crystalline powder. It has a bitter taste.

It is sparingly soluble in acetone, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

Melting point: about 188°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Fenbufen in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Fenbufen as directed in the potassium bromide disc method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Take 2.0 g of Fenbufen, add 2 mL of sulfuric acid, and carbonize by gentle heating, proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Fenbufen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.1 g of Fenbufen in 20 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel
Fentanyl Citrate / Official Monographs

with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (80:20:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.4% Not more than 0.3% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.4% Not more than 0.1% (1 g).

**Assay** Weigh accurately about 75 mg of Fentanyl Citrate, previously dried, dissolve in 100 mL of ethanol (99.5), and titrate with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 25.43 mg of C_{16}H_{14}O_{3}

**Containers and storage** Containers—Tight containers.

Fentanyl Citrate contains not less than 98.0% of C_{22}H_{28}N_{2}O.C_{6}H_{8}O_{7}, calculated on the dried basis.

**Description** Fentanyl Citrate occurs as white crystals or crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in water and in ethanol (95), and very slightly soluble in diethyl ether.

**Identification** (1) Dissolve 0.05 g of Fentanyl Citrate in 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Fentanyl Citrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Fentanyl Citrate (1 in 100) responds to the Qualitative Tests <1.09> (1) for citrate.

**pH** ≤2.5 ≤Dissolve 0.10 g of Fentanyl Citrate in 10 mL of water: the pH of this solution is between 3.0 and 5.0.

**Melting point** 150 – 154°C

**Purity (1)** Heavy metals ≤0.7—Proceed with 0.5 g of Fentanyl Citrate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Fentanyl Citrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorf’s TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ≤2.4% Not more than 0.5% (0.2 g, in vacuum, silica gel, 60°C, 2 hours).

**Residue on ignition** ≤2.4% Not more than 0.2% (0.5 g).

**Assay** Weigh accurately about 75 mg of Fentanyl Citrate, dissolve in 50 mL of acetic acid (100), and titrate with 0.02 mol/L perchloric acid VS (potentiometric titration) and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS = 10.57 mg of C_{22}H_{28}N_{2}O.C_{6}H_{8}O_{7}

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

Ferrous Sulfate Hydrate

硫酸鉄水和物

FeSO₄·7H₂O: 278.01

Ferrous Sulfate Hydrate contains not less than 98.0% and not more than 104.0% of FeSO₄·7H₂O.

**Description** Ferrous Sulfate Hydrate occurs as pale green crystals or crystalline powder. It is odorless, and has an astringent taste. It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether. It is efflorescent in dry air, and its surface becomes yellowish brown in moist air.

**Identification** A solution of Ferrous Sulfate Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for ferrous salt for sulfate.

**Purity (1)** Clarity of solution—Dissolve 1.0 g of Ferrous Sulfate Hydrate in 20 mL of water and 1 mL of dilute sulfuric acid: the solution is clear.

(2) Acidity—To 5.0 g of powdered Ferrous Sulfate Hy-
Containers and storage

(3) Heavy metals (1.07)—Take 1.0 g of Ferrous Sulfate Hydrate in a porcelain dish, add 3 mL of aqua regia, and dissolve. Transfer this solution to a separator. Wash the porcelain dish with 2 to 5 mL portions of 6 mol/L hydrochloric acid TS, and combine the washings and the solution in the separator. Pour four 40-mL portions and one 20-mL portion of diethyl ether in the separator, shaking each time to mix. Allow to stand, and discard each separated diethyl ether layer. To the aqueous layer add 0.05 g of hydroxylammonium chloride, dissolve, and heat on a water bath for 10 minutes. Cool, adjust the solution to a pH of 3 to 4 by dropping strong ammonia solution, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: take 2.5 mL of Standard Lead Solution in a porcelain dish, add 3 mL of aqua regia, and proceed as directed for the preparation of the test solution (not more than 25 ppm).

(4) Arsenic (1.17)—Prepare the test solution with 1.0 g of Ferrous Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Assay

Dissolve about 0.7 g of Ferrous Sulfate Hydrate, accurately weighed, in a mixture of 20 mL of water and 20 mL of dilute sulfuric acid, add 2 mL of phosphoric acid, and immediately titrate with 0.02 mol/L potassium permanganate VS with 0.02 mol/L potassium permanganate VS. Each mL of 0.02 mol/L potassium permanganate VS = 27.80 mg of FeSO₄•7H₂O

Containers and storage

Containers—Tight containers.

Fexofenadine Hydrochloride

フェキソフェナジン塩酸塩

\[
\text{C}_{32}\text{H}_{39}\text{NO}_{4}\cdot\text{HCl}: 538.12 \\
2-(4-[(1RS)-1-Hydroxy-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butyl]phenyl)-2-methylpropanoic acid monohydrochloride [153439-40-8]
\]

Fexofenadine Hydrochloride contains not less than 98.0% and not more than 102.0% of \( \text{C}_{32}\text{H}_{39}\text{NO}_{4}\cdot\text{HCl} \), calculated on the anhydrous basis.

Description

Fexofenadine Hydrochloride occurs as a white crystalline powder. It is very soluble in methanol, soluble in ethanol (99.5), and slightly soluble in water.

A solution of Fexofenadine Hydrochloride in methanol (3 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Fexofenadine Hydrochloride in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum or the spectrum of Fexofenadine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Fexofenadine Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum or the spectrum of Fexofenadine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the RS according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

(3) A solution of Fexofenadine Hydrochloride in a mixture of water and methanol (1:1) (3 in 200) responds to the Qualitative Tests (1.09) (2) for chloride.

Purity (1) Heavy metals (1.07)—Proceed with 2.0 g of Fexofenadine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 7.51 g of sodium dihydrogen phosphate dihydrate and 0.84 g of sodium perchlorate in 1000 mL of water, and adjust to pH 2.0 with phosphoric acid. In a mixture of this solution and acetonitrile for liquid chromatography (1:1) dissolve 25 mg of Fexofenadine Hydrochloride to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak other than fexofenadine obtained from the sample solution is not larger than the peak area of fexofenadine from the standard solution. For these calculations use the areas of the peaks, having the relative retention time of about 1.8 and 3.3 to fexofenadine, after multiplying by their relative response factors, 1.5 and 0.9, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of fexofenadine, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fexofenadine are not less than 8000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fexofenadine is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water \(2.4a\) Not more than 0.5% (0.25 g, coulometric titration).

Residue on ignition \(2.4a\) Not more than 0.1% (1 g).

**Assay**

Dissolve 7.51 g of sodium dihydrogen phosphate dihydrate and 0.84 g of sodium perchlorate in 1000 mL of water, and adjust to pH 2.0 with phosphoric acid. In a mixture of this solution and acetonitrile for liquid chromatography (1:1) dissolve accurately weighed about 25 mg each of Fexofenadine Hydrochloride and Fexofenadine Hydrochloride RS (separately determine the water of each). Pipet 3 mL each of these solutions, add the mobile phase to make exactly 25 mL each. Pipet 3 mL each of these solutions, add the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(2.0\) determining the peak areas, \(A_T\) and \(A_S\), of fexofenadine obtained from each solution.

Amount (mg) of C\(_{27}\)H\(_{31}\)N\(_9\)Na\(_2\)O\(_{15}\)P\(_2\): \(M_S \times A_T/A_S\), calculated on the anhydrous basis.

Operating conditions—
- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: To 650 mL of a solution, prepared by dissolving 7.51 g of sodium dihydrogen phosphate dihydrate and 0.84 g of sodium perchlorate in 1000 mL of water and adjusting to pH 2.0 with phosphoric acid, add 350 mL of acetonitrile for liquid chromatography and 3 mL of triethylamine.

Flow rate: Adjust the flow rate so that the retention time of fexofenadine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fexofenadine are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fexofenadine is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

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**Flavin Adenine Dinucleotide Sodium**

フラビンアデニンジヌクレオチドナトリウム

\[C_{27}H_{31}N_9Na_2O_{15}P_2; \text{829.51} \]

Disodium adenosine 5’-[2R,3S,4S]-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[ep]teridin-10(2H)-yl)-2,3,4-trihydroxypentyl diphosphate

[84366-81-4]

Flavin Adenine Dinucleotide Sodium contains not less than 93.0% of C\(_{27}\)H\(_{31}\)N\(_9\)Na\(_2\)O\(_{15}\)P\(_2\), calculated on the anhydrous basis.

**Description**

Flavin Adenine Dinucleotide Sodium occurs as an orange-yellow to light yellow-brown powder. It is odorless or has a slight, characteristic odor, and has a slightly bitter taste.

It is freely soluble in water, and practically insoluble in methanol, in ethanol (95), in ethyleneglycol and in diethyl ether.

It is hygroscopic.

It is decomposed by light.

**Identification**

(1) A solution of Flavin Adenine Dinucleotide Sodium (1 in 100,000) is light yellow-green in color, and shows a strong yellow-green fluorescence. To 5 mL of the solution add 0.02 g of hydroxysulfite sodium: the color and the fluorescence of the solution disappear, and gradually reappear when the solution is shaken in air. Add dilute hydrochloric acid or sodium hydroxide TS dropwise: the fluorescence of the solution disappears.

(2) Determine the infrared absorption spectrum of Flavin Adenine Dinucleotide Sodium as directed in the potassium bromide disk method under Infrared Spectroscopy (2.2S), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Flavin Adenine Dinucleotide Sodium add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite. To the residue add 10 mL of diluted nitric acid (1 in 50), boil for 5 minutes, and after cooling, neutralize with ammonia TS, then filter the solution if necessary: the solution responds to the Qualitative Tests \(<1.09\) for sodium salt and the Qualitative Tests \(<1.09\) (1) and (3) for phosphate.

**Optical rotation** \(2.4a\) \([\alpha]_2^0\); 21.0 – 25.5° (0.3 g, calculated on the anhydrous basis, water, 20 mL, 100 mm).

**pH** \(2.5a\) Dissolve 1.0 g of Flavin Adenine Dinucleotide Sodium in...
Flavin Adenine Dinucleotide Sodium

Sodium in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Flavin Adenine Dinucleotide Sodium in 10 mL of water: the solution is clear and orange-yellow in color.

(2) Free phosphoric acid—Weigh accurately about 0.02 g of Flavin Adenine Dinucleotide Sodium, dissolve in 10 mL of water, and use this solution as the sample solution. Separately, measure exactly 2 mL of Standard Phosphoric Acid Solution, add 10 mL of water, and use this solution as the standard solution. To each of the sample solution and standard solution add 2 mL of diluted perchloric acid (100 in 117), then add 1 mL of hexaammonium heptamolybdate TS and 2 mL of 2,4-diaminophenol hydrochloride TS, respectively, shake, add water to make exactly 25 mL, and allow to stand at 20±1°C for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry.<ref>2.24</ref>, using a solution prepared in the same manner with 2 mL of water, as the blank, and determine the absorbances, \( A_1 \) and \( A_2 \), of the subsequent solutions of the sample solution and the standard solution at 730 nm, respectively: the amount of free phosphoric acid is less than 0.25%.

\[
\text{Amount (mg) of flavin adenine dinucleotide sodium, calculated on the anhydrous basis} = A_1/A_2 \times \frac{1}{M \times 5.16}
\]

\( M \): Amount (mg) of flavin adenine dinucleotide sodium, calculated on the anhydrous basis

(3) Heavy metals <1.07>—Proceed with 1.0 g of Flavin Adenine Dinucleotide Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.17>—Prepare the test solution with 2.0 g of Flavin Adenine Dinucleotide Sodium according to Method 3, and perform the test (not more than 1 ppm).

(5) Related substances—Dissolve 0.10 g of Flavin Adenine Dinucleotide Sodium in 200 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 20 \( \mu \)L of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine the peak area, \( A \), of flavin adenine dinucleotide and the total area, \( S \), of peaks other than the peak of flavin adenine dinucleotide by the automatic integration method: \( S/A (A + S) \) is not more than 0.10.

Operating conditions—
Column, column temperature, mobile phase, flow rate, and time span of measurement: Proceed as directed in the operating conditions in the Procedure (ii) under Assay (1).
Detector: An ultraviolet absorption spectrophotometer (wavelength: 260 nm).
System suitability—
Test for required detection: To exactly 2 mL of the sample solution add the mobile phase to make exactly 20 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of flavin adenine dinucleotide obtained from 20 \( \mu \)L of the solution for system suitability test is equivalent to 8 to 12% of that from 20 \( \mu \)L of the sample solution.
System performance: Proceed as directed in the system suitability in the Procedure (ii) under Assay (1).
System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of flavin adenine dinucleotide is not more than 1.0%.

Water <2.48>—Take 50 mL of a mixture of methanol for Karl Fischer method and ethyleneglycol for Karl Fischer method (1:1) into a dry titration flask, and titrate with Karl Fischer TS until end point. Weigh accurately about 0.1 g of Flavin Adenine Dinucleotide Sodium, transfer quickly to the flask and dissolve quickly with water in the titration flask, add an excess and constant volume of Karl Fischer TS, dissolve by stirring for 10 minutes, and perform the test: the water content is not more than 10.0%.

Assay (1) Procedure (i) Total flavin content—Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 0.1 g of Flavin Adenine Dinucleotide Sodium, dissolve in water to make exactly 200 mL. Pipet 5 mL of this solution, add 5 mL of zinc chloride TS, and heat in a water bath for 30 minutes. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Riboflavin RS, previously dried at 105°C for 2 hours, dissolve in 200 mL of diluted acetic acid (100) (1 in 100) by warming, cool, add water to make exactly 500 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_1 \) and \( A_2 \), of the sample solution and standard solution at 450 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Total amount (mg) of flavin = \( M_5 \times A_1/A_2 \times 4/5 \)

\( M_5 \): Amount (mg) of Riboflavin RS

(ii) Peak area ratio of flavin adenine dinucleotide—Dissolve 0.1 g of Flavin Adenine Dinucleotide Sodium in 200 mL of water, and use this solution as the sample solution. Perform the test with 5 \( \mu \)L of the sample solution as directed under the Liquid Chromatography <2.07> according to the following conditions. Determine the peak area, \( A \), of flavin adenine dinucleotide, and the total area, \( S \), of the peaks other than flavin adenine dinucleotide by the automatic integration method.

Peak area ratio of flavin adenine dinucleotide = \( 1.08A/(1.08A + S) \)

Operating conditions—
Detector: A visible spectrophotometer (wavelength: 450 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecysilized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: A mixture of a solution of potassium dihydrogen phosphate (1 in 500) and methanol (4:1).
Flow rate: Adjust the flow rate so that the retention time of flavin adenine dinucleotide is about 10 minutes.
Time span of measurement: About 4.5 times as long as the retention time of flavin adenine dinucleotide.

System suitability—
Test for required detection: To exactly 2 mL of the sample solution add water to make exactly 20 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of...
the solution for system suitability test, and add water to make exactly 20 mL. Confirm that the peak area of flavin adenine dinucleotide obtained from 5 μL of this solution is equivalent to 8 to 12% of that from 5 μL of the solution for system suitability test.

System performance: Dissolve 20 mg each of Flavin Adenine Dinucleotide Sodium and riboflavin sodium phosphate in 100 mL of water. When the procedure is run with 5 μL of this solution under the above operating conditions, flavin adenine dinucleotide and riboflavin phosphate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of flavin adenine dinucleotide is not more than 1.0%.

(2) Calculation

\[
\text{Amount (mg) of } C_{27}H_{31}N_9Na_2O_{15}P_2 = f_1 \times f_2 \times 2.2040
\]

\[
f_1: \text{Total amount (mg) of flavin in Flavin Adenine Dinucleotide Sodium obtained from the procedure (i)}
\]

\[
f_2: \text{Peak area ratio of flavin adenine dinucleotide in Flavin Adenine Dinucleotide Sodium obtained from the procedure (ii)}
\]

Containers and storage  Containers—Tight containers.
Storage—Light-resistant.

**Flavoxate Hydrochloride**

![Flavoxate Hydrochloride](image)

\[C_{24}H_{25}NO_4\cdot HCl: 427.92\]

2-(Piperidin-1-yl)ethyl 3-methyl-4-oxo-2-phenyl-4H-chromene-8-carboxylate monohydrochloride [3717-88-2]

Flavoxate Hydrochloride, when dried, contains not less than 99.0% of \(C_{24}H_{25}NO_4\cdot HCl\).

**Description**  Flavoxate Hydrochloride occurs as white crystals or crystalline powder.

It is sparingly soluble in acetic acid (100) and in chloroform, slightly soluble in water and in ethanol (95), and practically insoluble in acetonitrile and in diethyl ether.

**Identification**  (1) Determine the absorption spectrum of a solution of Flavoxate Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.2>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flavoxate Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Flavoxate Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

**Purity**  (1) Heavy metals <1.07>—Proceed with 2.0 g of Flavoxate Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Flavoxate Hydrochloride according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 80 mg of Flavoxate Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 20 mL, then pipet 1 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) 3:1:1 to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41>—Not more than 1.0% (1 g, reduced pressure, silica gel, 2 hours).

**Residue on ignition** <2.44>—Not more than 0.1% (1 g).

**Assay**  Weigh accurately about 0.6 g of Flavoxate Hydrochloride, previously dried, add 10 mL of acetic acid (100) and 40 mL of acetonitrile to dissolve, add 50 mL of acetic anhydride, and titrate <2.25> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS 42.79 mg of \(C_{27}H_{31}N_9Na_2O_{15}P_2\).

Containers and storage  Containers—Tight containers.

**Flecainide Acetate**

![Flecainide Acetate](image)

\[C_{17}H_{20}F_2N_8O_2\cdot C_3H_4O_2: 474.39\]

\[N\cdot(2RS)-Piperidin-2-ylmethyl]-2,5-bis(2,2,2-trifluoroethoxy)benzamide monocacetate [54143-56-5]

Flecainide Acetate, when dried, contains not less than 98.0% and not more than 101.0% of...
C17H20F6N2O3.C2H4O2.

**Description**  
Flecainide Acetate occurs as a white crystalline powder, having slightly a characteristic or acetic acid like odor.

It is freely soluble in methanol, in ethanol (95) and in acetic acid (100), and sparingly soluble in water.

A solution of Flecainide Acetate in methanol (1 in 25) shows no optical rotation.

Melting point: about 150°C (with decomposition).

**Identification (1)**  
Dissolve 20 mg of Flecainide Acetate in 1 mL of water, add 1 mL of a solution of acetaldehyde (1 in 20), and shake. To this solution add dropwise at the same time 1 – 2 drops each of sodium pentacyanonitrosylferrate (III) dihydrate solution (1 in 10) and sodium hydrogen carbonate TS: a blue precipitate is formed.

(2) Determine the absorption spectrum of a solution of Flecainide Acetate in ethanol (95) (13 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Flecainide Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Flecainide Acetate responds to the Qualitative Tests <1.09> (1) for acetate.

**pH**  
The pH of a solution of 0.5 g of Flecainide Acetate in 20 mL of water is 6.7 to 7.1.

**Purity (1)**  
Clarity and color of solution—Dissolve 0.25 g of Flecainide Acetate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Transfer 1.0 g of Flecainide Acetate in a porcelain crucible, and heat gently to carbonize. After cooling, add 2 mL of sulfuric acid, heat carefully until white fumes are no longer evolved, then proceed according to Method 2 to prepare the test solution, and perform the test. Prepare the control solution as follows: Place 2 mL each of sulfuric acid and hydrochloric acid in a porcelain crucible, evaporate on a water bath, then evaporate to dryness on a sand bath, add to the residue 3 drops of hydrochloric acid, then proceed in the same manner as for the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(3) 2-Aminomethylpiperidine—Dissolve exactly 0.25 g of Flecainide Acetate in exactly 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve exactly 50 mg of 2-aminomethylpiperidine in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and ammonia solution (28) (20:1) to a distance of about 10 cm, and dry the plate. Spray evenly a solution of ninhydrin in methanol (1 in 500), and heat at 105°C for 2 to 5 minutes: the spot obtained from the sample solution, corresponding to the spot from the standard solution, is not more intense than the spot from the standard solution.

(4) Related substances—Dissolve 0.25 g of Flecainide Acetate in 25 mL of a mixture of water and acetonitrile (71:29), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (71:29) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile (71:29) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of the peak other than flecainide obtained from the sample solution is not larger than the peak area of flecainide from the standard solution, and the total area of the peaks other than flecainide from the sample solution is not larger than 2.5 times the peak area of flecainide from the standard solution. For these calculations use the areas of the peaks, having the relative retention time of about 1.5 and about 2.9 with respect to flecainide, after multiplying by their relative response factors, 0.3 and 1.7, respectively.

**Operating conditions**—
- **Detector:** An ultraviolet absorption photometer (wavelength: 254 nm).
- **Column:** A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature:** A constant temperature of about 40°C.
- **Mobile phase:** A mixture of water, acetonitrile, acetic acid (100) and tetrabutylammonium hydroxide-methanol TS (142:58:2:1), adjusted to pH 5.8 with ammonia solution (28).
- **Flow rate:** Adjust the flow rate so that the retention time of flecainide is about 4 minutes.
- **Time span of measurement:** About 5 times as long as the retention time of flecainide, beginning after the solvent peak.

**System suitability**—
Test for required detectability: To exactly 1 mL of the standard solution add a mixture of water and acetonitrile (71:29) to make exactly 10 mL. Confirm that the peak area of flecainide obtained from 20 μL of this solution is equivalent to 7 – 13% of that from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of flecainide are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flecainide is not more than 2.0%.

(5) Residual solvent—Being specified separately.

**Loss on drying** <2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Assay**  
Weigh accurately about 0.6 g of Flecainide Acetate, previously dried, dissolve in 100 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentio-
metric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

\[ \text{Each mL} = 47.44 \text{ mg of } C_{17}H_{20}F_{6}N_{2}O_{3}.C_{2}H_{4}O_{2} \]

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Flecainide Acetate Tablets

フルカイニド酢酸塩

Flecainide Acetate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of flecainide acetate (C17H20F6N2O3.C2H4O2: 474.39).

Method of preparation Prepare as directed under Tablets, with Flecainide Acetate.

Identification To an amount of powdered Flecainide Acetate Tablets, equivalent to 0.2 g of Flecainide Acetate according to the labeled amount, add 4 mL of methanol, shake for 20 minutes, then centrifuge and use the supernatant liquid as the sample solution. Separately, dissolve 0.1 g of flecainide acetate in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone and ammonia solution (28) (20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot from standard solution show the same Rf value.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Completely disintegrate 1 tablet of Flecainide Acetate Tablets in 41/5 mL of a solution of lactic acid (1 in 500) with the aid of ultrasonic waves. After allowing to stand for 30 minutes while swirling occasionally, add a solution of lactic acid (1 in 500) to make exactly V mL so that each mL contains about 1 mg of flecainide acetate (C17H20F6N2O3.C2H4O2), shake thoroughly, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 56 μg of flecainide acetate (C17H20F6N2O3.C2H4O2) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of flecainide acetate for assay, previously dried under reduced pressure not exceeding 0.67 kPa at 60°C for 2 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, Af and As, of the sample solution and standard solution at 296 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of flecainide acetate (C17H20F6N2O3.C2H4O2)

\[ M_S = \frac{A_f}{A_S} \times V \times 1/C \times 180 \]

MS: Amount (mg) of flecainide acetate for assay
C: Labeled amount (mg) of flecainide acetate (C17H20F6N2O3.C2H4O2) in 1 tablet

Assay Accurately weigh the mass of not less than 20 Flecainide Acetate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of flecainide acetate (C17H20F6N2O3.C2H4O2), add 80 mL of a solution of lactic acid (1 in 500), agitate for 5 minutes with the aid of ultrasonic waves, then add a solution of lactic acid (1 in 500) to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add a solution of lactic acid (1 in 500) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of flecainide acetate for assay, previously dried under reduced pressure not exceeding 0.67 kPa at 60°C for 2 hours, dissolve in a solution of lactic acid (1 in 500) to make exactly 50 mL. Pipet 10 mL of this solution, add a solution of lactic acid (1 in 500) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A5, of the sample solution and standard solution at 296 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of flecainide acetate (C17H20F6N2O3.C2H4O2)

\[ M_S = \frac{A_f}{A_S} \times V \times M \]

MS: Amount (mg) of flecainide acetate for assay

Containers and storage Containers—Tight containers.
Flomoxef Sodium

Flomoxef Sodium contains not less than 870 μg (potency) and not more than 985 μg (potency) per mg, calculated on the anhydrous basis. The potency of Flomoxef Sodium is expressed as mass (potency) of flomoxef (C₁₅H₁₈F₂N₆O₇S₂: 496.47).

**Description** Flomoxef Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol, and sparingly soluble in ethanol (99.5).

**Identification (1)** Decompose 0.01 g of Flomoxef Sodium as directed under Oxygen Flask Combustion Method 1.06, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. To 2 mL of the test solution so obtained add 1.5 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1): blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Flomoxef Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Flomoxef Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Determine the 'H spectrum of a solution of Flomoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy 2.21, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 3.5 ppm, a single signal or a sharp multiple signal B at around δ 3.7 ppm, and a single signal C at around δ 5.2 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:2:1.

(5) Flomoxef Sodium responds to the Qualitative Tests 1.09 for sodium salt.

**Optical rotation** \[\alpha_D^{20} = -8 - 13^\circ (1\text{ g calculated on the anhydrous basis}, a\text{ mixture of water and ethanol (99.5): (4:1)}, 50\text{ mL, 100 mm})\]

**pH** \[pH < 2.5\] The pH of a solution obtained by dissolving 0.5 g of Flomoxef Sodium in 5 mL of water is between 4.0 and 5.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Flomoxef Sodium in 10 mL of water: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 3.0 mL of Cobalt (II) Chloride CS and 12 mL of Iron (III) Chloride CS add 35 mL of diluted dilute hydrochloric acid (1 in 10). To 5.0 mL of this solution add 5.0 mL of diluted dilute hydrochloric acid (1:10).

(2) Heavy metals \[< 1.07\]—Proceed with 1.0 g of Flomoxef Sodium in a quartz crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic \[< 1.12\]—To 1.0 g of Flomoxef Sodium 5 mL of sulfuric acid and 5 mL of nitric acid, heat carefully until the solution changes to colorless to light yellow with occasional addition of 2 mL of nitric acid. After cooling, add 10 mL of ammonium oxalate TS, heat until white fumes evolve, and concentrate to 2 to 3 mL. After cooling, add water to make 10 mL, and perform the test using this solution as the test solution: the color is not darker than that of the control solution.

Control solution: Proceed to prepare a solution in the same manner as the test solution without Flomoxef Sodium, and transfer 10 mL of the solution so obtained to the generator bottle, add exactly 2 mL of Standard Arsenic Solution, and proceed in the same manner as the test solution (not more than 2 ppm).

(4) 1-(2-Hydroxyethyl)-1H-tetrazol-5-thiol—Use the sample solution obtained in the Assay as the sample solutions. Weigh accurately about 20 mg of 1-(2-hydroxyethyl)-1H-tetrazol-5-thiol, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 5 μL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01, according to the following conditions, and calculate the ratios, Q₉ and Q₈, of the peak area of 1-(2-hydroxyethyl)-1H-tetrazol-5-thiol to that of the internal standard: the amount of 1-(2-hydroxyethyl)-1H-tetrazol-5-thiol is not more than 1.0% of the amount of Flomoxef Sodium calculated on the anhydrous basis.

Amount (mg) of 1-(2-hydroxyethyl)-1H-tetrazol-5-thiol (C₉H₉N₅O₅S)

\[= M_S \times Q_7/Q_8 \times 1/10\]

M₅: Amount (mg) of 1-(2-hydroxyethyl)-1H-tetrazol-5-thiol

**Internal standard solution**—A solution of m-cresol (3 in 1000).

**Operating conditions**—Proceed as directed in the operating conditions in the Assay.

**System suitability**—Proceed as directed in the system suitability in the Assay.
**Flomoxef Sodium for Injection**

注射用フロモキセフナトリウム

Flomoxef Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of Flomoxef (C₁₅H₁₈F₂N₆O₇S₂: 496.47).

**Method of preparation** Prepare as directed under Injections, with Flomoxef Sodium.

**Description** Flomoxef Sodium for Injection occurs as white to light yellowish white, friable masses or powder.

**Identification** Proceed as directed in the Identification (3) under Flomoxef Sodium.

**pH** Not more than 1.5. Not more than 1.5% (0.5 g, volumetric titration, back titration).

**Assay** Weigh accurately an amount of Flomoxef Sodium and Flomoxef Triethylammonium RS, equivalent to about 50 mg (potency), and dissolve each in exactly 50 mL of the internal standard solution, add water to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 mL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of flomoxef to that of the internal standard.

\[ M_5 = \frac{\text{Amount} \times Q_1/ Q_2 \times 1000}{m} \]

Where:
- \( M_5 \): Amount [mg (potency)] of Flomoxef Triethylammonium RS
- \( m \): Amount [µg (potency)] of flomoxef (C₁₅H₁₈F₂N₆O₇S₂)

Internal standard solution—A solution of m-cresol (3 in 1000).

**Operating conditions**
- Detector: An ultraviolet absorption photometer (wavelength: 246 nm).
- Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadeclisilanized silica gel for liquid chromatography (5 - 10 µm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.60 g of tetra-n-butylanlaminon bromide in water to make 1000 mL. To 750 mL of this solution add 250 mL of methanol.
- Flow rate: Adjust the flow rate so that the retention time of flomoxef is about 9 minutes.

**System suitability**
- System performance: When the procedure is run with 5 µL of the standard solution under the above operating conditions, flomoxef and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
- System repeatability: When the test is repeated 3 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of flomoxef to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

**Storage**—Not exceeding 5°C.

**Containers and storage**

 Containers and storage—Tight containers.

**Storage**—Not exceeding 5°C.

**System suitability**
- System performance: When the procedure is run with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of flomoxef to that of the internal standard is not more than 1.0%.

**Containers and storage**

 Containers and storage—Tight containers.

**Storage**—Not exceeding 5°C.

**Flomoxef Sodium for Injection**

注射用フロモキセフナトリウム

Flomoxef Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of Flomoxef (C₁₅H₁₈F₂N₆O₇S₂: 496.47).

**Method of preparation** Prepare as directed under Injections, with Flomoxef Sodium.
Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Flomoxef Sodium for Injection, and calculate the average mass of the contents. Spread out thinly about 1 g of the content in a petri dish, allow the dish to stand in a desiccator containing a saturated solution of magnesium bromide without light exposure to equilibrate the sample to constant water content. Determine the water content, separately, with about 0.1 g of the sample according to the method described in Water. Weigh accurately an amount of the sample, equivalent to about 50 mg (potency) of Flomoxef Sodium according to the labeled amount, add exactly 50 mL of the internal standard solution to dissolve, add water to make 100 mL, and use this solution as the sample solution. Separately weigh accurately about 50 mg (potency) of Flomoxef Triethylammonium RS, add exactly 50 mL of the internal standard solution to dissolve, add water to make 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Flopropione Sodium.

Amount [µg (potency)] of flomoxef (C₁₅H₁₈F₂N₆O₇S₂) = Mₛ × Qₛ/Qₛ × 1000

Mₛ: Amount [mg (potency)] of Flomoxef Triethylammonium RS

Internal standard solution—A solution of m-cresol (3 in 1000).

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injection may be used.

Flopropione フロプロピオン

C₁₀H₁₀O₄: 182.17
1-(2,4,6-Trihydroxyphenyl)propan-1-one [2295-58-1]

Flopropione contains not less than 98.0% and not more than 101.0% of C₁₀H₁₀O₄, calculated on the anhydrous basis.

Description Flopropione occurs as a white to pale yellow-brown crystalline powder. It is very soluble in N₂N-dimethylformamide, freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Flopropione in ethanol (99.5) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flopropione as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 177 – 181°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Flopropione according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Flopropione in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of flopropione obtained from the sample solution is not larger than 1/10 times the peak area of flopropione from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 267 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile, water and phosphoric acid (114:86:1).

Flow rate: Adjust the flow rate so that the retention time of flopropione is about 3 minutes.

Time span of measurement: About 7 times as long as the retention time of flopropione.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of flopropione obtained from 20 µL of this solution is equivalent to 7 to 13% of that from 20 µL of the standard solution.

System performance: Dissolve 25 mg of ethyl parahydroxybenzoate in 30 mL of acetonitrile, and add the mobile phase to make 50 mL. To 2.5 mL of this solution add 2 mL of the sample solution and the mobile phase to make 50 mL. When the procedure is run with 20 µL of this solution under the above operating conditions, flopropione and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flopropione is not more than 1.0%. Water <2.45> Not more than 4.0% (0.5 g, volumetric titra-
tion, direct titration).

**Residue on ignition** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Flopropione, dissolve in 30 mL of N,N-dimethylformamide, and titrate with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L tetramethylammonium hydroxide VS} = 18.22 \text{ mg of } C_9H_{10}O_4
\]

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

### Flopropione Capsules

**Flopropione Capsules**

Flopropione Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of flopropione \((C_9H_{10}O_4): 182.17\).

**Method of preparation** Prepare as directed under the Capsules, with Flopropione.

**Identification** (1) Powder the contents of Flopropione Capsules. To a portion of the powder, equivalent to 60 mg of Flopropione according to the labeled amount, add 40 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of iron (III) nitrate TS: a red-purple color appears.

(2) Powder the contents of Flopropione Capsules. To a portion of the powder, equivalent to 90 mg of Flopropione according to the labeled amount, add 100 mL of ethanol (99.5), shake well, and filter. To 5 mL of the filtrate add ethanol (99.5) to make 50 mL. To 5 mL of this solution add ethanol (99.5) to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry \(2.24\), it exhibits a maximum between 283 nm and 287 nm.

**Uniformity of dosage units** Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 capsule of Flopropione Capsules add 43 mL of a mixture of water and phosphoric acid \((86:1)\), and disintegrate the capsule in a water bath at 50°C. After cooling, add a suitable amount of acetonitrile to make exactly \(V\) mL of a solution containing about 0.4 mg of flopropione \((C_9H_{10}O_4)\) per mL. Stir the solution for 10 minutes, centrifuge a part of the solution at 3000 rpm for 5 minutes, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of flopropione (} C_9H_{10}O_4\text{)} = M_S \times A_T/A_S \times V/100
\]

\(M_S\): Amount (mg) of flopropione for assay, calculated on the anhydrous basis

**Dissolution** When the test is performed at 100 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Flopropione Capsules is not less than 80%.

Start the test with 1 capsule of Flopropione Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly \(V\) mL so that each mL contains about 8.8 μg of flopropione \((C_9H_{10}O_4)\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of flopropione for assay (separately determine the water \(2.44\) in the same manner as Flopropione), and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), at 284 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(2.24\), using 0.1 mol/L hydrochloric acid TS as the blank.

\[
\text{Dissolution rate (％) with respect to the labeled amount of flopropione (} C_9H_{10}O_4\text{)} = M_S \times A_T/A_S \times V/V \times 1/C \times 36
\]

\(M_S\): Amount (mg) of flopropione for assay, calculated on the anhydrous basis

**Storage** Light-resistant.

**A. Assay** Weigh accurately about 0.3 g of Flopropione, dissolve in 30 mL of water, filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly \(V\) mL so that each mL contains about 8.8 μg of flopropione \((C_9H_{10}O_4)\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of flopropione for assay (separately determine the water \(2.44\) in the same manner as Flopropione), and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), at 284 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(2.24\), using 0.1 mol/L hydrochloric acid TS as the blank.

**B. Residue on ignition** Not more than 0.1% (1 g).

**C. After cooling, add** 70 mL of a solvent (methanol), dissolve by exposure for 10 minutes to ultrasonic vibration. Add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid chromatography \(2.07\) according to the following conditions, and determine the peak areas, \(A_T\) and \(A_S\), of flopropione.

\[
\text{Amount (mg) of flopropione (} C_9H_{10}O_4\text{)} = M_S \times A_T/A_S
\]

\(M_S\): Amount (mg) of flopropione for assay, calculated on the anhydrous basis

**Operating conditions**

- **Detector:** An ultraviolet absorption photometer (wavelength: 267 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography \((5 \mu m \text{ in particle diameter})\).
- **Column temperature:** A constant temperature of about 35°C.
- **Mobile phase:** A mixture of acetonitrile, water and phosphoric acid \((114:86:1)\)
  - **Flow rate:** Adjust the flow rate so that the retention time of flopropione is about 3 minutes.

**System suitability**
Fluconazole

**Description** Fluconazole occurs as a white to pale yellowish white crystalline powder.

It is soluble in ethanol (99.5), and slightly soluble in water. It dissolves in dilute hydrochloric acid.

**Identification** (1) Dissolve 0.1 g of Fluconazole in 10 mL of dilute hydrochloric acid, and add 1 mL of Reineke’s salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Fluconazole in 0.01 mol/L hydrochloric acid-methanol TS: a light red precipitate is formed.

(3) Determine the infrared absorption spectrum of Fluconazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Fluconazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point** 2.60° 137 – 141°C

**Purity** (1) Clarity and color of solution—A solution obtained by dissolving 0.10 g of Fluconazole in 50 mL of water is clear and colorless.

(2) Heavy metals \(<1.07\)—Proceed with 1.0 g of Fluconazole according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 30 mg of Fluconazole in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.26> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the peak area of related substance I, having the relative retention time about 0.60 to fluconazole obtained from the sample solution is not larger than 6 times the peak area of fluconazole from the standard solution, the area of the peak other than fluconazole and the related substance I obtained from the sample solution is not larger than the peak area of fluconazole from the standard solution, and the total of the areas of the peak other than fluconazole obtained from the sample solution is not larger than 8 times the peak area of fluconazole from the standard solution.

**Residual solvent**—Being specified separately.

**Loss on drying** <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

**Assay** Weigh accurately about 0.25 g of Fluconazole, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 15.31 mg of C_{13}H_{12}F_{2}N_{6}O.

**Containers and storage** Containers—Tight containers.
Flucytosine

**Flucytosine**  

\[
\text{C}_4\text{H}_4\text{FN}_3\text{O} : 129.09 \\ 5-\text{Fluorocytosine} \\
[2022-85-7]
\]

Flucytosine, when dried, contains not less than 98.5% of \(\text{C}_4\text{H}_4\text{FN}_3\text{O}\), and not less than 14.0% and not more than 15.5% of fluoride (F: 19.00).

**Description**  
Flucytosine occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in water, slightly soluble in methanol, in ethanol (95), in acetic anhydride and in acetic acid (100), and practically insoluble in diethyl ether.

It dissolves in 0.1 mol/L hydrochloric acid TS.

The pH of a solution of Flucytosine (1 in 100) is between 5.5 and 7.5.

It is slightly hygroscopic.

Melting point: about 295°C (with decomposition).

**Identification (1)**  
Add 0.2 mL of bromine TS to 5 mL of a solution of Flucytosine (1 in 500); a yellow-brown color of bromine TS is immediately discharged. Further add 2 mL of barium hydroxide TS: a purple precipitate is formed.

(2) Proceed with 0.1 g of Flucytosine as directed under Oxygen Flask Combustion Method \(<1.06>\), using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. The solution responds to the Qualitative Tests \(<1.09>\) (2) for fluoride.

(3) Determine the absorption spectrum of a solution of Flucytosine in 0.1 mol/L hydrochloric acid TS (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)**  
Clarity and color of solution—Dissolve 1.0 g of Flucytosine in 100 mL of water: the solution is clear and colorless.

(2) Chloride \(<1.03>\)—Dissolve 1.0 g of Flucytosine in 80 mL of water by heating on a water bath. After cooling, to 40 mL of this solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Fluoride—Dissolve 0.10 g of Flucytosine in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution to a 20-mL volumetric flask, add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerrous nitrate TS (1:1:1), and add water to make 20 mL. Allow the mixture to stand for 1 hour, and use this solution as the sample solution. Separately, transfer 4.0 mL of Standard Fluorescein Solution to a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerrous nitrate TS (1:1:1). Proceed in the same manner as directed in the preparation of the sample solution, and use this solution as the standard solution. Transfer 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) to a 20-mL volumetric flask, proceed in the same manner as directed in the preparation of the standard solution, and use this solution as the blank solution. Determine the absorbances, \(A_{1}\) and \(A_{2}\) of the sample solution and standard solution at 600 nm, using the blank solution as the control as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\): \(A_{1}\) is not larger than \(A_{2}\) (not more than 0.048%).

(4) Heavy metals \(<1.07>\)—Proceed with 1.0 g of Flucytosine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic \(<1.11>\)—Prepare the test solution with 1.0 g of Flucytosine according to Method 2, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 50 mg of Flucytosine in 5 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Measure accurately 1 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 25 mL. Measure accurately 1 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.02>\). Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the chromatogram with a mixture of ethyl acetate, methanol and water (5:3:2) to a distance of about 12 cm, air-dry the plate, and observe the spots under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying \(<2.41>\)** Not more than 1.0% (1 g, 105°C, 4 hours).

**Residue on ignition \(<2.44>\)** Not more than 0.1% (1 g).

**Assay (1)**  
Flucytosine—Weigh accurately about 0.2 g of Flucytosine, previously dried, dissolve in 40 mL of acetic acid (100), add 100 mL of acetic anhydride, and titrate \(<2.50>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 12.91 mg of \(\text{C}_4\text{H}_4\text{FN}_3\text{O}\)

(2) Fluorine—Weigh accurately about 10 mg of Flucytosine, previously dried, and proceed as directed in the determination of fluorine under Oxygen Flask Combustion Method \(<1.06>\), using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide VS and 20 mL of water as the absorbing liquid.

**Containers and storage**  
Containers—Tight containers.  
Storage—Light-resistant.
Fludiazepam

フルジアゼパム

C₁₆H₁₂ClFN₂O: 302.73
7-Chloro-5-(2-fluorophenyl)-1-methyl-1,3-dihydro-
2H-1,4-benzodiazepin-2-one
[3900-31-0]

Fludiazepam, when dried, contains not less than 99.0% of C₁₆H₁₂ClFN₂O.

Description Fludiazepam occurs as white to light yellow crystals or crystalline powder.

It is very soluble in chloroform, freely soluble in methanol, in ethanol (95), in acetic acid (100) and in diethyl ether, and practically insoluble in water.

Identification (1) Prepare the test solution with 0.01 g of Fludiazepam as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Fludiazepam in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Fludiazepam in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fludiazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Fludiazepam as directed under Flame Coloration Test <1.06> (2): a green color appears.

Melting point <2.60> 91 – 94°C

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Fludiazepam in 50 mL of diethyl ether, add 50 mL of water, and shake. Separate the water layer, wash it with two 20-mL portions of diethyl ether, and filter the water layer. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Fludiazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Fludiazepam in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 50 mL. Pipet 2 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 20 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethyl acetate (10:7) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Fludiazepam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 30.28 mg of C₁₆H₁₂ClFN₂O

Containers and storage Containers—Tight containers.

Fludrocortisone Acetate

フルドロコルチゾン酢酸エステル

C₂₃H₃₁FO₆: 422.49
9-Fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione
21-acetate
[514-36-3]

Fludrocortisone Acetate, when dried, contains not less than 97.5% and not more than 102.5% of C₂₃H₃₁FO₆.

Description Fludrocortisone Acetate occurs as a white to pale yellow crystals or crystalline powder.

It is soluble in acetone, sparingly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 220°C (with decomposition).

Identification (1) Prepare the test solution by proceeding with 10 mg of Fludrocortisone Acetate according to the Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide VS and 20 mL of water as the absorbing liquid: the test solution responds to
the Qualitative Tests <1.09> for fluoride.

(2) Determine the absorption spectrum of a solution of Fludrocortisone Acetate in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fludrocortisone Acetate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fludrocortisone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Fludrocortisone Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D 25° 2.24 + 131° ~ + 138° (after drying, 0.1 g, acetone, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 0.5 g of Fludrocortisone Acetate according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 20 mg of Fludrocortisone Acetate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.60> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of fludrocortisone acetate obtained from the sample solution is not larger than 1/4 times the area of fludrocortisone acetate obtained from the standard solution, and the total area of the peaks other than the peak of fludrocortisone acetate is not larger than 1/2 times the area of fludrocortisone acetate obtained from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of water and tetrahydrofuran (13:7).
Flow rate: Adjust the flow rate so that the retention time of fludrocortisone acetate is about 10 minutes.
Time span of measurement: About 2 times as long as the retention time of fludrocortisone acetate, beginning after the solvent peak.
System suitability—
Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of fludrocortisone acetate obtained from 20 µL of this solution is equivalent to 4.0 to 6.0% of that from 20 µL of the standard solution.
System performance: Dissolve 2 mg each of Fludrocortisone Acetate and hydrocortisone acetate in 50 mL of the mobile phase. When the procedure is run with 20 µL of this solution under the above operating conditions, hydrocortisone acetate and fludrocortisone acetate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fludrocortisone acetate is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, 100°C, 2 hours).
Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 25 mg each of Fludrocortisone Acetate and Fludrocortisone Acetate RS, previously dried, and dissolve separately in ethanol (95) to make exactly 100 mL. Pipet 4 mL each of these solutions, add ethanol (95) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A1 and A2, at 238 nm.

Amount (mg) of fludrocortisone acetate (C23H31FO6) = M2 × A1/A2
M2: Amount (mg) of Fludrocortisone Acetate RS

Flunitrazepam
フルニトラゼパム

Flunitrazepam, when dried, contains not less than 99.0% of C16H12F3N2O3.

Description Flunitrazepam occurs as a white to pale yellow crystalline powder. It is freely soluble in acetic acid (100), soluble in acetic anhydride and in acetone, slightly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Flunitrazepam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Flunitrazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point 2.60 168 – 172°C

Purity (1) Chloride 1.03—To 1.0 g of Flunitrazepam add 50 mL of water, allow to stand for 1 hour with occasional stirring, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.02%).

(2) Heavy metals 1.07—Proceed with 2.0 g of Flunitrazepam according to Method 4 using a platinum crucible, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Flunitrazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add acetone to make exactly 20 mL. Pipet 1 mL of this solution, add acetone to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.60. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, diethyl ether and ammonia solution (28:200:100:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): number of the spots other than the principal spot from the sample solution is not more than 2, and they are not more intense than the spot from the standard solution.

Loss on drying 2.41 Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition 2.44 Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Flunitrazepam, previously dried, dissolve in 20 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate 2.50 with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS 31.33 mg of C₆H₁₂F₅O₃

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Fluocinolone Acetonide

フルオシノロンアセトニド

C₂₄H₃₀F₂O₆: 452.49
6α,9-Difluoro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione [67-73-2]

Fluocinolone Acetonide, when dried, contains not less than 97.0% and not more than 102.0% of C₂₄H₂₅F₂O₆.

Description Fluocinolone Acetonide occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in acetone, soluble in ethanol (95) and in ethanol (99.5), sparingly soluble in methanol and in chloroform, slightly soluble in acetonitrile, very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: 266 – 274°C (with decomposition).

Identification (1) To 2 mg of Fluocinolone Acetonide add 2 mL of sulfuric acid: a yellow color is produced.

(2) Dissolve 0.01 g of Fluocinolone Acetonide in 1 mL of methanol, add 1 mL of Fehling’s TS, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Fluocinolone Acetonide as directed under Oxygen Flask Combustion Method 1.06, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests 1.09 for fluoride.

(4) Determine the infrared absorption spectrum of Fluocinolone Acetonide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Fluocinolone Acetonide RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Fluocinolone Acetonide and Fluocinolone Acetonide RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation 2.49 [α]D: +98 – +108° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity Related substances—Dissolve 15 mg of Fluocinolone Acetonide in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions. Determine each peak area of each solution by the automatic integration method:
the total area of the peaks other than the peak of fluocinolone acetonide from the sample solution is not larger than that of the peak area of fluocinolone acetonide from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: A mixture of water-saturated chloroform, methanol and acetic acid (100) (200:3:2).
Flow rate: Adjust the flow rate so that the retention time of fluocinolone acetonide is about 12 minutes.
Time span of measurement: About twice as long as the retention time of fluocinolone acetonide beginning after the solvent peak.
System suitability—
Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of fluocinolone acetonide obtained from 20 μL of this solution is equivalent to 4 to 6% of that from 20 μL of the standard solution.
System performance: Dissolve 5 mg each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 50 mL of acetonitrile, and add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, isopropyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.9.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of fluocinolone acetonide to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.
Storage—Light-resistant.

Fluocinonide

フルオシノニド

C_{26}H_{32}F_{2}O_{7}: 494.52
6α,9-Difluoro-11β,21-dihydroxy-16α,17β-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione 21-acetate
[356-12-7]

Fluocinonide, when dried, contains not less than 97.0% and not more than 103.0% of C_{26}H_{32}F_{2}O_{7}.

Description—Fluocinonide occurs as white crystals or crystalline powder.
It is sparingly soluble in chloroform, slightly soluble in acetonitrile, in methanol, in ethanol (95%) and in ethyl acetate, very slightly soluble in diethyl ether, and practically insoluble in water.

Identification—(1) To 0.01 g of Fluocinonide add 4 mL of water and 1 mL of Fehling’s TS, and heat: a red precipitate is formed.
(2) Prepare the test solution with 0.01 g of Fluocinonide as directed under Oxygen Flask Combustion Method <1.09>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests <1.09> for fluoride.
(3) Determine the absorption spectrum of a solution of Fluocinonide in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluocinonide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectra of Fluocinonide and Fluocinonide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare both spectra: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears in the absorption spectra, dissolve the sample and the RS in ethyl acetate, respectively, evaporate the ethyl acetate, and perform the test with the residue in the same manner.

Optical rotation <2.49> [α]D: +81 – +89° (after drying, 0.2 g, chloroform, 20 mL, 100 mm).

Purity Related substances—Dissolve 10 mg of Fluocinonide in 2 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.42>, and compare both solutions: both spectra exhibit similar intensities of absorption at the same wavelengths.

Columns: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of fluocinonide is about 8 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, fluocinonide and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of fluocinonide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Fluorescein Sodium

フルオレセインナトリウム

\[
\text{C}_{20}\text{H}_{10}\text{Na}_{2}\text{O}_{5}, \text{376.27}
\]

Disodium 2-(6-oxido-3-oxo-3H-xanthen-9-yl)benzoate

[518-47-8]

Fluorescein Sodium contains not less than 98.5% of \(\text{C}_{20}\text{H}_{10}\text{Na}_{2}\text{O}_{5}\), calculated on the dried basis.

Description Fluorescein Sodium occurs as an orange powder. It is odorless, and tasteless.

It is freely soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) To a solution of Fluorescein Sodium (1 in 100) having a strong green fluorescence, add a large quantity of water: the fluorescence remains. Acidify the solution with hydrochloric acid: the fluorescence disappears. Then render the solution alkaline with sodium hydroxide TS: the fluorescence reappears.

(2) Place 1 drop of a solution of Fluorescein Sodium (1 in 2000) on a piece of filter paper: a yellow spot develops. Expose the spot, while moist, to the vapor of bromine for 1 minute and then to ammonia vapor: the yellow color of the spot changes to red.

(3) Char 0.5 g of Fluorescein Sodium by ignition, cool, mix the residue with 20 mL of water, and filter: the filtrate responds to the Qualitative Tests <1.00> for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1 g of Fluorescein Sodium in 10 mL of water: the solution is clear, and shows a red color.

(2) Chloride <1.03>—Dissolve 0.15 g of Fluorescein Sodium in 20 mL of water, add 6 mL of dilute nitric acid and water to make 30 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric
acid VS (not more than 0.355%).

(3) Sulfate <1.14>—Dissolve 0.20 g of Fluorescein Sodium in 30 mL of water, add 2.5 mL of dilute hydrochloric acid and water to make 40 mL, and filter. To 20 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Zinc—Dissolve 0.10 g of Fluorescein Sodium in 10 mL of water, add 2 mL of hydrochloric acid, and filter. To the filtrate add 0.1 mL of potassium hexacyanoferrate (II) TS: no turbidity is produced immediately.

(5) Related substances—Dissolve 0.20 g of Fluorescein Sodium in 10 mL of methanol, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.13>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28:30:1) to a distance of about 10 cm, and air-dry the plate: any colored spot other than the principal spot does not appear.

**Spots**

**A** Fluorometholone

**B** Fluorometholone RS

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, constant mass).

**Assay** Transfer about 0.5 g of Fluorescein Sodium, accurately weighed, to a separator. Dissolve in 20 mL of water, add 5 mL of dilute hydrochloric acid, and extract with four 20-mL portions of a mixture of 2-methyl-1-propanol and dichloromethane, acetone and methanol (45:5:1) to a distance of about 10 cm, and air-dry the plate: any colored spot other than the principal spot does not appear.

**Containers and storage** Containers—Tight containers.

**Fluorometholone**

フルオロメトロン

C_{22}H_{29}FO_4: 376.46
9-Fluoro-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione
[426-13-1]

Fluorometholone, when dried, contains not less than 97.0% and not more than 103.0% of C_{22}H_{29}FO_4.

**Description** Fluorometholone occurs as a white to light yellowish white, odorless, crystalline powder.

It is freely soluble in pyridine, slightly soluble in methanol, in ethanol (99.5) and in tetrahydrofuran, and practically insoluble in water and in diethyl ether.

**Identification** (1) Proceed with 7 mg of Fluorometholone as directed under Oxygen Flask Combustion Method <1.067>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the liquid responds to the Qualitative Tests <1.097> (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Fluorometholone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluorometholone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fluorometholone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Fluorometholone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [α]_D^2 = +52 - +60° (after drying, 0.1 g, pyridine, 10 mL, 100 mm).

**Purity** (1) Heavy metals <1.077>—Proceed with 1.0 g of Fluorometholone according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Fluorometholone in 10 mL of tetrahydrofuran, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add tetrahydrofuran to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, acetone and methanol (45:5:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 1.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.2% (0.2 g, platinum crucible).

**Assay** Weigh accurately about 0.1 g each of Fluorometholone and Fluorometholone RS, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, and add diluted methanol (7 in 10) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and diluted methanol (7 in 10) to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of fluorometholone to that of the internal standard.

Amount (mg) of fluorometholone (C_{22}H_{29}FO_4) = M_s × Q_T/Q_S
**Fluorouracil**

フルオロウラシル

C$_7$H$_7$FN$_2$O$_2$: 130.08

5-Fluorouracil  
[51-21-8]

Fluorouracil, when dried, contains not less than 98.5% of C$_7$H$_7$FN$_2$O$_2$, and not less than 13.1% and not more than 16.1% of fluorine (F: 19.00).

**Description**  
Fluorouracil occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in N,N-dimethylformamide, sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 282°C (with decomposition).

**Identification**  
(1) Add 0.2 mL of bromine TS to 5 mL of a solution of Fluorouracil (1 in 500): the color of bromine TS is discharged. Further add 2 mL of barium hydroxide TS: a purple precipitate is formed.

(2) Proceed with 0.01 g of Fluorouracil as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <1.09> for fluoride.

(3) Determine the absorption spectrum of a solution of Fluorouracil in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.2>4, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity**  
(1) Clarity and color of solution—Add 20 mL of water to 0.20 g of Fluorouracil, and dissolve by warming: the solution is clear and colorless.

(2) Fluoride—Dissolve 0.10 g of Fluorouracil in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution to a 20-mL volumetric flask, add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS (1:1:1), and add water to make 20 mL. Allow to stand for 1 hour, and use this solution as the sample solution. Separately, transfer 1.0 mL of Standard Fluorine Solution to a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), and add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS (1:1:1). Proceed in the same manner as directed for the preparation of the sample solution, and use this solution as the standard solution. Perform the test as directed under Ultraviolet-visible Spectrophotometry <2.2>, using a solution, prepared with 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in the same manner, as the blank: the absorbance of the sample solution at 600 nm is not larger than that of the standard solution (not more than 0.012%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Fluorouracil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 0.2 ppm).

(4) Arsenic <1.17>—To 1.0 g of Fluorouracil in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), ignite the ethanol to burn, and incinerate by strong heating at 750°C to 850°C. If a carbonized substance remains in this method, moisten with a small amount of nitric acid, and incinerate by strong heating. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve it by warming on a water bath, use this solution as the test solution, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Fluorouracil in 10 mL of water, and use this solution as the sample solution. Measure exactly 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.02>. Spot 10 µL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (7:4:1) to a distance of about 12 cm, air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying <2.41>**  
Not more than 0.5% (1 g, in vacuum, 80°C, 4 hours).

**Residue on ignition <2.42>**  
Not more than 0.1% (1 g).

**Assay**  
(1) Fluorouracil—Weigh accurately about 0.2 g of Fluorouracil, previously dried, dissolve in 20 mL of N,N-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through blue-green to blue (indicator: 3 drops of thymol blue-dimethylformamide TS).
Perform a blank determination.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
   = 13.01 mg of C\textsubscript{12}H\textsubscript{27}F\textsubscript{3}O\textsubscript{3}.

(2) Fluorine—Weigh accurately about 4 mg of Fluoro-
uracil, previously dried, and proceed as directed in the de-
mination of fluorine under Oxygen Flask Combustion
Method \(<1.00>\), using a mixture of 0.5 mL of 0.01 mol/L sodium hy-
droxide TS and 20 mL of water as the absorbing liquid.

Containers and storage Containers—Tight containers.

**Fluoxymesterone**

フルオキシメステロン

C\textsubscript{20}H\textsubscript{29}FO\textsubscript{3}: 336.44
9-Fluoro-11\beta,17β-dihydroxy-17-methylandrost-4-en-3-one \([76-43-7]\)

Fluoxymesterone, when dried, contains not less than
97.0% and not more than 102.0% of C\textsubscript{20}H\textsubscript{29}FO\textsubscript{3}.

**Description** Fluoxymesterone occurs as white crystals or
crystalline powder. It is odorless.

It is sparingly soluble in methanol, slightly soluble in
ethanol (95) and in chloroform, very slightly soluble in
diethyl ether, and practically insoluble in water.

**Identification** (1) Dissolve 5 mg of Fluoxymesterone in 2
mL of sulfuric acid: a yellow color develops.

(2) Prepare the test solution with 0.01 g of Fluoxymes-
terone as directed under Oxygen Flask Combustion
Method \(<1.00>\), using a mixture of 0.5 mL of 0.01 mol/L sodium hy-
droxide TS and 20 mL of water as an absorbing liquid: the
result of the test responds to the Qualitative Tests \(<1.00>\) (2) for
fluoride.

(3) Determine the absorption spectrum of a solution of
Fluoxymesterone in ethanol (95) (1 in 100,000) as directed
under Ultraviolet-visible Spectrophotometry \(<2.24>\), and
compare the spectrum with the Reference Spectrum or the
spectrum of a solution of Fluoxymesterone RS prepared in
the same manner as the sample solution: both spectra exhibit
similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of
Fluoxymesterone, previously dried, as directed in the potas-
sium bromide disk method under Infrared Spectrophotom-
etry \(<2.25>\), and compare the spectrum with the Reference Spectrum or
the spectrum of previously dried Fluoxymesterone RS: both spectra exhibit similar intensities of absorp-
tion at the same wave numbers. If any difference appears
between the spectra, dissolve Fluoxymesterone and Fluox-
ymesterone RS in ethanol (99.5), respectively, then evaporate
the ethanol to dryness, and repeat the test on the residues.

**Optical rotation** \(<2.49>\) \([\epsilon]_{D}^{20}: +104 - +112^\circ\) (after drying,
0.1 g, ethanol (95), 10 mL, 100 mm).

**Purity** (1) Heavy metals \(<1.07>\)—Proceed with 0.5 g of
Fluoxymesterone according to Method 2, and perform the
test. Prepare the control solution with 1.5 mL of Standard
Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 0.03 g of Fluoxymes-
terone in 10 mL of methanol, and use this solution as the
sample solution. Pipet 1 mL of the sample solution, add
methanol to make exactly 100 mL, and use this solution as
the standard solution. Perform the test with these solutions
as directed under Thin-layer Chromatography \(<2.05>\). Spot
10 \(\mu\)L each of the sample solution and standard solution on a
plate of silica gel with fluorescent indicator for thin-layer
chromatography. Develop the plate with a mixture of tolu-
en, ethanol (95) and ethyl acetate (3:1:1) to a distance of
about 12 cm, and air-dry the plate. Examine under ultravio-
let light (main wavelength: 254 nm): the spots other than the
principal spot from the sample solution are not more intense
than the spot from the standard solution.

**Loss on drying** \(<2.47>\) Not more than 1.0% (1 g, 105°C,
3 hours).

**Residue on ignition** \(<2.44>\) Not more than 0.2% (0.5 g,
platinum crucible).

**Assay** Weigh accurately about 25 mg each of Fluoxymes-
terone and Fluoxymesterone RS, previously dried, dissolve
each in the internal standard solution to make exactly 100
mL, and use these solutions as the sample solution and the
standard solution, respectively. Perform the test with 10 \(\mu\)L
each of the sample solution and standard solution as directed
under Liquid Chromatography \(<2.01>\) according to the fol-
lowing conditions, and calculate the ratios, \(Q_1\) and \(Q_2\), of
the peak area of fluoxymesterone to that of the internal
standard, respectively.

\[
\text{Amount (mg)} \text{ of C}_{20}\text{H}_{29}\text{FO}_{3} = M_{S} \times \frac{Q_{T}}{Q_{S}}
\]

\(M_{S}\): Amount (mg) of Fluoxymesterone RS

**Internal standard solution**—A solution of methylpredniso-
lone in a mixture of chloroform and methanol (19:1) (1 in
5000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wave-
lengh: 254 nm).

Column: A stainless steel column 4.6 mm in inside diame-
ter and 30 cm in length, packed with silica gel for liquid
chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about
25°C.

Mobile phase: A mixture of n-butyl chloride, water-satu-
rated n-butyl chloride, tetrahydrofuran, methanol and acetic
acid (100) (95:95:14:7:6).

Flow rate: Adjust the flow rate so that the retention time
of fluoxymesterone is about 9 minutes.

**System suitability**—

System performance: When the procedure is run with 10
\(\mu\)L of the standard solution under the above operating con-
ditions, fluoxymesterone and the internal standard are eluted
in this order with the resolution between these peaks being
not less than 6.

System repeatability: When the test is repeated 6 times
with 10 \(\mu\)L of the standard solution under the above operat-
Fluphenazine Enanthate

フルフェナジンエナント酸エステル

\[\text{C}_{29}\text{H}_{38}\text{F}_{3}\text{N}_{3}\text{O}_{2}\text{S}: 549.69}\]

2-(4-[[3-[2-(Trifluoromethyl)-10\text{-}H\text{-}phenothiazin-10\text{-}yl]propyl]piperazin-1-yl]ethyl heptanoate [2746-81-8]

Fluphenazine Enanthate, when dried, contains not less than 98.5% of \(\text{C}_{29}\text{H}_{38}\text{F}_{3}\text{N}_{3}\text{O}_{2}\text{S}\).

Description Fluphenazine Enanthate is a light yellow to yellowish orange viscous liquid. It is generally clear, and can be opaque by producing crystals.

It is freely soluble in methanol and in diethyl ether, soluble in ethanol (95) and in acetic acid (100), and practically insoluble in water.

Identification (1) Prepare the test solution with 0.01 g of Fluphenazine Enanthate as directed under Oxygen Flask Combustion Method <1.067>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <1.09> for fluoride.

(2) Dissolve 2 mg of Fluphenazine Enanthate in 200 mL of a solution of hydrochloric acid in methanol (17 in 2000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fluphenazine Enanthate as directed in the liquid firm method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Fluphenazine Enanthate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 0.25 g of Fluphenazine Enanthate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, hexane and ammonia solution (28) (16:6:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Then spray evenly diluted sulfuric acid (1 in 2) on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Fluphenazine Enanthate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 27.49 mg of \(\text{C}_{29}\text{H}_{38}\text{F}_{3}\text{N}_{3}\text{O}_{2}\text{S}\).

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Flurazepam

フルラゼパム

\[\text{C}_{21}\text{H}_{23}\text{ClF}_{7}\text{N}_{3}\text{O}: 387.88}\]

7-Chloro-1-[[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2\text{-}H\text{-}1,4-benzodiazepin-2-one [17617-23-1]

Flurazepam, when dried, contains not less than 99.0% of \(\text{C}_{21}\text{H}_{23}\text{ClF}_{7}\text{N}_{3}\text{O}\).

Description Flurazepam occurs as white to light yellow crystals or crystalline powder.

It is very soluble in chloroform, freely soluble in methanol, in ethanol (95), in acetic anhydride and in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 0.01 g of Flurazepam in 3 mL of sulfuric acid: the solution shows a greenish yellow fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Dissolve 0.01 g of Flurazepam in 3 mL of citric acid-acetic acid-TS, and heat in a water bath for 4 minutes: a dark red color develops.

(3) Prepare the test solution with 0.01 g of Flurazepam as directed under Oxygen Flask Combustion Method <1.067>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide...
TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(4) Determine the absorption spectrum of a solution of Flurazepam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1; both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Flurazepam in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2; both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Perform the test with Flurazepam as directed under Flame Coloration Test <1.04> (2): a green color appears.

**Flurazepam Capsules**

Flurazepam Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of flurazepam (C₂₁H₂₃ClFN₃O). Flurazepam Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of flurazepam (C₂₁H₂₃ClFN₃O: 387.88).

**Method of preparation** Prepare as directed under Capsules, with Flurazepam.

**Identification** (1) Powder the contents of Flurazepam Capsules. To a quantity of the powder, equivalent to 0.1 g of Flurazepam according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, stir, and filter. To 40 mL of the filtrate add 80 mL of a solution of sodium hydroxide (1 in 250) and 100 mL of hexane, extract by shaking well, and use the hexane layer as the sample solution. Evaporate 25 mL of the sample solution on a water bath to dryness. Dissolve the residue in 3 mL of sulfuric acid: the solution shows a greenish yellow fluorescence under ultraviolet light.

(2) Evaporate 25 mL of the sample solution obtained in (1) on a water bath to dryness. Dissolve the residue in 3 mL of citric acid-acetic acid TS, and heat in a water bath for 4 minutes: a dark red color develops.

(3) Determine the absorption spectrum of the sample solution obtained in (1) under ultraviolet-visible Spectrophotometry: it exhibits a maximum between 315 nm and 319 nm, and a minimum between 297 nm and 301 nm.

**Assay** Weigh accurately about 0.3 g of Flurazepam, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS to the second equivalence point (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 19.39 mg of C₂₁H₂₃ClFN₃O

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

Weigh accurately about 0.3 g of Flurazepam, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS to the second equivalence point (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 19.39 mg of C₂₁H₂₃ClFN₃O

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.
Flurazepam Hydrochloride

フルラゼパム塩酸塩

C₂₁H₂₃ClFN₃O.HCl: 424.34
7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one monohydrochloride [36105-20-1]

Flurazepam Hydrochloride, when dried, contains not less than 99.0% of C₂₁H₂₃ClFN₃O.HCl.

Description Flurazepam Hydrochloride occurs as white to yellowish white crystals or crystalline powder.

It is freely soluble in water, in ethanol (95), in ethanol (99.5) and in acetic acid (100).

Melting point: about 197°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Flurazepam Hydrochloride in sulfuric acid—ethanol TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.4>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flurazepam Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectroscopy <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Flurazepam Hydrochloride (1 in 20) responds to the Qualitative Tests <1.097> for chloride.

pH <2.5> Dissolve 1.0 g of Flurazepam Hydrochloride in 20 mL of water: the pH of this solution is between 5.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Flurazepam Hydrochloride in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Sulfate <1.14>—Perform the test with 1.5 g of Flurazepam Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Heavy metals <1.077>—Proceed with 1.0 g of Flurazepam Hydrochloride in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.05 g of Flurazepam Hydrochloride in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 50 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Use 20 mL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Place the plate in a chamber filled with ammonia vapor, allow to stand for about 15 minutes, and immediately develop the plate with a mixture of diethyl ether and diethylamine (39:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 3 spots other than the principal spot and the spot on the starting point from the sample solution appear, and are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Flurazepam Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate to 2.50 with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 21.22 mg of C₂₁H₂₃ClFN₃O.HCl

Containers and storage Containers—Tight containers.

Flurbiprofen

フルルビプロフェン

C₁₅H₁₄FO₂: 244.26
(2RS)-2-(2-Fluorobiphenyl-4-yl)propionic acid [5104-49-4]

Flurbiprofen, when dried, contains not less than 98.0% of C₁₅H₁₁FO₂.

Description Flurbiprofen occurs as a white, crystalline powder. It has a slightly irritating odor.

It is freely soluble in methanol, in ethanol (95), in acetone and in diethyl ether, soluble in acetonitrile, and practically insoluble in water.

A solution of Flurbiprofen in ethanol (95) (1 in 50) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Flurbiprofen in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flurbiprofen, previously dried, as directed in the potassium
bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelength numbers.

**Purity** (1) Chloride 1.03—Dissolve 0.6 g of Flurbiprofen in 40 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.015%).

(2) Heavy metals 1.07—Dissolve 2.0 g of Flurbiprofen in 30 mL of acetone, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the sample solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Flurbiprofen in 10 mL of a mixture of water and acetonitrile (11:9), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (11:9) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 mL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than the peak of flurbiprofen from the sample solution is not larger than twice the peak area of flurbiprofen from the standard solution.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (12:7:1).
Flow rate: Adjust the flow rate so that the retention time of flurbiprofen is about 20 minutes.

**System suitability**—
Test for required detection: To exactly 5 mL of the standard solution add a mixture of water and acetonitrile (11:9) to make exactly 25 mL. Confirm that the peak area of flurbiprofen obtained from 20 \( \mu \)L of this solution is equivalent to 16 to 24% of that from 20 \( \mu \)L of the standard solution.

System performance: Dissolve 0.04 g of flurbiprofen and 0.02 g of butyl parahydroxybenzoate in 100 mL of a mixture of water and acetonitrile (11:9). To 5 mL of this solution add a mixture of water and acetonitrile (11:9) to make 50 mL. When the procedure is run with 20 \( \mu \)L of this solution under the above operating conditions, butyl parahydroxybenzoate and flurbiprofen are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flurbiprofen is not more than 2.0%.

**Loss on drying** 2.41—Not more than 0.10% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, silica gel, 4 hours).

**Residue on ignition** 2.44—Not more than 0.1% (1 g, platinum crucible).

**Assay** Weigh accurately about 0.6 g of Flurbiprofen, previously dried, dissolve in 50 mL of ethanol (95), and titrate 2.50 with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 24.43 mg of C\(_{11}\)H\(_{15}\)F\(_{3}\)NO\(_{3}\).

**Containers and storage** Containers—Well-closed containers.

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**Flutamide**

フルタミド

C\(_{11}\)H\(_{15}\)F\(_{3}\)N\(_{2}\)O\(_{3}\): 276.21
2-Methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide [13311-84-7]

Flutamide, when dried, contains not less than 98.5% and not more than 101.5% of C\(_{11}\)H\(_{15}\)F\(_{3}\)N\(_{2}\)O\(_{3}\).

**Description** Flutamide occurs as a light yellow crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Flutamide in ethanol (95) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Flutamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flutamide as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum or the spectrum of Flutamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** 2.60 109 – 113°C

**Purity** (1) Heavy metals 1.07—Proceed with 2.0 g of Flutamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Sol-
lution (not more than 10 ppm).

(2) Related substances—Dissolve 40 mg of Flutamide in 50 mL of methanol, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography (2.01) according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amounts of them by the area percentage method: the amount of each peak other than flutamide is not more than 0.3%, and the total amount of the peaks other than flutamide is not more than 0.5%.

Operating conditions—
Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Time span of measurement: About 2 times as long as the retention time of flutamide, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add methanol to make exactly 20 mL. Confirm that the peak area of flutamide obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of flutamide is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying (2.48) Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition (2.44) Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 40 mg each of Flutamide and Flutamide RS, previously dried, and dissolve separately in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, Q3 and Q5, of the peak height of flutamide to that of the internal standard.

Amount (mg) of flutamide (C19H16ClFN2O): M = M5 × Q5/Q3

M5: Amount (mg) of Flutamide RS

Internal standard solution—A solution of testosterone in methanol (9 in 10,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.05 mol/L potassium dihydrogen phosphate TS (7:4).
Flow rate: Adjust the flow rate so that the retention time of flutamide is about 12 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, flutamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flutamide is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Flutoprazepam

フルトプラゼパム

C19H16ClFN2O: 342.79
7-Chloro-1-cyclopropylmethyl-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one [25967-29-7]

Flutoprazepam, when dried, contains not less than 99.0% and not more than 101.0% of C19H16ClFN2O.

Description Flutoprazepam occurs as a white to light yellow crystals or crystalline powder.

It is freely soluble in ethyl acetate, soluble in ethanol (99.5) and in acetic anhydride, and practically insoluble in water.

Identification (1) Dissolve 2 mg of Flutoprazepam in 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flutoprazepam as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Flutoprazepam as directed under Flame Coloration Test (1.04) (2): a green color appears.
Flutoprazepam Tablets

Flutoprazepam Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of flutoprazepam \((C_{19}H_{16}ClFN_2O)\). Prepare as directed under Tablets, with Flutoprazepam.

Method of preparation

Identification

To a quantity of powdered Flutoprazepam Tablets, equivalent to 10 mg of Flutoprazepam according to the labeled amount, add 20 mL of a solution of sulfuric acid in ethanol (99.5) \((3 \text{ in } 1000)\), shake well, and add a solution of sulfuric acid in ethanol (99.5) \((3 \text{ in } 1000)\) to make 100 mL. Centrifuge this solution, to 10 mL of the supernatant liquid add a solution of sulfuric acid in ethanol (99.5) \((3 \text{ in } 1000)\) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry. It exhibits maxima between 240 nm and 244 nm, between 279 nm and 285 nm, and between 369 nm and 375 nm.

Uniformity of dosage units

When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Flutoprazepam Tablets is not less than 70%.

Start the test with 1 tablet of Flutoprazepam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \(\mu m\). Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test as directed in the Assay.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 \(\mu L\) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of flutoprazepam are not less than 4000 and not more than 1.5, respectively.
Fluvoxamine Maleate

フルボキサミンマレイン酸塩

C_{15}H_{21}F_{3}N_{2}O_{2}.C_{4}H_{4}O_{4}: 434.41
5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one (E)-O-(2-aminoethyl)oxime monomaleate [61718-82-9]

Fluvoxamine Maleate contains not less than 98.0% and not more than 101.0% of C_{15}H_{21}F_{3}N_{2}O_{2}.C_{4}H_{4}O_{4}, calculated on the dried basis.

Description Fluvoxamine Maleate occurs as a white crystalline powder.
It is freely soluble in ethanol (99.5), and sparingly soluble in water.

Identification (1) Dissolve 10 mg of Fluvoxamine Maleate in 5 mL of water, neutralize with dilute sodium hydroxide TS, then add 1 mL of ninhydrin TS, and heat in a water bath at 60 – 70°C for 5 minutes: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Fluvoxamine Maleate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluvoxamine Maleate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fluvoxamine Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum or the spectrum of Fluvoxamine Maleate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 5 mL of a solution of Fluvoxamine Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the TS disappears immediately.

Melting point 2.60° 120 – 124°C.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 0.5 g of Fluvoxamine Maleate in 50 mL of water is clear and colorless.

(2) Chloride 1.03—Perform the test with 1.0 g of Fluvoxamine Maleate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).

(3) Sulfate 1.14—Perform the test with 1.0 g of Fluvoxamine Maleate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.017%).

(4) Heavy metals 1.07—Proceed with 1.0 g of Fluvoxamine Maleate according to Method 2, using alumina ceramic crucible, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(5) Related substances—Dissolve 20 mg of Fluvoxamine Maleate in 20 mL of a mixture of methanol for liquid chromatography and water (7:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol for liquid chromatography and water (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks, having the relative retention time of about 0.76, about 0.82, about 0.89, about 1.58 and about 1.66 to fluvoxamine, obtained from the sample solution are not larger than 1/15 times, 3/10 times, 7/10 times, 1/10 times and 1/10 times the peak area of fluvoxamine from the standard solution, respectively, and the total area of the peaks other than fluvoxamine from the sample solution is not larger than 1.5 times the peak area of fluvoxamine from the standard solution. For these calculations use the areas of the peaks, having the relative retention time of about 0.76, about 0.89, about 1.58 and about 1.66, after multiplying by their relative response factors, 0.87, 2.00, 0.67 and 2.76, respectively.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 12.67 g of diammonium hydrogen phosphate and 0.85 g of sodium 1-heptanesulphonate in 900 mL of water, adjust to pH 2.0 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 700 mL of methanol for liquid chromatography.
Flow rate: Adjust the flow rate so that the retention time of fluvoxamine is about 9 minutes.
Time span of measurement: About 2 times as long as the retention time of fluvoxamine, beginning after the peak of maleic acid.
System suitability—
Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of methanol for liquid chromatography and water (7:3) to make exactly 20 mL. Confirm that the peak area of fluvoxamine with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fluvoxamine are not less than 5000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fluvoxamine is not more than 2.0%.

(6) Residual solvent—Being specified separately.
Loss on drying <2.41> Not more than 0.1% (1 g, in vacuum, 50°C, 4 hours).
Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 20 mg each of Fluvoxamine Maleate and Fluvoxamine Maleate RS (separately determine the loss on drying <2.41> in the same condition as Fluvoxamine Maleate), dissolve each in 10 mL of the mobile phase, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of fluvoxamine to that of the internal standard.

\[ M_s = \frac{Q_1}{Q_2} \]

Internal standard solution—A solution of diphenylamine in methanol (7 in 2000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 3.8 g of diammonium hydrogen phosphate and 0.8 g of sodium 1-heptanesulphonate in water to make 300 mL, add 700 mL of methanol, and adjust to pH 3.5 with phosphoric acid.
Flow rate: Adjust the flow rate so that the retention time of fluvoxamine is about 9 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, fluvoxamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of fluvoxamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Fluvoxamine Maleate Tablets
フルボキサミンマレイン酸塩錠

Fluvoxamine Maleate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of fluvoxamine maleate (C15H21F3N2O2.C4H4O4; 434.41).

Method of preparation Prepare as directed under Tablets, with Fluvoxamine Maleate.

Identification Powder Fluvoxamine Maleate Tablets. To a
portion of the powder, equivalent to 0.1 g of Fluvoxamine Maleate according to the labeled amount, add 50 mL of water, shake, then allow to stand, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 µm. To 0.5 mL of the filtrate add 50 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 243 nm and 247 nm.

**Uniformity of dosage units**<6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Fluvoxamine Maleate Tablets add 4 mL of water, disintegrate the tablet with the aid of ultrasonic waves, add a mixture of methanol for liquid chromatography and water (7:3) to make exactly 50 mL, and filter. Pipet V mL of the filtrate, equivalent to about 6 mg of fluvoxamine maleate (C\textsubscript{15}H\textsubscript{21}F\textsubscript{3}N\textsubscript{2}O\textsubscript{2}.C\textsubscript{4}H\textsubscript{4}O\textsubscript{4}), add exactly 2 mL of the internal standard solution, then add a mixture of methanol for liquid chromatography and water (7:3) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

**Internal standard solution**—A solution of diphenylamine in methanol for liquid chromatography (3 in 1000).

**Dissolution**<6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Fluvoxamine Maleate Tablets is not less than 80%.

Start the test with 1 tablet of Fluvoxamine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 20 µg of fluvoxamine maleate (C\textsubscript{15}H\textsubscript{21}F\textsubscript{3}N\textsubscript{2}O\textsubscript{2}.C\textsubscript{4}H\textsubscript{4}O\textsubscript{4}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Fluvoxamine Maleate RS (separately determine the loss on drying <2.41> in the same condition as Fluvoxamine Maleate), and dissolve in a mixture of methanol for liquid chromatography and water (7:3) to make exactly 25 mL. Pipet 3 mL of this solution, add exactly 2 mL of the internal standard solution, then add a mixture of methanol for liquid chromatography and water (7:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q\textsubscript{1} and Q\textsubscript{2}, of the peak area of fluvoxamine to that of the internal standard.

**Internal standard solution**—A solution of diphenylamine in methanol for liquid chromatography (3 in 1000).

**Operating conditions**—

Proceed as directed in the operating conditions in the Assay under Fluvoxamine Maleate.

**System suitability**—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, fluvoxamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of fluvoxamine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

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**Folic Acid**

\[
\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6, \quad 441.40 \\
\text{N-}[4-\{(2-\text{Amino}-4-\text{hydroxypteridin-6-ylmethyl)}\text{amino}\}\text{benzoyl}-1-\text{glutamic acid}} [59-30-3]
\]

Folic Acid contains not less than 98.0% and not more than 102.0% of C\textsubscript{19}H\textsubscript{19}N\textsubscript{7}O\textsubscript{6}, calculated on the
anhydrous basis.

**Description**  Folic Acid occurs as a yellow to orange-yellow, crystalline powder. It is odorless.

It is practically insoluble in water, in methanol, in ethanol (95), in pyridine and in diethyl ether.

It dissolves in hydrochloric acid, in sulfuric acid, in dilute sodium hydroxide TS and in a solution of sodium carbonate decahydrate (1 in 100), and these solutions are yellow in color.

It is slowly affected by light.

**Identification (1)**  Dissolve 1.5 mg of Folic Acid in dilute sodium hydroxide TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Folic Acid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)  To 10 mL of the solution obtained in (1) add 1 drop of potassium permanganate TS, and mix well until the color changes to blue, and immediately observe under ultraviolet light (main wavelength: 365 nm): a blue fluorescence is produced.

**Purity (1)**  Clarity and color of solution—Dissolve 0.10 g of Folic Acid in 10 mL of dilute sodium hydroxide TS: the solution is clear and yellow in color.

(2)  Free amines—Pipet 30 mL of the sample solution obtained in the Assay, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of p-Aminobenzoylglutamic Acid RS, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, dissolve in diluted ethanol (2 in 5) to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 1000 mL, and use this solution as the standard solution. Pipet 4 mL each of solutions of the sample solution and standard solution at 550 nm: the content of free amines is not more than 1.0%.

\[
\text{Content (\% of free amines) = } \frac{M_5}{M_4} \times \frac{A_T}{A_S}
\]

\[
M_4: \text{Amount (mg) of Folic Acid, calculated on the anhydrous basis}
\]

\[
M_5: \text{Amount (mg) of p-Aminobenzoylglutamic Acid RS}
\]

**Water**  <2.48>  Not more than 8.5% (10 mg, coulometric titration).

**Residue on ignition**  <2.48>  Not more than 0.5% (1 g).

**Assay**  Weigh accurately about 50 mg each of Folic Acid and Folic Acid RS (separately, determine the water <2.48> in the same manner as Folic Acid). To each add 50 mL of dilute sodium hydroxide TS, mix well to dissolve, add dilute sodium hydroxide TS to make exactly 100 mL, and use these solutions as the sample solution and standard solution. To 30 mL each of these solutions, accurately measured, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 60 mL each of these solutions add 0.5 g of zinc powder, and allow to stand with frequent shaking for 20 minutes. Filter each mixture through a dry filter paper, and discard the first 10 mL of the filtrate. Pipet 10 mL each of the subsequent filtrate, and add water to make exactly 100 mL. To 4 mL each of solutions, accurately measured, add 1 mL of water, 1 mL of dilute hydrochloric acid and 1 mL of a solution of sodium nitrite (1 in 1000), mix well, and allow to stand for 2 minutes. To each solution add 1 mL of a solution of ammonium amidosulfate (1 in 200), mix thoroughly, and allow to stand for 2 minutes. To each of these solutions, add 1 mL of a solution of N-(1-naphthyl)-N’-diethylthylethylenediamine oxalate (1 in 1000), shake, allow to stand for 10 minutes, and add water to make exactly 20 mL. Separately, to 30 mL of the sample solution, accurately measured, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 18 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 4 mL of this solution, and prepare the blank solution in the same manner as the sample solution. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 4 mL of water in the same manner as a blank. Determine the absorbances, \(A_T\), \(A_S\), and \(A_C\), of the subsequent solution of the sample solution, the standard solution and the blank solution at 550 nm.

\[
\frac{\text{Amount (mg) of C}_{19}\text{H}_{19}\text{N}_7\text{O}_6}{\text{M}_5} = \frac{M_5}{M_4} \times \frac{A_T}{A_S} / \frac{A_T}{A_S}
\]

**Containers and storage**  Containers—Tight containers.

Storage—Light-resistant.

**Folic Acid Injection**

葉酸注射液

Folic Acid Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 115.0% of the labeled amount of folic acid (C\(_{19}\)H\(_{19}\)N\(_7\)O\(_6\): 441.40).

**Method of preparation**  Dissolve Folic Acid in water with the aid of Sodium Hydroxide or Sodium Carbonate, and prepare as directed under Injections.

**Description**  Folic Acid Injection is a yellow to orange-yellow, clear liquid.

pH: 8.0 – 11.0

**Identification (1)**  To a volume of Folic Acid Injection, equivalent to 1.5 mg of Folic Acid according to the labeled amount, add dilute sodium hydroxide TS to make 100 mL. Proceed as directed in the Identification (2) under Folic Acid, using this solution as the sample solution.

(2)  Determine the absorption spectrum of the sample solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 255 nm and 257 nm, between 281 nm and 285 nm and between 361 nm and 369 nm. Separately, determine the maximal absorbances of the sample solution, \(A_1\) and \(A_2\), between 255 nm and 257 nm and between 361 nm and 369 nm, respectively: the ratio of \(A_1/A_2\) is between 2.80 and 3.00.

(3)  Folic Acid Injection responds to the Qualitative Tests
Extractable volume \(<6.05\) It meets the requirement.

Foreign insoluble matter \(<6.06\) Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter \(<6.07\) It meets the requirement.

Sterility \(<4.06\) Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** To an exactly measured volume of Folic Acid Injection, equivalent to about 50 mg of folic acid \((C_{19}H_{19}N_{7}O_{6})\) add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Folic Acid RS, dissolve in dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Proceed with exactly 30 mL each of the sample solution and standard solution as directed in the Assay under Folic Acid.

\[
\text{Amount (mg) of folic acid} (C_{19}H_{19}N_{7}O_{6}) = M_5 \times (A_1 - A_2)/A_3
\]

\(M_5\): Amount (mg) of Folic Acid RS, calculated on the anhydrous basis

**Containers and storage** Containers—Hermetic containers, and colored containers may be used. Storage—Light-resistant.

**Folic Acid Tablets**

Folic Acid Tablets contain not less than 90.0% and not more than 115.0% of the labeled amount of folic acid \((C_{19}H_{19}N_{7}O_{6}): 441.40\).

**Method of preparation** Prepare as directed under Tablets, with Folic Acid.

**Identification** (1) Take a quantity of powdered Folic Acid Tablets, equivalent to 1.5 mg of Folic Acid according to the labeled amount, add 100 mL of dilute sodium hydroxide TS, shake, and filter. Discard the first 10 mL of the filtrate, use the subsequent filtrate as the sample solution, and proceed as directed in the Identification (2) under Folic Acid.

(2) Determine the absorption spectrum of the filtrate obtained in (1) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\): it exhibits maxima between 255 nm and 257 nm, between 281 nm and 285 nm and between 361 nm and 369 nm. Separately, determine the maximal absorbances of the filtrate, \(A_1\) and \(A_2\), between 255 nm and 257 nm and between 361 nm and 369 nm, respectively: the ratio of \(A_1/A_2\) is between 2.80 and 3.00.

**Uniformity of dosage units** \(<6.02\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Folic Acid Tablets add 50 mL of dilute sodium hydroxide TS, shake frequently, and filter. Wash the residue with dilute sodium hydroxide TS, combine the filtrate and the washings, then add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample stock solution. Pipet 30 mL of the sample stock solution, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 60 mL of this solution, add 0.5 g of zinc powder, shake frequently, and filter the solution through a dried filter paper. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains about 15 \(\mu\)g of folic acid \((C_{19}H_{19}N_{7}O_{6})\), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Folic Acid RS (separately determine the water \(<2.48\) in the same manner as Folic Acid), and dissolve in dilute sodium hydroxide TS to make exactly 100 mL. Pipet 60 mL of this solution, add 0.5 g of zinc powder, shake frequently, and filter the solution through a dried filter paper. Discard the first 10 mL of the filtrate, pipet \(10\) mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution, add 1 mL of water, 1 mL of dilute hydrochloric acid and 1 mL of sodium nitrite solution (1 in 1000) to them, mix, and allow to stand for 2 minutes. To these solutions add 1 mL of a solution of ammonium amidosulfate (1 in 200), shake, and allow them to stand for 2 minutes. To these solutions add 1 mL of a solution of \(N,N\)-diethyl-\(N^1\)-napthylheteroarylenediamine oxalate (1 in 1000), shake, allow to stand for 10 minutes, and add water to make exactly 20 mL. Separately, pipet 30 mL of the sample stock solution, add 20 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet \(V\) mL of this solution, and add water to make exactly \(V\) mL so that each mL contains about 15 \(\mu\)g of folic acid \((C_{19}H_{19}N_{7}O_{6})\). With exactly 4 mL of this solution perform the same procedure described above for obtaining the sample solution, and use the solution so obtained as the blank solution. Determine the absorbances at 550 nm, \(A_3\), \(A_4\) and \(A_5\), of the solutions obtained from the sample solution and standard solution, and the blank solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), using a control solution obtained with 4 mL of water in the same manner as described above.

\[
\text{Amount (mg) of folic acid} (C_{19}H_{19}N_{7}O_{6}) = M_5 \times (A_1 - A_2)/A_3 \times V/V \times 1/10
\]

\(M_5\): Amount (mg) of Folic Acid RS, calculated on the anhydrous basis

**Dissolution** \(<6.10\) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Folic Acid Tablets is not less than 75%.

Start the test with 1 tablet of Folic Acid Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains about 5.6 \(\mu\)g of folic acid \((C_{19}H_{19}N_{7}O_{6})\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Folic Acid RS (separately determine the water \(<2.48\) in the same manner as Folic Acid), and dissolve in the 2nd fluid for dissolution test to make exactly 100 mL. Pipet 2.5 mL of this solution, add
the 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, at 280 nm of the sample solution

Dissolution rate ($z$) with respect to the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$) $= M_S \times A_T / A_S \times V / V' \times 1/ C \times 45/2$

$M_S$: Amount (mg) of Folic Acid RS, calculated on the anhydrous basis

$C$: Labeled amount (mg) of folic acid ($C_{19}H_{19}N_7O_6$) in 1 tablet

**Assay**  Weigh accurately and powder not less than 20 Folic Acid Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of folic acid ($C_{19}H_{19}N_7O_6$). Add 50 mL of dilute sodium hydroxide TS, shake frequently, then filter into a 100-mL volumetric flask, and wash with dilute sodium hydroxide TS. To the combined filtrate and washings add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Folic Acid RS, dissolve in dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Proceed with exactly 30 mL each of the sample solution and standard solution as directed in the Assay under Folic Acid.

Amount (mg) of folic acid ($C_{19}H_{19}N_7O_6$) $= M_S \times (A_T - A_C) / A_S$

$M_S$: Amount (mg) of Folic Acid RS, calculated on the anhydrous basis

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

### Formalin

ホルマリン

Formalin contains not less than 35.0% and not more than 38.0% of formaldehyde ($CH_2O$: 30.03.)

It contains 5% to 13% of methanol to prevent polymerization.

**Description**  Formalin is a clear, colorless liquid. Its vapor is irritating to the mucous membrane.

It is miscible with water and with ethanol (95).

When stored for a long time, especially in a cold place, it may become cloudy.

**Identification** (1) Dilute 2 mL of Formalin with 10 mL of water in a test tube, and add 1 mL of silver nitrate-ammonia TS: a gray precipitate is produced, or a silver mirror is formed on the wall of the test tube.

(2) To 5 mL of sulfuric acid in which 0.1 g of salicylic acid has been dissolved add 2 drops of Formalin, and warm the solution: a persistent, dark red color develops.

**Purity**  Acidity—Dilute 20 mL of Formalin with 20 mL of water, and add 5.0 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of bromothymol blue TS: a blue color develops.

### Residue on ignition <2.44>  Not more than 0.06 w/v% (5 mL, after evaporation).

**Assay**  Weigh accurately a weighing bottle containing 5 mL of water, add about 1 g of Formalin, and weigh accurately again. Add water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 50 mL of 0.05 mol/L iodine VS and 20 mL of potassium hydroxide TS, and allow to stand for 15 minutes at an ordinary temperature. To this mixture add 15 mL of dilute sulfuric acid, and titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS $= 1.501$ mg of $CH_2O$

**Containers and storage**  Containers—Tight containers.

Storage—Light-resistant.

### Formalin Water

ホルマリン水

Formalin Water contains not less than 0.9 w/v% and not more than 1.1 w/v% of formaldehyde ($CH_2O$: 30.03).

**Method of preparation**

<table>
<thead>
<tr>
<th>Formalin Water</th>
<th>30 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td>To make</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Prepare by mixing the above ingredients.

**Description**  Formalin Water is a clear, colorless liquid. It has a slight odor of formaldehyde.

It is almost neutral.

**Assay**  Transfer 20 mL of Formalin Water, measured exactly, to a 100-mL volumetric flask containing 2.5 mL of 1 mol/L sodium hydroxide VS, and add water to make 100 mL. Pipet 10 mL of this solution, and proceed as directed in the Assay under Formalin.

Each mL of 0.05 mol/L iodine VS $= 1.501$ mg of $CH_2O$

**Containers and storage**  Containers—Tight containers.
Formoterol Fumarate Hydrate

ホルモテロールフマル酸塩水和物

\[
\begin{align*}
\text{C}_{19}	ext{H}_{24}	ext{N}_2\text{O}_4 & : 804.88, \\
\text{z} & = \text{calculated on the anhydrous basis.}
\end{align*}
\]

Formoterol Fumarate Hydrate contains not less than 98.5% of formoterol fumarate [(C19H24N2O4)2].

**Description** Formoterol Fumarate Hydrate occurs as a white to yellowish white, crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Formoterol Fumarate Hydrate in methanol (1 in 100) shows no optical rotation.

Melt point: about 138°C (with decomposition).

**Identification (1)** Dissolve 0.5 g of Formoterol Fumarate Hydrate in 20 mL of 0.5 mol/L sulfuric acid TS, and extract with three 25-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 10 mL of 0.5 mol/L sulfuric acid TS, and evaporate the ether layer under reduced pressure, and dry the residue at 105°C for 3 hours: the residue melts \( < 260^\circ C \) at about 290°C (with decomposition, in a sealed tube).

(2) Determine the absorption spectrum of a solution of Formoterol Fumarate Hydrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry \( < 2.25 > \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Formoterol Fumarate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry \( < 2.25 > \), and compare with the spectrum of Fosfomycin Calcium Hydrate: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity (1)** Heavy metals \( < 0.07 \)—Proceed with 1.0 g of Formoterol Fumarate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related Substances—Dissolve 0.20 g of Formoterol Fumarate Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( < 2.05 > \). Spot 5 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, 1,4-dioxane, ethanol (99.5) and ammonia solution (28) (20:20:10:3) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 5 minutes in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Water** \( < 2.45 > \ 4.0 - 5.0\% (0.5 \text{ g}, \text{ volumetric titration, direct titration}).

**Residue on ignition** \( < 2.44 > \) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.7 g of Formoterol Fumarate Hydrate, dissolve in 50 mL of acetic acid (100), and titrate \( < 2.50 > \) with 0.1 mol/L perchloric acid TS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 40.24 mg of \( \text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_4 \).

**Containers and storage** Containers—Tight containers.

**Fosfomycin Calcium Hydrate**

ホスホマイシンカルシウム水和物

\[
\begin{align*}
\text{C}_{11}\text{H}_{12}\text{CaO}_{4}\text{P}.\text{H}_2\text{O} & : 194.14 \\
\text{S} & = \text{Monocalcium (2R,3S)-3-methyloxiran-2-ylphosphonate monohydrate}
\end{align*}
\]

Fosfomycin Calcium Hydrate is the calcium salt of a substance having antibacterial activity produced by the growth of \textit{Streptomyces fradiae} or by the chemical synthesis.

It contains not less than 725 \( \mu \text{g} \) (potency) and not more than 805 \( \mu \text{g} \) (potency) per mg, calculated on the anhydrous basis. The potency of Fosfomycin Calcium Hydrate is expressed as mass (potency) of fosfomycin (C7H4CaO4P: 138.06).

**Description** Fosfomycin Calcium Hydrate occurs as a white crystalline powder.

It is slightly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

**Identification (1)** Determine the infrared absorption spectrum of Fosfomycin Calcium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry \( < 2.25 > \), and compare with the spectrum of Fosfomycin Calcium Hydrate: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the \(^1\)H spectrum of a solution of Fosfomycin Calcium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 300), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy \( < 2.25 > \): it exhibits a double signal at around \( \delta 1.5 \) ppm, a double double...
signal at around $\delta$ 2.9 ppm, a multiple signal at around $\delta$ 3.3 ppm, and no signal at around $\delta$ 1.4 ppm.

(3) A solution of Fosfomycin Calcium Hydrate (1 in 500) responds to the Qualitative Tests $<$1.09$>$ (3) for calcium salt.

Optical rotation $<$2.49$>$ [α]$_{D}$: $-2.5$ to $-5.4^\circ$ (0.5 g calculated on the anhydrous bases, 0.4 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, pH 8.5, 10 mL, 100 mm).

Phosphorus Content Weigh accurately about 0.1 g of Fosfomycin Calcium Hydrate, add 40 mL of sodium periodate (107 in 10,000) and 2 mL of perchloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 200 mL. Pipet 10 mL of this solution, and add 1 mL of potassium iodide TS. To this solution add sodium thiosulfate TS until the solution is colorless, add water to make exactly 100 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 70 mg of potassium dihydrogenphosphate, proceed with this solution in the same manner as directed for the preparation of the sample stock solution, and use the solution so obtained as the standard stock solution. Proceed and prepare a solution in the same manner for the preparation of the sample stock solution without using Fosfomycin Calcium Hydrate, and use the solution so obtained as the blank stock solution. Pipet 5 mL each of the sample stock solution, the standard stock solution, and the blank stock solution, add 2.5 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, mix, and add water to make exactly 100 mL, and use this solution as the sample stock solution.

Water

Water $<$2.48$>$ Not more than 12.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics $<$4.02$>$ according to the following conditions.

(i) Test organism—*Proteus* sp. (MB838) (ii) Culture medium—Dissolve 5.0 g of peptone, 3.0 g of meat extract, 2.0 g of yeast extract, and 15 g of agar in 1000 mL of water, sterilize, and use as the agar media for base layer and seed layer with the pH of between 6.5 and 6.6 after sterilization.

(ii) Seeded agar layer—Incubate the test organism on the slant of the agar medium for transferring test organisms at 37°C for 40 – 48 hours. Subcultures at least three times. Inoculate the grown organisms onto the surface of 300 mL of the agar medium for transferring test organisms in a Roux bottle, incubate at 37°C for 40 – 48 hours, and suspend the grown organisms in about 30 mL of water. To the suspension add water, and use this as the stock suspension of test organism. The amount of the water to be added is adjust so that the percent transmission at 560 nm of the suspension diluted ten times with water is 17%. Keep the stock suspension at 10°C or below and use within 7 days. Add 1.0 – 2.0 mL of the stock suspension of test organism to 100 mL of the agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the deeded agar layer.

Calculated Content Weigh accurately about 0.2 g of Fosfomycin Calcium Hydrate, add 4 mL of 1 mol/L Hydrochloric acid TS, and shake well until the sample is completely dissolved. To this solution add 100 mL of water, 9 mL of sodium hydroxide TS and 0.1 g of methylthymol blue-sodium chloride indicator, and titrate $<$2.50$>$ with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from clear blue to gray or gray-purple: calcium content is 19.6 – 21.7%. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.004 mg of Ca

Purity (1) Heavy metals $<$1.07$>$—To 1.0 g of Fosfomycin Calcium Hydrate add 40 mL of 0.25 mol/L acetic acid TS and water to make 50 mL. Proceed with this solution according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic $<$1.17$>$—Prepare the test solution with 1.0 g of Fosfomycin Calcium Hydrate according to Method 3, and perform the test (not more than 2 ppm).

Containers and storage Containers—Tight containers.
Fosfomycin Sodium

ホスホマイシンナトリウム

C₂H₃NO₃P: 182.02
Disodium (2R,3S)-3-methyloxiran-2-ylphosphonate [26016-99-9]

Fosfomycin Sodium is the sodium salt of a substance having antibacterial activity produced by the growth of *Streptomyces fradiae* or by the chemical synthesis.

It contains not less than 725 μg (potency) and not more than 770 μg (potency) per mg, calculated on the anhydrous basis. The potency of Fosfomycin Sodium is expressed as mass (potency) of fosfomycin (C₉H₇O₄P: 138.06).

**Description**

Fosfomycin Sodium occurs as a white crystalline powder.

It is very soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

**Identification (1)**

Determine the infrared absorption spectrum of Fosfomycin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry (1 in 300), using sodium 3-trimethylsilyl-2-fumarate TS until the solution is colorless, add water to make exactly 25 mL, and use these solutions as the sample solution, the standard solution, and the blank solution, respectively. After allowing these solutions to stand for 30 minutes at 20 ± 1°C, perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry (1 < 2.24), using water as a blank, and determine the absorbances at 740 nm, A₁, A₂, A₃, and A₄, of the sample solution, the standard solution and the blank solution: the content of phosphorus is 16.2 - 17.9%.

Amount (mg) of phosphorus (P)

\[ M = \frac{M \times (A_1 - A_3)/(A_3 - A_4)}{0.228} \]

**Purity (1)**

Clarity and color of solution—Dissolve 1.0 g of Fosfomycin Sodium in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals ≤ 1.0 mg/kg—Proceed with 1.0 g of Fosfomycin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ≤ 1.0 mg/kg—Prepare the test solution with 1.0 g of Fosfomycin Sodium according to Method 3, and perform the test (not more than 2 ppm).

**Water** ≤ 3.0% (0.2 g, volumetric titration, direct titration).

**Assay**

Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ≤ 0.02% according to the following conditions.

(i) Test organism—*Proteus* sp. (MB838)

(ii) Culture medium—Mix 5.0 g of peptone, 3.0 g of meat extract, 2.0 g of yeast extract, and 15 g of agar in 1000 mL of water, sterilize, and use as the agar media for base layer and seed layer with the pH of between 6.5 and 6.6 after sterilization.

(ii) Seeded agar layer—Incubate the test organism on the slant of the agar medium for transferring test organisms at 37°C for 40 – 48 hours. Subcultures at least three times. Inoculate the grown organisms onto the surface of 300 mL of the agar medium for transferring test organisms in a Roux bottle, incubate at 37°C for 40 – 48 hours, and suspend the grown organisms in about 30 mL of water. To the suspension add water, and use this as the stock suspension of test organism. The amount of the water to be added is adjust so that the percent transmission at 560 nm of the suspension diluted ten times with water is 17%. Keep the stock suspension at 10°C or below and use within 7 days. Add 1.0 – 2.0 mL of the stock suspension of test organism to 100 mL of the agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the dedeed agar layer.

(iv) Standard solutions—Weigh accurately about 0.1 g of Fosfomycin Sodium, add 40 mL of a solution of sodium periodate (107 in 10,000) and 2 mL of perchloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 200 mL. Pipet 10 mL of this solution, and add 1 mL of potassium iodide TS. To this solution add sodium thiosulfate TS until the solution is colorless, add water to make exactly 100 mL, and use this solution as the sample stock solution. Separate, weigh accurately about 70 mg of potassium dihydrogenphosphate, proceed with this solution in the same manner as directed for the preparation of the sample stock solution, and use the solution so obtained as the standard stock solution. Proceed and prepare a solution in the same manner for the preparation of the sample stock solution without using Fosfomycin Sodium, and use the solution so obtained as the blank stock solution. Pipet 5 mL each of the sample stock solution, the standard stock solution, and the blank stock solution, add 2.5 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, mix, and add water to make exactly 25 mL, and use these solutions as the sample solution, the standard solution, and the blank solution, respectively. After allowing these solutions to stand for 30 minutes at 20 ± 1°C, perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry (2.24), using water as a blank, and determine the absorbances at 420 nm, A₁, A₂, A₃, and A₄, of the sample solution, the standard solution and the blank solution: the content of phosphorus is 16.2 - 17.9%.
mol/L tris buffer solution, pH 7.0 to make solutions so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(v) Sample solutions—Weigh accurately an amount of Fosfomycin Sodium equivalent to about 20 mg (potency), and dissolve in 0.05 mol/L tris buffer solution, pH 7.0 to make exactly 50 mL. To exactly a suitable amount of this solution add 0.05 mol/L tris buffer solution, pH 7.0 to make solutions so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Fosfomycin Sodium for Injection

Fosfomycin Sodium for Injection is a preparation for injection which is dissolved before use. It contains not less than 90.0% and not more than 110.0% of the labeled amount of fosfomycin (C₃H₇O₄P: 138.06).

Method of preparation Prepare as directed under Injections, with Fosfomycin Sodium.

Description Fosfomycin Sodium for Injection occurs as a white crystalline powder.

Identification (1) Dissolve about 0.1 g of Fosfomycin Sodium for Injection in 3 mL of a solution of perchloric acid (1 in 4), add 1 mL of 0.1 mol/L sodium periodate solution, and heat in a water bath at 60°C for 30 minutes. After cooling, add 50 mL of water, neutralize with saturated sodium carbonate solution, and add 1 mL of potassium iodide TS; the solution does not reveal a red color, while the blank solution reveals a red color.

(2) To 2 mL of a solution of Fosfomycin Sodium for Injection (1 in 250) add 1 mL of perchloric acid and 2 mL of 0.1 mol/L sodium periodate solution, and heat in a water bath for 10 minutes. After cooling, add 1 mL of hexammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, and allow to stand for 30 minutes: a blue color develops.

(3) Dissolve an amount of Fosfomycin Sodium for Injection, equivalent to 0.1 g (potency) of Fosfomycin Sodium, in 50 mL of water. Perform the test with this solution as directed in the Identification (3) under Fosfomycin Sodium.

pH <2.5> The pH of a solution prepared by dissolving an amount of Fosfomycin Sodium for Injection, equivalent to 1.0 g (potency) of Fosfomycin Sodium according to the labeled amount, in 20 mL of water is between 6.5 and 8.5.

Purity Clarity and color of solution—Dissolve an amount of Fosfomycin Sodium for Injection, equivalent to 1.0 g (potency) of Fosfomycin Sodium according to the labeled amount, in 10 mL of water: the solution is clear and colorless.

Water <2.4> Not more than 4.0% (0.1 g, coulometric titration).

Bacterial endotoxins <4.0> Less than 0.025 EU/mg (potency).

Uniformity of dosage unit <6.0> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.0> Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter <6.0> It meets the requirement.

Sterility <4.0> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.0> according to the following conditions.

(i) Test organism, culture medium, seeded agar layer, and standard solutions—Proceed as directed in the Assay under Fosfomycin Sodium.

(ii) Sample solutions—Weigh accurately the mass of the contents of not less than 10 Fosfomycin Sodium for Injection. Weigh accurately an amount of the content, equivalent to about 20 mg (potency) of Fosfomycin Sodium according to the labeled amount, and dissolve in 0.05 mol/L tris buffer solution, pH 7.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.05 mol/L tris buffer solution, pH 7.0 to make solutions so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.
Fradiomycin Sulfate

Neomycin Sulfate

Fradiomycin Sulfate is the sulfate of a mixture of aminoglycoside substances having antibacterial activity produced by the growth of *Streptomyces fradiae*.

It, when dried, contains not less than 623 μg (potency) and not more than 740 μg (potency) per mg. The potency of Fradiomycin Sulfate is expressed as mass (potency) of fradiomycin (C_{23}H_{46}N_{6}O_{13}.3H_{2}SO_{4}: 908.88

Fradiomycin Sulfate B
2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)-
[2,6-diamino-2,6-dideoxy-β-L-idopyranosyl-(1→3)-β-D-ribofuranosyl-(1→5)]-2-deoxy-β-D-streptamine trisulfate

[119-04-0, Neomycin B]

Fradiomycin Sulfate C
2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)-
[2,6-diamino-2,6-dideoxy-β-D-glucopyranosyl-(1→3)-
β-D-ribofuranosyl-(1→5)]-2-deoxy-β-D-streptamine trisulfate

[66-86-4, Neomycin C]
[1405-10-3, Neomycin Sulfate]

**Description** Fradiomycin Sulfate occurs as a white to light yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

It is hygroscopic.

**Identification (1)** Dissolve 50 mg each of Fradiomycin Sulfate and Fradiomycin Sulfate RS in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and dichloromethane (3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 110°C for 15 minutes: the Rf values of the principal spots from the sample solution and the standard solution are not different each other.

(2) A solution of Fradiomycin Sulfate (1 in 20) responds to the Qualitative Tests <1.09> (1) for sulfate.

**Optical rotation** <2.49> [α]D: +53.5° to +59.0° (1 g calculated on the dried basis, water, 10 mL, 100 mm).

**pH** <2.54> The pH of a solution obtained by dissolving 1.0 g of Fradiomycin Sulfate in 10 mL of water is between 5.0 and 7.5.

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Fradiomycin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Fradiomycin Sulfate according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.63 g of Fradiomycin Sulfate in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and dichloromethane (3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 110°C for 15 minutes: the spot at around Rf value 0.4 from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 8.0% (0.2 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.3% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Agar medium for seed and base layer

Glucose 1.0 g

Peptone 6.0 g

Meat extract 1.5 g

Yeast extract 3.0 g

Sodium chloride 2.5 g

Agar 15.0 g

Water 1000 mL

Mix all the ingredients and sterilize. Adjust the pH after sterilization to 7.8 – 8.0 with sodium hydroxide TS.

(iii) Standard solutions—Weigh accurately an amount of Fradiomycin Sulfate RS, previously dried, equivalent to about 50 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution, re-
Fructose / Official Monographs

Fructose

Fructose Injection

Fructose Injection is an aqueous solution for injection. It contains not less than 95.0% and not more than 105.0% of the labeled amount of fructose (C₆H₁₂O₆: 180.16).
before the test.

**Purity**  (1) Heavy metals <1.07>—Take a volume of Fructose Injection, equivalent to 5.0 g of Fructose, to the labeled amount, and evaporate on a water bath to dryness. With the residue, proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution.

(2) Arsenic <1.11>—Take a volume of Fructose Injection, equivalent to 1.5 g of Fructose, to the labeled amount, dilute with water or concentrate on a water bath to 5 mL, if necessary, and add 5 mL of dilute sulfuric acid and 1 mL of bromine TS. Proceed as directed in the purity (8) under Fructose.

**Residue on ignition** <2.44> Measure exactly a volume of Fructose Injection, equivalent to 2 g of Fructose according to the labeled amount, evaporate on a water bath to dryness, and perform the test: the residue weighs not more than 2 mg.

**Bacterial endotoxins** <4.01> Less than 0.5 EU/mL.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Measure exactly a volume of Fructose Injection equivalent to about 4 g of fructose (C₆H₁₂O₆), add 0.2 mL of ammonia TS, dilute with water to make exactly 100 mL, shake well, and after allowing to stand for 30 minutes, determine the optical rotation, α₂₀, in a 100-mm cell at 20 ± 1°C as directed under Optical Rotation Determination <2.49>.

Amount (mg) of fructose (C₆H₁₂O₆) = |α₂₀| × 1087.0

**Containers and storage** Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

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**Furosemide**

フロセミド

C₁₂H₁₁ClN₂O₅S: 330.74
4-Chloro-2-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid
[54-31-9]

Furosemide, when dried, contains not less than 98.0% and not more than 101.0% of C₁₂H₁₁ClN₂O₅S.

**Description** Furosemide occurs as white, crystals or crystalline powder.

It is freely soluble in N,N-dimethylformamide, soluble in methanol, sparingly soluble in ethanol (99.5), slightly soluble in acetonitrile and in acetic acid (100), and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

Melting point: about 205°C (with decomposition).

**Identification** (1) Dissolve 25 mg of Furosemide in 10 mL of methanol. To 1 mL of this solution add 10 mL of 2 mol/L hydrochloric acid TS. Heat the solution under a reflux condenser on a water bath for 15 minutes, cool, and add 18 mL of sodium hydroxide TS to make weakly acidic: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines, producing a red to red-purple color.

(2) Determine the absorption spectrum of a solution of Furosemide in dilute sodium hydroxide TS (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Furosemide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Furosemide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Furosemide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Furosemide in 10 mL of a solution of sodium hydroxide (1 in 50): the solution is clear and colorless.

(2) Chloride <1.09>—Dissolve 2.6 g of Furosemide in 90 mL of dilute sodium hydroxide TS, add 2 mL of nitric acid, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.40 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.020%).

(3) Sulfate <1.14>—To 20 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.030%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Furosemide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 25 mg of Furosemide in 25 mL of the dissolving solution, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the dissolving solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak appeared ahead of the peak of furosemide obtained from sample solution is not larger than 2.5 times the peak area of furosemide from the standard solution, the area of each peak appeared behind the peak of furosemide is not larger than 1/4 times the peak area of furosemide from the standard solution, and the total area of these peaks is not larger than 2 times the peak area of furosemide from the
standard solution.

Dissolving solution—To 22 mL of acetic acid (100) add a mixture of water and acetonitrile (1:1) to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, tetrahydrofuran and acetic acid (100) (70:30:1).

Flow rate: Adjust the flow rate so that the retention time of furosemide is about 18 minutes.

Time span of measurement: About 2.5 times as long as the retention time of furosemide beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the dissolving solution to make exactly 50 mL. Confirm that the peak area of furosemide obtained from 20 μL of this solution is equivalent to 3.2 to 4.8% of that from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of furosemide is not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of furosemide is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Furosemide, previously dried, dissolve in 50 mL of N,N-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow to blue (indicator: 3 drops of bromothymol blue TS). Perform a blank determination with a mixture of 50 mL of N,N-dimethylformamide and 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 33.07 mg of C₁₂H₁₁ClN₂O₅S

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Furosemide Injection

フロセミド注射液

Furosemide Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of furosemide (C₁₂H₁₁ClN₂O₅S: 330.74).

Method of preparation Prepare as directed under Injection, with Furosemide.

Description Furosemide Injection is a colorless, clear liquid.

Identification (1) To a volume of Furosemide Injection, equivalent to 2.5 mg of Furosemide according to the labeled amount, add 10 mL of 2 mol/L hydrochloric acid TS, heat under a reflux condenser on a water bath for 15 minutes. After cooling, render the solution slightly acid with 18 mL of sodium hydroxide TS: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines. The color of the solution is red to red-purple.

(2) To a volume of Furosemide Injection, equivalent to 20 mg of Furosemide according to the labeled amount, add water to make 100 mL. To 2 mL of this solution add 0.01 mol/L sodium hydroxide TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 227 nm and 231 nm, between 269 nm and 273 nm, and between 330 nm and 336 nm.

Osmotic pressure ratio Being specified separately.

pH Being specified separately.

Purity Pipet a volume of Furosemide Injection, equivalent to 40 mg of Furosemide according to the labeled amount, add 30 mL of acetone, shake well, and add acetone to make exactly 50 mL. Centrifuge this solution, to 1.0 mL of the supernatant liquid add 3.0 mL of water, cool in a ice bath, add 3.0 mL of dilute hydrochloric acid and 0.15 mL of sodium nitrite TS, shake, and allow to stand for 1 minute. To this solution add 1.0 mL of ammonium amidosulfate TS, shake well, allow to stand for 3 minutes, add 1.0 mL of N,N-diethyl-N’-1-naphthylethylendiamine oxalate TS, shake well, and allow to stand for 5 minutes. Determine the absorbance of this solution at 530 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution, prepared in the same manner with 1.0 mL of acetone, as the blank: the absorbance is not more than 0.10.

Bacterial endotoxins <4.07> Less than 1.25 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Furosemide Injection, equivalent to about 20 mg of Furosemide (C₁₂H₁₁ClN₂O₅S), add water to make exactly 100 mL. Pipet 3 mL of this solution, add 0.01 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Furosemide RS, previously dried at 105°C for 4 hours, add 0.01 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 3 mL of this solution, add 0.01 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible
Spectrophotometry <2.24>, and determine the absorbances, \( A_T \) and \( A_S \), at 271 nm.

\[
\text{Amount (mg) of furosemide (C12H11ClN2O5S)} = M_S \times A_T / A_S \\
M_S: \text{Amount (mg) of Furosemide RS}
\]

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Furosemide Tablets フロセミド錠

Furosemide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of furosemide (C12H11ClN2O5S: 330.74).

Method of preparation Prepare as directed under Tablets, with Furosemide.

Identification (1) Shake well a quantity of powdered Furosemide Tablets, equivalent to 0.2 g of Furosemide according to the labeled amount, with 40 mL of acetic acid, and filter. To 0.5 mL of the filtrate add 10 mL of 2 mol/L hydrochloric acid TS, and heat under a reflux condenser on a water bath for 15 minutes. After cooling, add 18 mL of sodium hydroxide TS to make the solution slightly acetic: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines, producing a red to red-purple color.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 272 nm and 231 nm, between 269 nm and 275 nm, and between 330 nm and 336 nm.

Purity To a quantity of powdered Furosemide Tablets, equivalent to 40 mg of Furosemide according to the labeled amount, add about 30 mL of acetic acid, shake well, and add acetic acid to make exactly 50 mL. Centrifuge the solution, add 3.0 mL of water to 1.0 mL of the supernatant liquid, cool in ice, add 3.0 mL of dilute hydrochloric acid and 0.15 mL of sodium nitrite TS, shake, and allow to stand for 1 minute. Add 1.0 mL of ammonium amidosulfate TS, shake well, and allow to stand for 3 minutes, add 1.0 mL of N,N-diethyl-N'-1-naphthylthelyenediamine oxalate TS, shake well, and allow to stand for 5 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared in the same manner with 1.0 mL of acetic acid as the blank: the absorbance at 530 nm is not more than 0.10.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Furosemide Tablets add a suitable amount of 0.05 mol/L sodium hydroxide TS, shake to disintegrate, then add 0.05 mol/L sodium hydroxide TS to make exactly V’ mL so that each mL contains about 0.4 mg of furosemide (C12H11ClN2O5S). Filter the solution, discard the first 10 mL of the filtrate, pipet the subsequent 2 mL of the filtrate, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of furosemide (C12H11ClN2O5S)} = M_S \times A_T / A_S \times V'/50 \\
M_S: \text{Amount (mg) of Furosemide RS}
\]

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rates of a 20-mg tablet in 15 minutes and a 40-mg tablet in 30 minutes are not less than 80%.

Start the test with 1 tablet of Furosemide Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \)m. Discard the first 10 mL of the filtrate, pipet V’ mL of the subsequent filtrate, add the dissolution medium to make exactly V’ mL so that each mL contains about 10 \( \mu \)g of furosemide (C12H11ClN2O5S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Furosemide RS, previously dried at 105°C for 4 hours, and dissolve in 5 mL of methanol, and add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 277 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Dissolution rate (\%) with respect to the labeled amount of furosemide (C12H11ClN2O5S)} = M_S \times A_T / A_S \times V'/V \times 1/C \times 45 \\
M_S: \text{Amount (mg) of Furosemide RS}
\]

C: Labeled amount (mg) of furosemide (C12H11ClN2O5S)

Assay Weigh accurately the mass of not less than 20 Furosemide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 40 mg of furosemide (C12H11ClN2O5S), add about 70 mL of 0.05 mol/L sodium hydroxide TS, shake well, and add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL. Filter, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Furosemide RS, previously dried at 105°C for 4 hours, and dissolve in 5 mL of methanol, and add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 271 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Amount (mg) of furosemide (C12H11ClN2O5S)} = M_S \times A_T / A_S \times 2 \\
M_S: \text{Amount (mg) of Furosemide RS}
\]

Containers and storage Containers—Tight containers. Storage—Light-resistant.
Fursultiamine Hydrochloride

フルスルチアミン塩酸塩

C_{17}H_{26}N_{4}O_{3}S_{2}.HCl: 435.00
N-(4-Amino-2-methylpyrimidin-5-ylmethyl)-N-
[(1Z)-4-hydroxy-1-methyl-2-[(2RS)-tetrahydrofuran-
2-ylmethyldisulfanyl]but-1-en-1-yl]formamide
monohydrochloride

[804-30-8, Fursultiamine]

Fursultiamine Hydrochloride contains not less than 98.5% of C_{17}H_{26}N_{4}O_{3}S_{2}.HCl, calculated on the dried basis.

Description Fursultiamine Hydrochloride occurs as white crystals or crystalline powder. It is odorless or has a characteristic odor, and has a bitter taste.

It is freely soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Dissolve 5 mg of Fursultiamine Hydrochloride in 6 mL of 0.1 mol/L hydrochloric acid. Add 0.1 g of zinc powder, allow to stand for several minutes, and filter. To 3 mL of the filtrate, add 3 mL of sodium hydroxide and 0.5 mL of potassium hexacyanoferrate (III) (3 in 400). The blue-purple fluorescence of the solution appears again by alkalifying.

(2) Determine the infrared absorption spectrum of a solution of Fursultiamine Hydrochloride, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum, or with the spectrum of Fursultiamine Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours: both spectra exhibit similar intensities of absorption at the same wave numbers. If any differences appear, dissolve the Fursultiamine Hydrochloride in water, evaporate the water, and dry the residue in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, and repeat the test.

(3) A solution of Fursultiamine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Clarity of solution—Dissolve 1.0 g of Fursultiamine Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Proceed with 1.5 g of Fursultiamine Hydrochloride, and perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Fursultiamine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.10 g of Fursultiamine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of fursultiamine from the sample solution is not larger than the peak area of fursultiamine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detector sensitivity: Adjust the detection sensitivity so that the peak height of fursultiamine from 10 μL of the standard solution is between 20 mm and 30 mm.

Time span of measurement: About 3 times as long as the retention time of fursultiamine.

Water <2.48> Not more than 5.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 55 mg each of Fursultiamine Hydrochloride and Fursultiamine Hydrochloride RS (previously determined the water <2.48> in the same manner as Fursultiamine Hydrochloride) and dissolve each in 50 mL of water, and add exactly 10 mL each of the internal standard solution, then add water to make exactly 100 mL. To 8 mL each of the solution add water to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{T} and Q_{S}, of the peak area of fursultiamine to that of the internal standard, respectively.

Amount (mg) of C_{17}H_{26}N_{4}O_{3}S_{2}.HCl

\[
M \times Q_{T}/Q_{S}
\]

M: Amount (mg) of Fursultiamine Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl 4-aminobenzoate in ethanol (95) (3 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 1.01 g of sodium 1-heptane sulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 675 mL of this solution add 325 mL of a mixture of methanol and acetonitrile (3:2).
Flow rate: Adjust the flow rate so that the retention time of Pursultiamine is about 9 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions and calculate the resolution. Use a column giving elution of pursultiamine and the internal standard in this order with the resolution between these peaks being not less than 10.

Containers and storage  Containers—Tight containers.

Gabexate Mesilate

Gabexate Mesilate occurs as white crystals or crystalline powder.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1)  To 4 mL of a solution of Gabexate Mesilate (1 in 2000) add 2 mL of 1-naphthol TS and 1 mL of diacetyl TS, and allow to stand for 10 minutes: a red color develops.

(2)  Dissolve 1 g of Gabexate Mesilate in 5 mL of water, add 2 mL of sodium hydroxide TS, and heat in a water bath for 5 minutes. After cooling, add 2 mL of dilute nitric acid and 5 mL of ethanol (95), shake, add 5 drops of iron (III) chloride TS, and shake: a purple color develops.

(3)  Determine the absorption spectrum of a solution of Gabexate Mesilate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum or the spectrum of a solution prepared in the same manner with the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4)  A 0.1 g portion of Gabexate Mesilate responds to the Qualitative Tests (1.08) (1) for mesilate.

pH (2.54)  Dissolve 1.0 g of Gabexate Mesilate in 10 mL of water: the pH of the solution is between 4.5 and 5.5.

Melting point (2.60)  90 – 93°C.

Purity (1)  Clarity and color of solution—Dissolve 1.0 g of Gabexate Mesilate in 10 mL of water: the solution is clear and colorless.

(2)  Heavy metals  (1.07) —Proceed with 2.0 g of Gabexate Mesilate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3)  Arsenic  (1.11) —Dissolve 2.0 g of Gabexate Mesilate in 20 mL of 1 mol/L hydrochloric acid TS by heating in a water bath, and continue the heating for 20 minutes. After cooling, centrifuge, and use 10 mL of the supernatant liquid as the test solution. Perform the test (not more than 2 ppm).

(4)  Ethyl parahydroxybenzoate—Weigh 50 mg of Gabexate Mesilate, previously dried, and dissolve in dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, dissolve 5.0 mg of ethyl parahydroxybenzoate in dilute ethanol to make exactly 100 mL. Pipet 1 mL of this solution, and add dilute ethanol to make exactly 20 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ethyl parahydroxybenzoate to that of the internal standard: Q_T is not larger than Q_S.

Internal standard solution—A solution of butyl parahydroxybenzoate in dilute ethanol (1 in 5000).

Operating conditions— Proceed as directed in the operating conditions in the Assay.

System suitability— Proceed as directed in the system suitability in the Assay.

(5)  Related substances—Dissolve 0.20 g of Gabexate Mesilate in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.02). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate until it has no acetic odor. Spray evenly a solution of 8-quinolinol in acetone (1 in 1000) on the plate, and after air-drying, spray evenly bromine-sodium hydroxide TS: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying (2.41)  Not more than 0.30% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition (2.44)  Not more than 0.1% (1 g).

Assay  Weigh accurately about 50 mg each of Gabexate Mesilate and Gabexate Mesilate RS, previously dried, and dissolve each in dilute ethanol to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of gabexate to that of the internal standard.

Amount (mg) of C_{16}H_{23}N_{3}O_{4}.CH_{4}O_{3}S

\[ Q_T = M_S \times \frac{Q_S}{Q_T} \]

M_S: Amount (mg) of Gabexate Mesilate RS

Internal standard solution—A solution of butyl parahy-
β-Galactosidase (Aspergillus)

β-ガラクトシダーゼ（アスペルギルス）

β-Galactosidase (Aspergillus) contains an enzyme produced by Aspergillus oryzae. It is an enzyme drug having lactose decomposition activity.

It contains 8000 to 12000 units per g.

Generally, it is a white or light yellowish powder.

Stability It is slightly soluble in water with a turbidity, and practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 25 mg of β-Galactosidase (Aspergillus) in 100 mL of water, then to 1 mL of this solution add 9 mL of lactose substrate TS, and stand at 30°C for 10 minutes. To 1 mL of this solution add 6 mL of glucose detection TS, and stand at 30°C for 10 minutes: a red to red-purple color develops.

(2) Dissolve 0.1 g of β-Galactosidase (Aspergillus) in 100 mL of water, and filter the solution if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry 2.4.2, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Odor—β-Galactosidase (Aspergillus) has no any rancid odor.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 245 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of methanol, a solution of sodium lauryl sulfate (1 in 1000), a solution of sodium 1-heptane sulfonate (1 in 200) and acetic acid (100) (540:200:20:1).
Flow rate: Adjust the flow rate so that the retention time of gabexate is about 13 minutes.

System suitability—
System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the internal standard and gabexate are eluted in this order with the resolution between these peaks being not less than 5.

System sensitivity: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of gabexate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

β-Galactosidase (Penicillium)

β-ガラクトシダーゼ（ペニシリウム）

β-Galactosidase (Penicillium) contains an enzyme, having lactose decomposition activity, produced by Penicillium multicolor.

It contains not less than 8500 units and not more than 11,500 units in each g.
Usually, it is diluted with D-Mannitol.

**Description**  
β-Galactosidase (Penicillium) occurs as a white to pale yellowish white, crystalline powder or powder.

It is soluble in water with a turbidity, and practically insoluble in ethanol (95%).

It is hygroscopic.

**Identification (1)**  
Dissolve 0.05 g of β-Galactosidase (Penicillium) in 100 mL of water, then to 0.2 mL of this solution add 0.2 mL of lactose substrate TS, and allow to stand at 30°C for 10 minutes. To this solution add 3 mL of glucose detection TS, and allow to stand at 30°C for 10 minutes: a red to red-purple color develops.

(2) Dissolve 0.15 g of β-Galactosidase (Penicillium) in 100 mL of water, filter if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.247: it exhibits a maximum between 278 nm and 282 nm.

**Purity (1)**  
Odor—β-Galactosidase (Penicillium) has no any rancid odor.

(2) Heavy metals 1.077—Proceed with 1.0 g of β-Galactosidase (Penicillium) according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic 1.115—Prepare the test solution with 1.0 g of β-Galactosidase (Penicillium) according to Method 3, and perform the test (not more than 2 ppm).

(4) Nitrogen—Weigh accurately about 0.1 g of β-Galactosidase (Penicillium), and perform the test as directed under Nitrogen Determination 1.082: not more than 3 mg of nitrogen (N: 14.01) is found for each labeled 1000 Units.

(5) Protein contaminants—Dissolve 0.15 g of β-Galactosidase (Penicillium) in 4 mL of water, and use this solution as the sample solution. Perform the test with 15 μL of the sample solution as directed under Liquid Chromatography 2.017 according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak having retention time of about 19 minutes is not more than 75% of the total area of all peaks, and the areas of peaks other than the peaks having retention times of about 3, 16 and 19 minutes are not more than 15% of the total area of all peaks.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 7.5 mm in inside diameter and about 75 mm in length, packed with strongly acidic ion-exchange resin for liquid chromatography of sulfopropyl group-binding hydrophilic polymer (10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A solution obtained by dissolving 2.83 g of sodium acetate in 1000 mL of water, and adjusting to pH 4.5 with acetic acid (100) (mobile phase A), and a solution obtained by dissolving 29.2 g of sodium chloride in 1000 mL of mobile phase A (mobile phase B).

Flow system: Adjust a linear concentration gradient from the mobile phase A to the mobile phase B immediately after injection of the sample so that the retention times of non-retaining protein and the enzyme protein are about 3 minutes and 19 minutes, respectively, when the flow runs 0.8 mL per minute, and then continue the running of the mobile phase B.

Selection of column: Dissolve 15 mg of β-lactoglobulin in 4.5 mL of water, add 0.5 mL of a solution of cytosine (1 in 5000), and use this solution as the column-selecting solution. Proceed with 15 μL of the column-selecting solution under the above operating conditions, and calculate the resolution. Use a column giving elution of cytosine and β-lactoglobulin in this order with the resolution between these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of β-lactoglobulin from 15 μL of the column-selecting solution is between 5 cm and 14 cm.

Time span of measurement: About 1.4 times as long as the retention time of β-lactoglobulin.

**Loss on drying 2.41**  
Not more than 5.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition 2.44**  
Not more than 2% (1 g).

**Assay** (i) Substrate solution—Dissolve 0.603 g of 2-nitrophenyl-β-D-galactopyranoside in disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 to make 100 mL.

(ii) Procedure—Weigh accurately about 0.15 g of β-Galactosidase (Penicillium), dissolve in water with thorough shaking to make exactly 100 mL, and allow to stand at room temperature for an hour. Pipet 2 mL of this solution, add disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 to make exactly 100 mL, and use this solution as the sample solution. Transfer exactly 0.5 mL of the sample solution to a test tube, stand at 30 ± 0.1°C for 10 minutes, add exactly 0.5 mL of the substrate solution previously kept at 30 ± 0.1°C, then mix immediately, and stand at 30 ± 0.1°C for exactly 10 minutes. Then add exactly 1 mL of sodium carbonate TS, mix immediately to stop the reaction. To this solution add exactly 8 mL of water, mix, and use as the colored sample solution. Separately, pipet 0.5 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 4.5, then proceed in the same manner as the sample solution, and use the solution so obtained as the colored blank solution. Perform the test with the colored sample solution and the colored blank solution as directed under Ultraviolet-visible Spectrophotometry 2.247, using water as the blank, and determine the absorbances, Aₜ and Aₜₐₕ, at 420 nm.

Units per g of β-Galactosidase (Penicillium)  
\[ = \frac{1}{M} \times (Aₜ - Aₜₐₕ) \times 0.459 \times 1/10 \]

0.459: Absorbance of 1 μmol/10 mL of o-nitrophenol  
M: Amount (g) of the sample in 0.5 mL of the sample solution

Unit: One unit indicates an amount of the enzyme which decomposes 1 μmol of 2-nitrophenyl-β-D-galactopyranoside in 1 minute under the above conditions.

**Containers and storage**  
Containers—Tight containers.
**Gallium (67Ga) Citrate Injection**

クエン酸ガリウム (67Ga) 注射液

Gallium (67Ga) Citrate Injection is an aqueous solution for injection containing gallium-67 (67Ga) in the form of gallium citrate.

It conforms to the requirements of Gallium (67Ga) Citrate Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

**Description** Gallium (67Ga) Citrate Injection is a colorless or light red liquid.

**Gas Gangrene Antitoxin, Equine**

ガスえそウマ抗毒素

Gas Gangrene Antitoxin, Equine, is a liquid for injection containing Clostridium perfringens (C. welchii) Type A antitoxin, Clostridium septicum (Vibrion septicum) antitoxin and Clostridium oedematiens (C. novyi) antitoxin in immunoglobulin of horse origin.

It may contain also Clostridium histolyticum antitoxin.

It conforms to the requirements of Gas Gangrene Antitoxin, Equine, in the Minimum Requirements for Biological Products.

**Description** Gas Gangrene Antitoxin, Equine, is a colorless to light yellow-brown, clear liquid or a slightly whitish turbid liquid.

**Gefarnate**

ゲファルナート

Gefarnate is a mixture of 4E geometrical isomer.

It contains not less than 98.0% and not more than 101.0% of C$_{27}$H$_{44}$O$_2$.

**Description** Gefarnate is a light yellow to yellow, clear oily liquid.

It is miscible with acetonitrile, with ethanol (99.5) and with cyclohexane.

It is practically insoluble in water.

**Identification** Determine the infrared absorption spectrum of Gefarnate as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Gefarnate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Specific gravity** $<2.56>\ d_2^0: 0.906 - 0.914.$

**Purity (1)** Acidity—To 1.0 g of Gefarnate add 30 mL of neutralized ethanol. To this solution add 1 drop of phenolphthalein TS and 0.40 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Heavy metals $<1.07>\text{—Proceed with 2.0 g of Gefarnate according to Method 2, and perform the test.} \text{ Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).}$

(3) Related substances—Use a solution of Gefarnate in acetonitrile (1 in 500) as the sample solution. Pipet 2 mL of this solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.02>$ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of gefarnate obtained from the sample solution is not larger than 1/2 times the peak area of gefarnate from the standard solution, and the total area of the peaks other than the peak of gefarnate obtained from the sample solution is not larger than the peak area of gefarnate from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of gefarnate, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 2 mL of the standard solution, add acetonitrile to make exactly 20 mL. Confirm that the peak area of gefarnate obtained from 2 μL of this solution is equivalent to 7 to 13% of that of gefarnate from 2 μL of the standard solution.

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of gefarnate are not less than 4000, and between 0.9 and 1.2, respectively.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of gefarnate is not more than 1.0%.

(4) Residual solvent—Being specified separately.

**Isomer ratio** To 1 mL of Gefarnate add 100 mL of ethanol (99.5), and use this solution as the sample solution. Perform the test with 4 μL of the sample solution as directed under Gas Chromatography $<2.02>$ according to the following conditions. Determine the areas of two adjacent peaks, A$_a$ and A$_b$, having the retention time of about 37 minutes, where A$_a$ is the peak area of shorter retention time and A$_b$ is the peak area of longer retention time: A$_a$ / (A$_a$ + A$_b$) is between 0.2 and 0.3.

**Operating conditions**

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 160 cm in length, packed with polyethylene glycol 20M for gas
column chromatography coated at the ratio of 5% on acid-treated and silanized siliceous earth for gas chromatography (149 to 177 μm in particle diameter).

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the reaction time of the peak showing earlier elution of the two peaks of gefarnate is about 35 minutes.

System suitability—

System performance: When the procedure is run with 4 μL of the sample solution under the above conditions: the resolution between the two peaks of gefarnate is not less than 1.0.

System repeatability: When the test is repeated 6 times with 4 μL of the sample solution under the above operating conditions: the relative standard deviation of the peak area of gefarnate with the shorter retention time of the two peaks is not more than 2.0%.

Assay Weigh accurately about 50 mg each of Gefarnate and Gefarnate RS, add exactly 5 mL of the internal standard solution and 20 μL of acetonitrile, and use these solutions as the sample solution and standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0> according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of gefarnate to that of the internal standard.

\[
\text{Amount (mg) of gefarnate (C}_{27}\text{H}_{44}\text{O}_{2}) = M_S \times \frac{Q_1}{Q_2}
\]

\[
M_S \text{ Amount (mg) of Gefarnate RS}
\]

Internal standard solution—A solution of tris (4-tert-butylphenyl) phosphate in acetonitrile (1 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with phenylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile for liquid chromatography, water and phosphoric acid (700:300:1).

Flow rate: Adjust the flow rate so that the retention time of gefarnate is about 19 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and gefarnate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of gefarnate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and under nitrogen atmosphere.

Gelatin

ゼラチン

Gelatin is a product prepared from aqueous extract of raw collagen by heating. The raw collagen is obtained by acid or alkali treatment of the bone, skin, ligament or tendon of animals.

Description Gelatin occurs as colorless or white to light yellow-brown sheets, shreds, granules or powder. It is odorless and tasteless.

It is very soluble in hot water, and practically insoluble in ethanol (95) and in diethyl ether.

It does not dissolve in water, but slowly swells and softens when immersed in it, gradually absorbing water 5 to 10 times its own mass.

It derived from an acid-treated collagen exhibits an isoelectric point between pH 7.0 and 9.0, and Gelatin derived from an alkali-treated collagen exhibits an isoelectric point between pH 4.5 and 5.0.

Identification (1) To 5 mL of a solution of Gelatin (1 in 100) add chromium (VI) oxide TS or 2,4,6-trinitrophenol TS dropwise: a precipitate is formed.

(2) To 5 mL of a solution of Gelatin (1 in 5000) add tannic acid TS dropwise: the solution becomes turbid.

Purity (1) Foreign odor and water-insoluble substances—Dissolve 1.0 g of Gelatin in 40 mL of water by heating: the solution has no disagreeable odor. It is clear, or only slightly opalescent. The solution has no more color than Matching Fluid A.

(2) Sulfite—Take 20.0 g of Gelatin in a round-bottomed flask, dissolve in 150 mL of hot water, and add 3 to 5 drops of silicone resin, 5 mL of phosphoric acid and 1 g of sodium hydrogen carbonate. Attach a condenser, immediately distil the solution, immersing the end of the condenser into a receiver containing 50 mL of iodine TS, and continue the distillation until 50 mL of distillate is obtained. Acidify the distillate with 2 to 3 drops of hydrochloric acid, add 2 mL of barium chloride TS, and heat on a water bath until the color of iodine TS is discharged. Collect the precipitates, wash with water, and ignite: the mass of the residue is not more than 4.5 mg, but the mass of the residue obtained from Gelatin for use in the preparation of capsules and tablets is not more than 75 mg. Perform a blank determination, and make any necessary correction.

(3) Heavy metals <1.07>—Proceed with 0.5 g of Gelatin according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(4) Arsenic <1.11>—Take 15.0 g of Gelatin in a flask, add 60 mL of diluted hydrochloric acid (1 in 5), and heat until solution is effected. Add 15 mL of bromine TS, heat until the excess of bromine is expelled, neutralize with ammonia TS, add 1.5 g of disodium hydrogen phosphate dodecahydrate, and allow to cool. To this solution add 30 mL of magnesia TS, allow to stand for 1 hour, and collect the precipitates. Wash the precipitates with five 10-mL portions of diluted ammonia TS (1 in 4), and dissolve in diluted hydrochloric acid (1 in 4) to make exactly 50 mL. Perform the test with 5 mL of this solution: the solution has no more
color than the following color standard.

Color standard: Proceed with 15 mL of Standard Arsenic Solution, instead of Gelatin, in the same manner (not more than 1 ppm).

(5) Mercury—Place 2.0 g of Gelatin in a decomposition flask, add 20 mL of diluted sulfuric acid (1 in 2) and 100 mL of a solution of potassium permanganate (3 in 50), heat gently under a reflux condenser, and boil for 2 hours. If the solution becomes clear during boiling, reduce the temperature of the solution to about 60°C, add further 5 mL of a solution of potassium permanganate (3 in 50), boil again, and repeat the above-mentioned procedure until the precipitate of manganese dioxide remains for about 20 minutes. Cool, add a solution of hydroxylammonium chloride (1 in 5) until the precipitate of manganese dioxide disappears, add water to make exactly 150 mL, and use the solution as the sample solution. Perform the test as directed under Atomic Absorption Spectrophotometry (Cold vapor type) using the sample solution. Place the sample solution in a sample water bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the atomic absorption spectrophotometer, add 10 mL of water, allow to stand for 30 minutes, and collect the precipitate. Wash with water, and ignite: the mass of the residue is not more than 1.5 mg. Perform a blank determination, and make any necessary correction.

(3) Sulfite—Take 20.0 g of Gelatin in a round-bottomed flask, dissolve in 150 mL of hot water, and add 3 to 5 drops of silicone resin, 5 mL of phosphoric acid and 1 g of sodium hydrogen carbonate. Attach a condenser, immediately distil the solution, immersing the end of the condenser into a receiver containing 50 mL of iodine TS, and continue the distillation until 50 mL of distillate is obtained. Acidify the distillate by dropwise addition of hydrochloric acid, add 2 mL of barium chloride TS, and heat on a water bath until the color of iodine TS is discharged. Collect the precipitates, wash with water, and ignite: the mass of the residue is not more than 1.5 mg. Perform a blank determination, and make any necessary correction.

(4) Arsenic—Place 15.0 g of Gelatin in a flask, add 60 mL of diluted hydrochloric acid (1 in 5), and heat until solution is effected. Add 15 mL of bromine TS, heat until the excess of bromine is expelled, neutralize with ammonia TS, add 1.5 g of disodium hydrogen phosphate dodecahydrate, and allow to cool. To this solution add 30 mL of magnesia TS, allow to stand for 1 hour, and collect the precipitates. Wash the precipitates with five 10-mL portions of diluted ammonia TS (1 in 4), and dissolve in diluted hydrochloric acid (1 in 4) to make exactly 50 mL. Perform the test with 5 mL of this solution: the solution has no more color than the following standard solution.

Standard solution: Proceed with 15 mL of Standard Arsenic Solution, instead of Gelatin, in the same manner (not more than 1 ppm).

(5) Mercury—Place 2.0 g of Gelatin in a decomposition flask, add 20 mL of diluted sulfuric acid (1 in 2) and 100 mL of a solution of potassium permanganate (3 in 50), heat gently under a reflux condenser, and boil for 2 hours. If the solution becomes clear during boiling, reduce the temperature of the solution to about 60°C, add further 5 mL of a solution of potassium permanganate (3 in 50), boil again, and repeat the above-mentioned procedure until the precipitate of manganese dioxide remains for about 20 minutes. Cool, add a solution of hydroxylammonium chloride (1 in 5) until the precipitate of manganese dioxide disappears, add water to make exactly 150 mL, and use the solution as the sample solution. Perform the test as directed under Atomic Absorption Spectrophotometry (Cold vapor type) using the sample solution. Place the sample solution in a sample water bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the atomic absorption spectro-
photometer, and circulate air. Determine the absorbance $A_1$ of the sample solution at 253.7 nm when the indication of the recorder has risen rapidly and become constant. On the other hand, place 2.0 mL of Standard Mercury Solution in a decomposition flask, add 20 mL of diluted sulfuric acid (1 in 2) and 100 mL of a solution of potassium permanganate (3 in 50), and proceed in the same manner as for the sample solution. Determine the absorbance $A_2$ of the solution as obtained: $A_2$ is not more than $A_1$ (not more than 0.1 ppm).

**Loss on drying** Not more than 15.0%. Take about 1 g of Purified Gelatin, accurately weighed, in a tared 200-mL beaker containing 10 g of sea sand (No. 1), previously dried at 110°C for 3 hours. Add 20 mL of water, allow to stand for 30 minutes with occasional shaking, evaporate on a water bath to dryness with occasional shaking, and dry the residue at 110°C for 3 hours.

**Residue on ignition** Not more than 2.0% (0.5 g).

**Containers and storage** Containers—Tight containers.

### Gentamicin Sulfate

ゲンタマイシン硫酸塩

Gentamicin Sulfate is the sulfate of a mixture of aminoglycoside substances having antibacterial activity produced by the growth of *Micromonospora purpurea* or *Micromonospora echinospora*.

It contains not less than 590 μg (potency) and not more than 775 μg (potency) per mg, calculated on the dried basis. The potency of Gentamicin Sulfate is expressed as mass (potency) of gentamicin C₁ (C₁₃H₂₁N₅O₇: 477.60).

**Description** Gentamicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

**Identification** (1) Dissolve 50 mg of Gentamicin Sulfate in 1 mL of water, and add 2 drops of a solution of 1-naphthol in ethanol (95) (1 in 500). Gently superimpose this solution on 1 mL of sulfuric acid: a blue-purple color develops at the zone of contact.

(2) Dissolve 50 mg each of Gentamicin Sulfate and Gentamicin Sulfate RS in 10 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 20 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm², and without putting a filter paper in the container, and air-dry the plate. Allow the plate to stand in iodine vapors: three principal spots obtained from the sample solution are the same with the corresponding spots from the standard solution in color tone and the $R_f$ value, respectively.

(3) Dissolve 50 mg of Gentamicin Sulfate in 5 mL of water, and add 0.5 mL of barium chloride TS: a white precipitate is formed.

**Optical rotation** $\left[\alpha\right]_{D}^{25}: +107 – 121°$ (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

**pH** $\left[\alpha\right]_{D}^{25}$ The pH of a solution obtained by dissolving 0.20 g of Gentamicin Sulfate in 5 mL of water is between 3.5 and 5.5.

**Content ratio of the active principle** Dissolve 50 mg of Gentamicin Sulfate in water to make 10 mL, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography 2.03. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm², without putting a filter paper in the container, and air-dry the plate. Allow the plate to stand in iodine vapor. Determine the integral absorbances, $A_a$, $A_b$, and $A_c$, of the colored spots of gentamicin C₁ ($R_f$ value: about 0.3), gentamicin C₂ ($R_f$ value: about 0.2) and gentamicin C₃ ($R_f$ value: about 0.1), respectively, by a densitometer (wavelength: 450 nm) while covering the plate with a glass plate, and calculate these amounts by the following formulae.
Gentamicin Sulfate Ophthalmic Solution / Official Monographs

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Gentamicin Sulfate Ophthalmic Solution

It contains not less than 90.0% and not more than 110.0% of the labeled potency of expressed as mass of gentamicin C₁ (C₁₂H₁₆N₄O₧·H₂O: 477.60).

Method of preparation  Prepare as directed under Ophthalmic Solution, with Gentamicin Sulfate.

Identification  Gentamicin Sulfate Ophthalmic Solution is a clear, colorless or pale yellow liquid.

Assay  Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.07> according to the following conditions.

(i) Test organism—Staphylococcus epidermidis ATCC 12228

(ii) Agar media for seed and base layer—
- Glucose 1.0 g
- Peptone 6.0 g
- Meat extract 1.5 g
- Yeast extract 3.0 g
- Sodium chloride 10.0 g
- Agar 15.0 g
- Water 1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

(iii) Agar medium for transferring test organisms—Use the medium ii in 2) Medium for other organisms under (2) Agar media for transferring test organisms.
**JP XVI**

**Official Monographs / Gliclazide**

**Glibenclamide**

![Glibenclamide structure](image)

C₂₃H₂₈ClN₃O₅S: 494.00
4-[2-(5-Chloro-2-methoxybenzoylamino)ethyl]-N-(cyclohexylcarbamoyl)benzenesulfonamide [10238-21-8]

Glibenclamide, when dried, contains not less than 98.5% of C₂₃H₂₈ClN₃O₅S.

**Description** Glibenclamide occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in dimethylformamide, sparingly soluble in chloroform, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

**Identification**

(1) Determine the absorption spectrum of a solution of Glibenclamide in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\) and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Glibenclamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\), and compare the spectrum with the Reference Spectrum.

(3) Perform the test with Glibenclamide as directed under Flame Coloration Test \(<1.04\) (2): a green color appears.

**Melting point \(<2.60\)** 169 - 174°C.

**Purity**

(1) Heavy metals \(<1.07\) — Proceed with 1.0 g of Glibenclamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances — Dissolve 0.20 g of Glibenclamide in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.60\). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, chloroform and diluted ammonia TS (4 in 5) (11:7:2) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying \(<2.41\)** Not more than 0.5% (1 g, 105°C, 4 hours).

**Assay** Weigh accurately about 0.9 g of Glibenclamide, previously dried, dissolve in 50 mL of N,N-dimethylformamide, and titrate \(<2.50\) with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination with a solution prepared by adding 18 mL of water to 50 mL of N,N-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 49.40 mg of C₂₃H₂₈ClN₃O₅S

**Containers and storage** Containers—Tight containers.

**Expiration date** 24 months after preparation.

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**Gliclazide**

![Gliclazide structure](image)

C₁₅H₁₉N₃O₆S: 323.41
1-(Hexahydrocyclopenta[c]pyrrol-2[H]-yl)-3-[(4-methylphenyl)sulfonyl]urea [21187-98-4]

Gliclazide, when dried, contains not less than 98.5% and not more than 101.0% of C₁₅H₁₉N₃O₆S.

**Description** Gliclazide is a white crystalline powder.

It is sparingly soluble in acetonitrile and in methanol, slightly soluble in ethanol (99.5) and practically insoluble in...
water.

**Identification (1)** Determine the absorption spectrum of a solution of Gliclazide in methanol (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Gliclazide as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** \(<2.60>\) 165 – 169°C.

**Purity (1)** Heavy metals \(<1.07>\)—Proceed with 2.0 g of Gliclazide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure within 2 hours after preparation of the sample solution. Dissolve 50 mg of Gliclazide in 23 mL of acetonitrile, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, and add a mixture of water and acetonitrile (11:9) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of water and acetonitrile (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of gliclazide is not more than 5.65. System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of gliclazide is not more than 2.0%.

(3) Residual solvent—Being specified separately.

**Loss on drying** \(<2.41>\) Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** \(<2.44>\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Gliclazide, previously dried, dissolve in 30 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate \(<2.50>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.34 mg of C17H17N2O2S

**Containers and storage** Containers—Well-closed containers.

### Glimepiride

**グリメピリド**

C_{24}H_{34}N_{4}O_{5}S: 490.62

1-(4-[2-[(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carbonyl)amino][ethyl]phenylsulfonyl]-3-(trans-4-methylcyclohexyl)urea [93479-97-1]

Glimepiride contains not less than 98.0% and not more than 102.0% of C_{24}H_{34}N_{4}O_{5}S, calculated on the anhydrous basis.

**Description** Glimepiride occurs as a white crystalline powder.

It is slightly soluble in dichloromethane, very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point: about 202°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Glimepiride in methanol (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Glimepiride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Glimepiride as directed in the potassium bromide disk
method under Infrared Spectrophotometry \(<2.25\)

Purity (1) Heavy metals \(<1.07\)—Proceed with 2.0 g of Glimepiride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) cis-Isomer—Dissolve 10 mg of Glimepiride in 5 mL of dichloromethane, add the mobile phase to make 20 mL, and use this solution as the sample solution. Perform the test with exactly 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\)> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.25 to 2.48, is not larger than 4 times the peak area of glimepiride from the standard solution, the area of the peak, having the relative retention time of about 1.1, is not larger than 2 times the peak area of glimepiride from the standard solution, the area of the peak, having the relative retention time of about 0.32, is not larger than 1.5 times the peak area of glimepiride from the standard solution, the area of peak other than glimepiride and above mentioned peak from the sample solution is not larger than the peak of glimepiride from the standard solution, and the total area of the peaks other than glimepiride and the peak, having the relative retention time of about 0.25 to 1.1, is not larger than 5 times the peak area of glimepiride from the standard solution.

**Operating conditions**—
- Detector: An ultraviolet absorption photometer (wavelength: 228 nm).
- Column: A stainless steel column 3 mm in inside diameter and 15 cm in length, packed with diol silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of heptane for liquid chromatography, 2-propanol for liquid chromatography, and acetic acid (100) (900:100:1).
- Flow rate: Adjust the flow rate so that the retention time of glimepiride is about 14 minutes.
- System suitability—
  - Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of glimepiride obtained with 10 \(\mu\)L of this solution is equivalent to 35 to 65% of that with 10 \(\mu\)L of the standard solution.
  - System performance: When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 3000 and not more than 1.5, respectively.
  - System repeatability: When the test is repeated 6 times with 20 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glimepiride is not more than 2.0%.

(3) Related substances—Keep the sample solution and the standard solution below 4°C after preparing. Dissolve 20 mg of Glimepiride in 100 mL of a mixture of acetonitrile for liquid chromatography and water (4:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\)> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.25 to glimepiride, obtained from the sample solution is not larger than 4 times the peak area of glimepiride from the standard solution, the area of the peak, having the relative retention time of about 1.1, is not larger than 2 times the peak area of glimepiride from the standard solution, the area of the peak, having the relative retention time of about 0.32, is not larger than 1.5 times the peak area of glimepiride from the standard solution, the area of peak other than glimepiride and above mentioned peak from the sample solution is not larger than the peak of glimepiride from the standard solution, and the total area of the peaks other than glimepiride and the peak, having the relative retention time of about 0.25 to glimepiride, from the sample solution is not larger than 5 times the peak area of glimepiride from the standard solution.

**System suitability**—
- Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of glimepiride obtained with 20 \(\mu\)L of this solution is equivalent to 35 to 65% of that with 20 \(\mu\)L of the standard solution.
- System performance: When the procedure is run with 20 \(\mu\)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 9000 and not more than 1.5, respectively.
- System repeatability: When the test is repeated 6 times with 20 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glimepiride is not more than 2.0%.

(4) Residual solvent—Being specified separately.

**Water** \(<2.49\> Not more than 0.5% (0.25 g, coulometric titration).

**Residue on ignition** \(<2.49\> Not more than 0.2% (1 g).

**Assay** Weigh accurately about 20 mg each of Glimepiride and Glimepiride RS (separately determine the water \(<2.49\>) in the same manner as Glimepiride), dissolve each substance in a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\> according to the following conditions, and determine the peak areas, \(A_1\) and \(A_5\), of glimepiride from each solution.

\[
\text{Amount (mg) of glimepiride } (C_{25}H_{23}N_4O_3S) = M_S \times A_1/A_5
\]

\(M_S\): Amount (mg) of Glimepiride RS, calculated on the anhydrous basis.

**Operating conditions**—
- Detector: An ultraviolet absorption photometer (wavelength: 228 nm).
- Column: A stainless steel column 4 mm in inside diameter.
and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.5 g of sodium dihydrogen phosphate dihydrate in 500 mL of water, adjust to pH 2.5 with phosphoric acid, and add 500 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of glimepiride is about 17 minutes.

System suitability—
System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 9000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glimepiride is not more than 1.0%.

Container and storage Containers—Well-closed containers.

Glimepiride Tablets
グリメピリド錠

Glimepiride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of glimepiride (C\(_{24}\)H\(_{34}\)N\(_4\)O\(_5\)S: 490.62).

Method of preparation Prepare as directed under Tablets, with Glimepiride.

Identification To a quantity of powdered Glimepiride Tablets, equivalent to 20 mg of Glimepiride according to the labeled amount, add 40 mL of acetonitrile, shake for 15 minutes, and centrifuge. Evaporate the supernatant liquid on a water bath under reduced pressure, suspend the residue with 1 mL of water, and filter under reduced pressure. Wash the residue with 1 mL of water, dry at 105°C for 1 hour. Determine the infrared absorption spectrum as directed in the Infrared Spectrophotometry \( \text{\<2.25\>1} \): it exhibits absorption at the wave numbers of about 3370 cm\(^{-1}\), 3290 cm\(^{-1}\), 2930 cm\(^{-1}\), 1708 cm\(^{-1}\), 1674 cm\(^{-1}\), 1347 cm\(^{-1}\), 1156 cm\(^{-1}\) and 618 cm\(^{-1}\).

Purity Related substances—Keep the sample solution and the standard solution below 4°C after preparation. To a quantity of powdered Glimepiride Tablets, equivalent to 9 mg of Glimepiride according to the labeled amount, wet with 0.5 mL of water, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make 50 mL, shake, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( \text{\<2.07\>1} \) according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.3 to glimepiride, obtained from the sample solution is not larger than 2.6 times the peak area of glimepiride from the standard solution, the area of the peak other than glimepiride and the peak mentioned above from the sample solution is not larger than 3/10 times the peak area of glimepiride from the standard solution, and the total area of the peaks other than glimepiride and the peak mentioned above from the sample solution is not larger than the peak area of glimepiride from the standard solution, and the total area of the peaks other than glimepiride from the sample solution is not larger than 3 times the peak area of glimepiride from the standard solution.

Separately, weigh accurately about 20 mg of Glimepiride RS (separately determine the water \( \text{\<2.48\>1} \) in the same manner as Glimepiride), and dissolve in a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL. Pipet 5 mL of this solution, add 2 mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL. Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To one tablet of Glimepiride Tablets add \( V/20 \) mL of water, disintegrate, add \( V/2 \) mL of a mixture of acetonitrile for liquid chromatography and water (4:1), and shake. To this solution add exactly \( V/10 \) mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly \( V \) mL so that each mL contains about 50 \( \mu \)g of glimepiride (C\(_{24}\)H\(_{34}\)N\(_4\)O\(_5\)S), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Glimepiride RS (separately determine the water \( \text{\<2.48\>1} \) in the same manner as Glimepiride), and dissolve in a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL. Pipet 5 mL of this solution, add 2 mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of glimepiride (C}_{24}\text{H}_{34}\text{N}_{4}\text{O}_{5}\text{S}) = M_S \times Q_1/\bar{Q}_2 \times V/400
\]

\( M_S \): Amount (mg) of Glimepiride RS, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of acetonitrile for liquid chromatography and water (4:1) (1 in 1000).

JP XVI
Dissolution<sup>C6.10D</sup> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 7.5, as the dissolution medium, the dissolution rate in 15 minutes of a 1-mg tablet is not less than 75%, and that in 30 minutes of a 3-mg tablet is not less than 70%.

Start the test with 1 tablet of Glimepiride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 1.1 mg (C24H34N4O5S), add 3 mL of water, and shake with 30 mL of dissolution of the powder, equivalent to about 3 mg of glimepiride Glimepiride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3 mg of glimepiride (C24H34N4O5S), add 3 mL of water, and shake with 30 mL of a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL. Pipet 15 mL of this solution, add exactly 6 mL of the internal standard solution, a mixture of acetonitrile for liquid chromatography and water (4:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography<sup>C2.01D</sup> according to the following conditions. Calculate the ratios, Q<sub>T</sub> and Q<sub>S</sub>, of the peak area of glimepiride to that of the internal standard.

\[
M_5: \text{Amount (mg) of Glimepiride RS, calculated on the anhydrous basis}
\]

\[
M_S: \text{Amount (mg) of Glimepiride RS, (separately determine the water <2.48> in the same manner as Glimepiride), and dissolve in acetonitrile for liquid chromatography to make exactly 100 mL. Pipet 2 mL of this solution, add 8 mL of acetonitrile for liquid chromatography, and add the dissolution medium to make exactly 200 mL. Pipet 10 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography<sup>C2.01D</sup> according to the following conditions, and determine the peak areas, A<sub>T</sub> and A<sub>S</sub>, of glimepiride of both solutions.}

\[
\text{Dissolution rate (%) with respect to the labeled amount of glimepiride (C}_{24}\text{H}_{34}\text{N}_{4}\text{O}_{5}\text{S}) = \frac{M_S}{M_5} \times \frac{A_T}{A_S} \times \frac{V/V^*}{1/C} \times \frac{1}{9/2}
\]

\[
C: \text{Labeled amount (mg) of glimepiride (C}_{24}\text{H}_{34}\text{N}_{4}\text{O}_{5}\text{S}) in 1 tablet}
\]

Operating conditions—

Detector, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glimepiride is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 Glimepiride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3 mg of glimepiride (C24H34N4O5S), add 3 mL of water, and shake with 30 mL of a mixture of acetonitrile for liquid chromatography and water (4:1). Add exactly 6 mL of the internal standard solution, and add a mixture of acetonitrile for liquid chromatography and water (4:1) to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Glimepiride RS, (separa-
Glucose

\[
\text{C}_6\text{H}_12\text{O}_6: 180.16
\]

\(\alpha\)-Glucopyranose


\[
\text{D-Glucopyranose}
\]


\[
\beta\)-Glucopyranose

Glucose is \(\alpha\)-d-glucopyranose, \(\beta\)-d-glucopyranose, or a mixture of them, and when dried, it contains not less than 99.5% of \(\text{C}_6\text{H}_12\text{O}_6\).

**Description** Glucose occurs as white crystals or crystalline powder. It is odorless, and has a sweet taste.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

**Identification** Add 2 to 3 drops of a solution of Glucose (1 in 20) to 5 mL of boiling Fehling’s TS: a red precipitate is produced.

**Purity** (1) Clarity and color of solution—Add 25 g of Glucose to 30 mL of water in a Nessler tube, warm at 60°C in a water bath until solution is effected, cool, and add water to make 50 mL: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 1.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS, and 2.0 mL of Copper (II) Sulfate CS, add water to make 10.0 mL. To 3.0 mL of this solution add water to make 50 mL.

(2) Acidity—Dissolve 5.0 g of Glucose in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphalein TS and 0.60 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Chloride \(<2.0\text{d}\>—\text{Perform the test with 2.0 g of Glucose. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).}

(4) Sulfate \(<1.14\>—\text{Perform the test with 2.0 g of Glucose. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).}

(5) Heavy metals \(<0.7\>—\text{Proceed with 5.0 g of Glucose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).}

(6) Arsenic \(<1.11\>—\text{Dissolve 1.5 g of Glucose in 5 mL of water, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, heat on a water bath for 5 minutes, and concentrate to 5 mL. After cooling, perform the test with this solution as the test solution (not more than 1.3 ppm).}

(7) Dextrin—To 1.0 g of Glucose add 20 mL of ethanol (95), and boil under a reflux condenser: the solution is clear.

(8) Soluble starch and sulfite—Dissolve 1.0 g of Glucose in 10 mL of water, and add 1 drop of iodine TS: a yellow color develops.

**Assay** Measure accurately a volume of Glucose Injection, equivalent to 2.5 g of Glucose according to the labeled amount, and add water to make exactly 100 mL. Determine the absorbance of this solution at 284 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>}: it is not more than 0.80.

**Bacterial endotoxins** \(<4.01\>—\text{Less than 0.50 EU/mL.}

**Extractable volume** \(<6.05\>—\text{It meets the requirement.}

**Foreign insoluble matter** \(<5.06\>—\text{Perform the test according to Method 1: it meets the requirement.}

**Insoluble particulate matter** \(<6.07\>—\text{It meets the requirement.}

**Sterility** \(<4.06\>—\text{Perform the test according to the Membrane filtration method: it meets the requirement.}

**Loss on drying** \(<2.41\>—\text{Not more than 1.0% (1 g, 105°C, 6 hours).}

**Residue on ignition** \(<2.44\>—\text{Not more than 0.1% (2 g).}

**pH** \(<2.54\>—3.5 – 6.5 In the case where the labeled concentration of the injection exceeds 5%, dilute to 5% with water before the test.

**Purity** 5-Hydroxymethylfurfural and related substances—Measure exactly a volume of Glucose Injection, equivalent to 2.5 g of Glucose according to the labeled amount, and add water to make exactly 100 mL. Determine the absorbance of this solution at 284 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>}: it is not more than 0.80.

**Containers and storage** Containers—Tight containers.

**Glucose Injection**

ブドウ糖注射液

Glucose Injection is an aqueous solution for injection. It contains not less than 95.0% and not more than 105.0% of the labeled amount of glucose (\(\text{C}_6\text{H}_12\text{O}_6: 180.16\)).

**Method of preparation** Prepare as directed under Injections, with Glucose. No preservative is added.

**Description** Glucose Injection is a clear, colorless liquid. It has a sweet taste. It occurs as a colorless to pale yellow, clear liquid when its labeled concentration exceeds 40%.

**Identification** Measure a volume of Glucose Injection, equivalent to 0.1 g of Glucose according to the labeled amount, and, if necessary, add water or evaporate on a water bath to a volume of 2 mL. Add 2 to 3 drops of the solution to 5 mL of boiling Fehling’s TS: a red precipitate is produced.

**pH** \(<2.54\>—3.5 – 6.5 In the case where the labeled concentration of the injection exceeds 5%, dilute to 5% with water before the test.

**Purity** 5-Hydroxymethylfurfural and related substances—Measure exactly a volume of Glucose Injection, equivalent to 2.5 g of Glucose according to the labeled amount, and add water to make exactly 100 mL. Determine the absorbance of this solution at 284 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>}: it is not more than 0.80.
cell as directed under Optical Rotation Determination 2.49.

Amount (mg) of glucose (C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}) = $a_0 \times 1895.4$

**Containers and storage** Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

### L-Glutamic Acid

#### L-グルタミン酸

L-Glutamic Acid occurs as white crystals or a white crystalline powder. It has a slightly characteristic taste and an acid taste.

It is slightly soluble in water, and practically insoluble in ethanol (99.5).

L-Glutamic Acid is soluble in 2 mol/L hydrochloric acid TS.

The pH of a solution prepared by dissolving 0.7 g of L-Glutamic Acid in 100 mL of water by warming and then cooling is 2.9 to 3.9.

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of L-Glutamic Acid in 10 mL of 2 mol/L hydrochloric acid TS: the solution is clear and colorless.

2. Chloride—Dissolve 0.5 g of L-Glutamic Acid in 6 mL of dilute nitric acid and 20 mL of water, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.01 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

3. Sulfate—Dissolve 0.6 g of L-Glutamic Acid in 5 mL of dilute hydrochloric acid and 30 mL of water, and add water to make 45 mL. Perform the test using this solution as the test solution. Prepare the control solution from 0.35 mL of 0.005 mol/L sulfuric acid VS and 5 mL of dilute hydrochloric acid, and dilute with water to 45 mL. Prepare the test solution and the control solution with 5 mL of barium chloride TS, respectively (not more than 0.028%).

4. Ammonium—Perform the test with 0.25 g of L-Glutamic Acid. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

5. Heavy metal—Dissolve 1.0 g of L-Glutamic Acid in 20 mL of water and 7 mL of a solution of sodium hydroxide (1 in 25) by warming, cool, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution from 1.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).

6. Iron—Prepare the test solution with 0.1 g of L-Glutamic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 1.0 ppm).

7. Related substances—Weigh accurately about 0.5 g of L-Glutamic Acid, and dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount, equivalent to 2.5 mmol, of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, glycine, L-alanine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine and L-arginine, dissolve them in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, and add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 6 mL of this solution, and add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the mass percentage of each amino acid, using the mass of amino acid other than glutamic acid in 1 mL of the sample solution obtained from the height of the peaks obtained from the sample solution and standard solution: the amount of each amino acid other than glutamic acid is not more than 0.2%, and the total amount of these amino acids is not more than 0.6%.

**Operating conditions**

Detector: A visible absorption photometer (wavelength: 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (Na type) composed with a sulfonated polystyrene (3 µm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Reaction time: About 1 minute.

Mobile phase: Prepare the mobile phases A, B, C, D and E according to the following table, and add 0.1 mL each of caprylic acid.
Mobile phase

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<tbody>
<tr>
<td>Citric acid monohydrate</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>12.80 g</td>
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<tr>
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<td>7.74 g</td>
<td>13.31 g</td>
<td>26.67 g</td>
<td>—</td>
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<tr>
<td>Sodium chloride</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>3.74 g</td>
<td>54.35 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>8.00 g</td>
</tr>
<tr>
<td>Ethanol (99.5)</td>
<td>130 mL</td>
<td>20 mL</td>
<td>4 mL</td>
<td>—</td>
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<tr>
<td>Thioglycol</td>
<td>5 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benzy alcohol</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5 mL</td>
<td>—</td>
</tr>
<tr>
<td>Lauromacrogol solution (1 in 4)</td>
<td>4 mL</td>
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Total amount

<table>
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<th></th>
<th>A</th>
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<td>1000 mL</td>
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</tbody>
</table>

Changing of mobile phase: Switch the mobile phases A, B, C, D and E sequentially so that when proceed with 20 μL of the standard solution under the above conditions, aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine and arginine are eluted in this order with the resolution between the peaks of isoleucine and leucine being not less than 1.2.

Reaction reagent: Dissolve 204 g of lithium acetate dihydrate in an appropriate amount of water, add 123 mL of acetic acid (100), 401 mL of 1-methoxy-2-propanol and water to make 1000 mL, introduce nitrogen for 10 minutes, and use this solution as Solution (I). Separately, to 979 mL of 1-methoxy-2-propanol add 39 g of ninhydrin, introduce nitrogen for 5 minutes, add 81 mg of sodium borohydride, introduce nitrogen for 30 minutes, and use this solution as Solution (II). Prepare a mixture with an equal volume of the Solution (I) and Solution (II) (Prepare before use).

Flow rate of mobile phase: 0.20 mL per minute.

Flow rate of reaction reagent: 0.24 mL per minute.

System suitability—

System performance: When the test is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and L-alanine is not less than 1.2.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak height of each amino acid in the standard solution is not more than 5.0%, and the relative standard deviation of the retention time of them is not more than 1.0%.

**Loss on drying** <2.41> Not more than 0.3% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.12 g of L-Glutamic Acid, dissolve in 40 mL of water by warming, cool, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 14.71 mg of C5H9NO4

**Containers and storage** Containers—Tight containers.

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**L-Glutamine**

L-グルタミン

C₅H₁₀N₂O₄; 146.14
(2S)-2,5-Diamino-5-oxopentanoic acid [56-85-9]

L-Glutamine, when dried, contains not less than 99.0% and not more than 101.0% of C₅H₁₀N₂O₄.

**Description** L-Glutamine occurs as white crystals or a crystalline powder. It has a slight characteristic taste.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (99.5).

**Identification** Determine the infrared absorption spectrum of L-Glutamine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [α]D 20° +6.3 – +7.3° Weigh accurately about 2 g of L-Glutamine, previously dried, add 45 mL of water, warm to 40°C to dissolve, and after cooling, add water to make exactly 50 mL. Determine the optical rotation of this solution in a 100-mm cell, within 60 minutes.

**pH** <2.54> The pH of a solution prepared by dissolving 1.0 g of L-Glutamine in 50 mL of water is between 4.5 and 6.0.

**Purity** (1) Clarity and color of solution—A solution obtained by dissolving 0.5 g of L-Glutamine in 20 mL of water is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of L-Glutamine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Glutamine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.10 g of L-Glutamine, using the distillation under reduced pressure. Prepare the control solution with 10.0 mL of Standard Ammonium Solution. The temperature of the water bath is 45°C (not more than 0.1%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Glutamine according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <1.10>—Perform the test solution with 1.0 g of L-Glutamine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Glutamine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed.
under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample-layer and standard solution on a plate of silica gel for thin-layer chromatography. Then develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

**Loss on drying** <2.41> Not more than 0.3% (1 g, 105°C, 3 hours).

**Residue on Ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.15 g of L-Glutamine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 14.61 mg of C₆H₁₀N₂O₃.

**Containers and storage** Containers—Tight containers.

### Glutathione

Glutathione occurs as a white crystalline powder.

- It is freely soluble in water, and practically insoluble in ethanol (99.5).
- Melting point: about 185°C (with decomposition).

**Description** Glutathione occurs as a white crystalline powder.

**Identification** Determine the infrared absorption spectrum of Glutathione, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [α]D: -15.5 to -17.5° (after drying, 2 g, water, 50 mL, 100 mm).

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Glutathione in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Glutathione according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.17>—Prepare the test solution with 1.0 g of Glutathione according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Glutathione in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 4 with respect to glutathione obtained from sample solution is not larger than 3/4 times the peak area of glutathione from the standard solution, and the total area of the peaks other than the peak of glutathione is not larger than the peak area of glutathione from the standard solution.

**Operating conditions**

- **Detector:** An ultraviolet absorption photometer (wavelength: 210 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature:** A constant temperature of about 30°C.
- **Mobile phase:** Dissolve 6.8 g of potassium dihydrogen phosphate and 2.02 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To 970 mL of this solution add 30 mL of methanol.
- **Flow rate:** Adjust the flow rate so that the retention time of glutathione is about 5 minutes.
- **Time span of measurement:** About 6 times as long as the retention time of glutathione beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of glutathione obtained from 10 μL of this solution is equivalent to 8 to 12% of that from 10 μL of the standard solution.

System performance: Dissolve 50 mg of glutathione, 10 mg of D-phenylglycine and 50 mg of ascorbic acid in 100 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, ascorbic acid, glutathione and D-phenylglycine are eluted in this order, and the resolutions between the peaks of ascorbic acid and glutathione and between the peaks of glutathione and D-phenylglycine are not less than 5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glutathione is not more than 1.5%.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Glutathione, previously dried, dissolve in 50 mL of a solution of metaphosphoric acid (1 in 50), and titrate <2.50> with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS). Perform a blank
Glycerin / Official Monographs

**Glycerin**

**Glycerol**

グリセリン

C₃H₈O₃: 92.09

Glycerin contains not less than 84.0% and not more than 87.0% of C₃H₈O₃.

**Description**

Glycerin is a clear, colorless, viscous liquid. It has a sweet taste.

It is miscible with water and with ethanol (99.5).

It is hygroscopic.

**Identification**

Determine the infrared absorption spectrum of Glycerin as directed in the liquid film method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Refractive index**

\[ n_d^2 = 1.449 - 1.454 \]

**Specific gravity**

\[ d_20 = 1.221 - 1.230 \]

**Purity (1)**

Color—Place 50 mL of Glycerin in a Nessler tube, and observe downward: the solution has no more color than the following control solution. Control solution: Place 50 mL of Glycerin in a Nessler tube, and add water to make 50 mL.

(2) Acidity or alkalinity—To 2 mL of Glycerin add 8 mL of water and mix: the solution is neutral.

(3) Chloride (1.05)—Take 10.0 g of Glycerin, and perform the test: Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.001%).

(4) Sulfate (1.14)—Take 10.0 g of Glycerin, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

(5) Ammonium—To 5 mL of Glycerin add 5 mL of a solution of sodium hydroxide (1 in 10), and boil: the gas evolved does not change, and no turbidity is produced.

(6) Heavy metals (1.07)—Proceed with 5.0 g of Glycerin according to Method 1, and perform the test: Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(7) Calcium—To 5 mL of the solution obtained in (2) add 3 drops of ammonium oxalate TS: the solution remains unchanged.

(8) Arsenic (1.11)—Prepare the test solution with 1.0 g of Glycerin according to Method 1, and perform the test (not more than 2 ppm).

(9) Acrolein, glucose, and other reducing substances—To 1.0 g of Glycerin add 1 mL of ammonia TS, mix, and warm in a water bath at 60°C for 5 minutes: no yellow color is produced. Take the solution out of the water bath, add 3 drops of silver nitrate TS immediately, and allow to stand in a dark place for 5 minutes: the color of the solution does not change, and no turbidity is produced.

(10) Fatty acids and esters—Mix 50 g of Glycerin with 50 mL of freshly boiled and cooled water, add exactly 10 mL of 0.1 mol/L sodium hydroxide VS, boil the mixture for 15 minutes, cool, and titrate (2.50) the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS: 0.1 mol/L sodium hydroxide VS consumed is not more than 3.0 mL (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

(11) Diethylene glycol and related substances—Weigh accurately about 5.88 g of Glycerin, mix in methanol, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of diethylene glycol, mix in methanol, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 µL each of the sample solution and standard solution as directed under Gas Chromatography (2.02) according to the following conditions. Determine each peak area of the sample solution and standard solution by the automatic integration method, and determine the peak areas, A₁ and A₂, of diethylene glycol in each solution. Calculate the amount of diethylene glycol by the following equation: not more than 0.1%. Calculate the amount of the related substances other than Glycerin and diethylene glycol is not more than 0.1% and the total amount of peaks other than Glycerin is not more than 1.0%.

\[ \text{Amount} \% = \frac{M_s}{M_d} \times \frac{A_1}{A_2} \times \frac{5}{0.85} \]

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (%) of diethylene glycol</th>
</tr>
</thead>
<tbody>
<tr>
<td>M_s: Amount (g) of diethylene glycol</td>
<td></td>
</tr>
<tr>
<td>M_d: Amount (g) of glycerin</td>
<td></td>
</tr>
</tbody>
</table>

**Operating conditions**

Detector: A hydrogen flame-ionization detector.

Column: A fused-silica column 0.32 mm in inside diameter and 30 m in length, coated the inner surface with 14% cyanopropylphenyl/86% dimethysilicon polymer for gas chromatography 1 µm in thickness.

Column temperature: Inject at a constant temperature of about 100°C, raise the ratio of 7.5°C per minute to 220°C, and maintain at a constant temperature of about 220°C.

Injection port temperature: A constant temperature of about 220°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: About 38 cm per second.

Split ratio: 1:20

Time span of measurement: About 3 times as long as the retention time of glycerin, beginning after the solvent peak.

**System suitability**

System performance: Weigh 0.05 g of diethylene glycol and glycerin, and mix in 100 mL of methanol. When the procedures is run with 1 µL of this solution under the above operating conditions, diethylene glycol and glycerin are eluted in this order with the resolution between these peaks being not less than 7.0.

System repeatability: When the test is repeated 6 times
with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diethylene glycol is not more than 15%.

(12) Readily carbonizable substances—To 5 mL of Glycerin add carefully 5 mL of sulfuric acid for readily carbonizable substances, mix gently at a temperature between 18°C and 20°C, and allow to stand for 1 hour between 15°C and 25°C: the solution has not more color than Matching Fluid H.

Water $<2.48\times$ 13–17% (0.1 g, volumetric titration, direct titration).

Residue on ignition $<2.48\times$ Weigh accurately about 10 g of Glycerin in a tared crucible, heat to boiling, and fire to burn immediately. After cooling, moisten the residue with 1 to 2 drops of sulfuric acid, and ignite cautiously to constant mass: the mass of the residue is not more than 0.01%.

Assay Weigh accurately about 0.2 g of Glycerin, transfer into a glass-stoppered flask, add 50 mL of water, mix, add exactly 50 mL of sodium periodate TS, shake, and allow to stand in a dark place at room temperature for about 30 minutes. Add 10 mL of a mixture of water and ethylene glycol (1:1), allow to stand for about 20 minutes, add 100 mL of water, and titrate $<2.50\times$ with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make the necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 9.209 mg of C$_3$H$_8$O$_3$.

Containers and storage Containers—Tight containers.

Concentrated Glycerin

Concentrated Glycerol

濃グリセリン

C$_3$H$_8$O$_3$: 92.09
Propane-1,2,3-triol [56-81-5]

Concentrated Glycerin contains not less than 98.0% and not more than 101.0% of glycerin (C$_3$H$_8$O$_3$), calculated of the anhydrous basis.

Description Concentrated Glycerin is a clear, colorless and viscous liquid. It has a sweet taste. It is miscible with water and with ethanol (99.5). It is hygroscopic.

Identification Determine the infrared absorption spectrum of Concentrated Glycerin as directed in the liquid film method under Infrared Spectrophotometry $<2.25\times$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index $<2.45\times$ $n_2^D$: Not less than 1.470

Specific gravity $<2.56\times$ $d_2^0$: Not less than 1.258
glycerin and diethylene glycol is not more than 0.1% and the total amount of peaks other than glycerin is not more than 1.0%.

Amount (%): diethylene glycol = \( M_2/M_1 \times A_2/A_1 \times 5 \)

- \( M_2 \): Amount (g) of diethylene glycol
- \( M_1 \): Amount (g) of concentrated glycerin

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A fused-silica column 0.32 mm in inside diameter and 30 m in length, coated the inner surface with 14% cyanopropylphenyl/86% dimethylsilicon polymer for gas chromatography 1 \( \mu \)m in thickness.
Column temperature: Inject at a constant temperature of about 100°C, raise at the ration of 7.5°C per minute to 220°C, and maintain at a constant temperature of about 220°C.
Injection port temperature: A constant temperature of about 220°C.
Detector temperature: A constant temperature of about 250°C.
Carrier gas: Helium.
Flow rate: About 38 cm per second.
Split ratio: 1:20
Time span of measurement: About 3 times as long as the retention time of glycerin, beginning after the solvent peak.
System suitability—
System performance: Weigh 0.05 g of diethylene glycol and glycerin, and mix in 100 mL of methanol. When the procedure is run with 1 \( \mu \)L of this solution under the above operating conditions, diethylene glycol and glycerin are eluted in this order with the resolution between these peaks being not less than 7.0.
System repeatability: When the test is repeated 6 times with 1 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diethylene glycol is not more than 15%.

(12) Readily carbonizable substances—To 5 mL of Concentrated Glycerin add carefully 5 mL of sulfuric acid for readily carbonizable substances, mix gently at a temperature between 18°C and 20°C, and allow to stand for 1 hour between 15°C and 25°C: the solution has no more color than Matching Fluid H.

Water <2.48> Not more than 2.0% (6 g, volumetric titration, direct titration).

Residue on ignition <2.44> Weigh accurately about 10 g of Concentrated Glycerin in a tared crucible, heat to boiling, and fire to burn immediately. Cool, moisten the residue with 1 to 2 drops of sulfuric acid, and ignite cautiously to constant mass: the mass of the residue is not more than 0.01%.

Assay Weigh accurately about 0.2 g of Concentrated Glycerin, transfer into a glass-stoppered flask, add 50 mL of water, mix, add exactly 50 mL of sodium periodate TS, shake, and allow to stand in a dark place at a room temperature for about 30 minutes. Add 10 mL of a mixture of water and ethylene glycol (1:1), allow to stand for about 20 minutes, add 100 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make the necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 9.209 mg of \( \text{C}_3\text{H}_8\text{O}_3 \).

Containers and storage Containers—Tight containers.

### Glycerin and Potash Solution

#### Method of preparation

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Hydroxide</td>
<td>3</td>
</tr>
<tr>
<td>Glycerin</td>
<td>200 mL</td>
</tr>
<tr>
<td>Ethanol</td>
<td>250 mL</td>
</tr>
<tr>
<td>Aromatic substance</td>
<td>a suitable quantity</td>
</tr>
<tr>
<td>Water, Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Dissolve Potassium Hydroxide in a portion of Water, Purified Water or Purified Water in Containers, add Glycerin, Ethanol, a suitable quantity of aromatic substance and another portion of Water, Purified Water or Purified Water in Containers to volume, and filter. Concentrated Glycerin may be used in place of Glycerin.

#### Description
Glycerin and Potash Solution is a clear, colorless liquid, having an aromatic odor. The pH of a solution of Glycerin and Potash Solution (1 in 5) is about 12.

Specific gravity \( d_{20}^\circ \), about 1.02

#### Identification

1. A solution of Glycerin and Potash Solution (1 in 2) is alkaline (potassium hydroxide).
2. Place 10 mL of a solution of Glycerin and Potash Solution (1 in 10) in a glass-stoppered test tube, add 2 mL of sodium hydroxide TS and 1 mL of copper (II) sulfate TS, and shake: a blue color is produced (glycerin).
3. Glycerin and Potash Solution responds to the Qualitative Tests <1.09> for potassium salt.

Containers and storage Containers—Tight containers.

### Glyceryl Monostearate

モノステアリン酸グリセリン

Glyceryl Monostearate is a mixture of \( \alpha \)- and \( \beta \)-glyceryl monostearate and other fatty acid esters of glycerin.

#### Description
Glyceryl Monostearate occurs as white to light yellow, waxy masses, thin flakes, or granules. It has a characteristic odor and taste. It is very soluble in hot ethanol (95), soluble in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water and in ethanol (95). It is slowly affected by light.

#### Identification

1. Heat 0.2 g of Glyceryl Monostearate with 0.5 g of potassium hydrogen sulfate until thoroughly charred: the irritative odor of acrolein is perceptible.
2. Dissolve 0.1 g of Glyceryl Monostearate in 2 mL of
ethanol (95) by warming, heat with 5 mL of dilute sulfuric acid in a water bath for 30 minutes, and cool: a white to yellow solid is produced. This separated solid dissolves when shaken with 3 mL of diethyl ether.

Melting point <1.13> Not below 55°C.

Acid value <1.13> Not more than 15.

Saponification value <1.13> 157 – 170

Iodine value <1.13> Not more than 3.0. Use chloroform instead of cyclohexane.

Purity <1.13> Acidity or alkalinity—To 1.0 g of Glyceryl Monostearate add 20 mL of boiling water, and cool with swirling: the solution is neutral.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Glycine

Aminoacetic Acid

\[
\text{C}_2\text{H}_4\text{NO}_2: 75.07
\]

Aminoacetic acid [56-40-6]

Glycine, when dried, contains not less than 98.5% of \(\text{C}_2\text{H}_4\text{NO}_2\).

Description Glycine occurs as white crystals or crystalline powder. It is odorless. It has a sweet taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95).

Identification Determine the infrared absorption spectrum of Glycine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.23>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Glycine in water, evaporate the water to dryness, and repeat the test with the residue.

pH <2.54> Dissolve 1.0 g of Glycine in 20 mL of water: the pH of the solution is between 5.6 and 6.6.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Glycine in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.09>—Perform the test with 0.5 g of Glycine. Prepare the control solution with 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of Glycine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test using 0.25 g of Glycine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of Glycine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.17>—Prepare the test solution with 1.0 g of Glycine according to Method 1, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of Glycine in 25 mL of water and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.63>. Spot 5 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of l-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50), and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 80 mg of Glycine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 7.507 mg of \(\text{C}_2\text{H}_4\text{NO}_2\)

Containers and storage Containers—Well-closed containers.

Gonadorelin Acetate

Gonadorelin Acetate occurs as a white to pale yellow powder. It is odorless or has a slight, acetic odor.

It is freely soluble in water, in methanol and in acetic acid (100), and sparingly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a
solution of Gonadorelin Acetate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Gonadorelin Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Gonadorelin Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 20 mg of Gonadorelin Acetate in 0.5 mL of ethanol (99.5), add 1 mL of sulfuric acid, and heat: the odor of ethyl acetate is perceptible.

Optical rotation <2.49> [α]D²⁰: −53.0° to −57.0° (0.1 g calculated on the anhydrous basis, diluted acetic acid (100) (1 in 100), 10 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Gonadorelin Acetate in 10 mL of water: the pH of this solution is between 4.8 and 5.8.

Constituent amino acids Put 10 mg of Gonadorelin Acetate in a test tube for hydrolysis, add 0.5 mL of hydrochloric acid and 0.5 mL of a solution of mercaptoacetic acid (2 in 25), seal the tube under reduced pressure, and heat at 110°C for 5 hours. After cooling, open the tube, transfer the hydrolyzate into a beaker, and evaporate to dryness on a water bath. Add exactly 100 mL of 0.02 mol/L hydrochloric acid TS to dissolve the residue, and use this solution as the sample solution. Separately, weigh exactly 0.105 g of L-serine, 0.147 g of L-glutamic acid, 0.115 g of L-proline, 75 mg of glycine, 0.131 g of L-leucine, 0.181 g of L-tyrosine, 0.210 g of L-histidine hydrochloride monohydrate, 0.204 g of L-tryptophan and 0.211 g of L-arginine hydrochloride, which are all previously dried at 105°C for 9 hours, add 50 mL of 1 mol/L hydrochloric acid TS to dissolve them, and add water to make exactly 1000 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to dissolve the residue, and use this solution as the standard solution. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the peaks of nine constituent amino acids are observed on the chromatogram, and their respective molar ratios with respect to arginine are 0.7–1.0 for serine and tryptophan, 0.8–1.2 for proline, 0.9–1.1 for glutamic acid, leucine, tyrosine and histidine, respectively, and 1.8–2.2 for glycine.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm for proline and 570 nm for others).

Column: A stainless steel column 4 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated polystyrene copolymer (5 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Mobile phase: Prepare the mobile phases A, B, C and D according to the following table.

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisodium citrate dihydrate</td>
<td>6.19 g</td>
<td>7.74 g</td>
<td>26.67 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>8.00 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>54.35 g</td>
<td>—</td>
</tr>
<tr>
<td>Citric acid monohydrate</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>6.10 g</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol (99.5)</td>
<td>130 mL</td>
<td>20 mL</td>
<td>—</td>
<td>100 mL</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>—</td>
<td>5 mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Thioglycolic acid</td>
<td>4 mL</td>
<td>4 mL</td>
<td>5 mL</td>
<td>—</td>
</tr>
<tr>
<td>Lauramocetyl solution in diethyl ether (1 in 4)</td>
<td>4 mL</td>
<td>4 mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Caprylic acid</td>
<td>0.1 mL</td>
<td>a sufficient amount</td>
<td>0.1 mL</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Water</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total volume</td>
<td>1000 mL</td>
<td>1000 mL</td>
<td>1000 mL</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Flowing of mobile phase: Control the gradient by mixing the mobile phases A, B, C and D as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
<th>Mobile phase C (vol%)</th>
<th>Mobile phase D (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 9</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9 – 25</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25 – 61</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>61 – 76</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>76 – 96</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Reaction reagent: Dissolve 204 g of lithium acetate dihydrate in 336 mL of water, add 123 mL of acetic acid (100) and 401 mL of 1-methoxy-2-propanol, and use as Solution A. Separately, dissolve 39 g of ninhydrin and 81 mg of sodium borohydride in 979 mL of 1-methoxy-2-propanol, and use as Solution B. Mix the same volume of Solution A and Solution B before use.

Flow rate of mobile phase: 0.25 mL per minute.

Flow rate of reaction reagent: 0.3 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, serine, glutamic acid, proline, glycine, leucine, tyrosine, histidine, tryptophan and arginine are eluted in this order with enough separation between these peaks.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 0.10 g of Gonadorelin Acetate in 10 mL of water is clear, and the absorbance of this solution at 350 nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.10.

(2) Related substances—Dissolve 50 mg of Gonadorelin Acetate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than gonadorelin from the sample solution is not larger than 3/5 times the peak area of gonadorelin from the standard solution, and the total area of the peaks other than the peak of gonadorelin is not larger than 3/5 times the peak area of gonadorelin from the standard solution.
**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of gonadorelin beginning after the solvent peak.

**System suitability**

- Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of gonadorelin obtained from 10 μL of this solution is equivalent to 1 to 3% of that from 10 μL of the standard solution.

- System performance: Dissolve 4 mg of Gonadorelin Acetate in a suitable amount of the mobile phase, add 5 mL of a solution of phenacetin in acetonitrile (1 in 1000) and the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, gonadorelin and phenacetin are eluted in this order with the resolution between these peaks being not less than 3.

- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of gonadorelin is not more than 5%.

**Water** Not more than 8.0% (0.15 g, volumetric titration, direct titration).

**Residue on ignition** Not more than 0.2% (0.1 g).

**Assay** Weigh accurately about 40 mg of Gonadorelin Acetate and Gonadorelin Acetate RS (separately determine the water in the same manner as Gonadorelin Acetate) and dissolve in diluted acetic acid (100) (1 in 1000) to make exactly 25 mL each. Pipet 5 mL of each of these solutions, add exactly 5 mL of the internal standard solution and water to make 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL of this solution under the above operating conditions. Inject 5.0 mL of the sample solution. The peak area of gonadorelin is not more than 5 days.

**System suitability**

- When the procedure is run with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of gonadorelin is not more than 5%.

- When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of gonadorelin to that of the internal standard is not more than 1.5%.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Human Chorionic Gonadotrophin**

**Chorionic Gonadotrophin**

ヒト絨毛性腺刺激ホルモン

Human Chorionic Gonadotrophin is a dried preparation of gonad-stimulating hormone obtained from the urine of healthy pregnant women after the manufacturing process to remove or inactivate the virus.

It contains not less than 2500 human chorionic gonadotrophin Units per mg, and contains not less than 3000 chorionic gonadotrophin Units per mg protein.

It contains not less than 80% and not more than 125% of the labeled human chorionic gonadotrophin Units.

**Description** Human Chorionic Gonadotrophin occurs as a white to light yellow-brown powder.

It is freely soluble in water.

**Identification** Calculate b by the following equation, using Y3 and Y4 obtained in the Assay: b is not less than 120.

\[
\begin{align*}
E &= Y_3 - Y_4 \\
I &= \log \left( \frac{T_H}{T_L} \right)
\end{align*}
\]

**Purity** (1) Clarity and color of solution—Dissolve 0.05 g of Human Chorionic Gonadotrophin in 5 mL of isotonic sodium chloride solution: the solution is clear and colorless or light yellow.

(2) Estrogen—Inject subcutaneously into each of three female albino rats or albino mice ovariectomized at least two weeks before the test, single dose of 100 units according to the labeled Units dissolved in 0.5 mL of isotonic sodium chloride solution. Take vaginal smear twice daily, on the third, fourth and fifth day. Place the smear thinly on a slide glass, dry, stain with Giemsa’s TS, wash with water, and again dry: no estrus figure is shown microscopically.

**Loss on drying** Not more than 5.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Bacterial endotoxins** Less than 0.03 EU/unit.

**Abnormal toxicity** Dilute Human Chorionic Gonadotrophin with isotonic sodium chloride solution so that each mL of the solution contains 120 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution
into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g, and observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

**Specific activity** When calculate from the results obtained by the Assay and the following test, the specific activity is not less than 3000 human chorialic gonadotrophin Units per mg protein.

(i) Sample solution—To an exactly amount of Human Chorialic Gonadotrophin add water to make a solution so that each mL contains about 300 Units of human chorialic gonadotrophin according to the labeled amount.

(ii) Standard solution—Weigh accurately about 10 mg of bovine serum albumin, and dissolve in water to make exactly 20 mL. To a suitable volume of this solution add water to make four solutions containing exactly 300, 200, 100 and 50 μg of the albumin per mL, respectively.

(iii) Procedure—Pipe 0.5 mL of each of the sample solution and standard solutions, put them in glass test tubes about 18 mm in inside diameter and about 130 mm in length, add exactly 5 mL of alkaline copper TS, mix, and allow the tubes to stand in a water bath at 30°C for 10 minutes. Then add exactly 0.5 mL of diluted Folin’s TS (1 in 2), mix, and warm in a water bath at 30°C for 20 minutes. Determine the absorbances of these solutions at 750 nm as directed under Ultraviolet-visible Spectrophotometry and standard solutions, put them in glass test tubes and their protein concentrations on the horizontal axis to prepare a calibration curve, and determine the protein content of the sample solution from its absorbance by using this curve. Then calculate the amount of the protein in the sample.

**Assay** (i) Test animals—Select healthy female albino rats weighing about 45 to 50 g.

(ii) Standard solution—Dissolve a quantity of Human Chorialic Gonadotrophin RS in bovine serum albumin-isotonic sodium chloride solution to prepare four kinds of solutions, having 7.5, 15, 30 and 60 Units per 2.5 mL, respectively. Inject subcutaneously 0.5 mL of each mL to each group for 5 days. On the sixth day, excise the ovaries, remove the fat and other unwonitored tissues attached to the ovaries, and remove the adhering body by lightly pressing between filter paper, and immediately weigh the ovaries.

(v) Calculation—Designate the mass of ovaries by S_H, S_L, T_H and T_L as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4.

Units per mg of Human Chorialic Gonadotrophin

\[ M = \frac{1}{4} \sum_{i=1}^{4} y_i \]

(iii) Sample solution—To an exactly amount of Human Chorialic Gonadotrophin add water to make a solution so that each mL contains about 300 Units of human chorialic gonadotrophin according to the labeled amount.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.5 mL of S_H, S_L, T_H and T_L in each group for 5 days. On the sixth day, excise the ovaries, remove the fat and other unwonitored tissues attached to the ovaries, and remove the adhering body by lightly pressing between filter paper, and immediately weigh the ovaries.

\[ Y = \frac{1}{4} \sum_{i=1}^{4} y_i \]

(v) Calculation—Designate the mass of ovaries by S_H, S_L, T_H and T_L as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4.

Units per mg of Human Chorialic Gonadotrophin

\[ M = \frac{1}{4} \sum_{i=1}^{4} y_i \]

(iii) Sample solution—To an exactly amount of Human Chorialic Gonadotrophin add water to make a solution so that each mL contains about 300 Units of human chorialic gonadotrophin according to the labeled amount.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.5 mL of S_H, S_L, T_H and T_L in each group for 5 days. On the sixth day, excise the ovaries, remove the fat and other unwonitored tissues attached to the ovaries, and remove the adhering body by lightly pressing between filter paper, and immediately weigh the ovaries.

\[ Y = \frac{1}{4} \sum_{i=1}^{4} y_i \]

(v) Calculation—Designate the mass of ovaries by S_H, S_L, T_H and T_L as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4.

Units per mg of Human Chorialic Gonadotrophin

\[ M = \frac{1}{4} \sum_{i=1}^{4} y_i \]

(iii) Sample solution—To an exactly amount of Human Chorialic Gonadotrophin add water to make a solution so that each mL contains about 300 Units of human chorialic gonadotrophin according to the labeled amount.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.5 mL of S_H, S_L, T_H and T_L in each group for 5 days. On the sixth day, excise the ovaries, remove the fat and other unwonitored tissues attached to the ovaries, and remove the adhering body by lightly pressing between filter paper, and immediately weigh the ovaries.

\[ Y = \frac{1}{4} \sum_{i=1}^{4} y_i \]

(v) Calculation—Designate the mass of ovaries by S_H, S_L, T_H and T_L as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4.

Units per mg of Human Chorialic Gonadotrophin

\[ M = \frac{1}{4} \sum_{i=1}^{4} y_i \]

(iii) Sample solution—To an exactly amount of Human Chorialic Gonadotrophin add water to make a solution so that each mL contains about 300 Units of human chorialic gonadotrophin according to the labeled amount.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.5 mL of S_H, S_L, T_H and T_L in each group for 5 days. On the sixth day, excise the ovaries, remove the fat and other unwonitored tissues attached to the ovaries, and remove the adhering body by lightly pressing between filter paper, and immediately weigh the ovaries.

\[ Y = \frac{1}{4} \sum_{i=1}^{4} y_i \]

(v) Calculation—Designate the mass of ovaries by S_H, S_L, T_H and T_L as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4.

Units per mg of Human Chorialic Gonadotrophin

\[ M = \frac{1}{4} \sum_{i=1}^{4} y_i \]
Human Chorionic Gonadotrophin for Injection

Chorionic Gonadotrophin for Injection

Human Chorionic Gonadotrophin for Injection is a preparation for injection which is dissolved before use. It contains not less than 80% and not more than 125% of the labeled human chorionic gonadotrophin Units.

Method of preparation
Prepare as directed under Injections with Human Chorionic Gonadotrophin.

Description
Human Chorionic Gonadotrophin for Injection occurs as a white to light yellow-brown powder or masses.

Identification
Proceed as directed in the Identification under Human Chorionic Gonadotrophin.

pH
Prepare a solution so that each mL of isotonic sodium chloride solution contains 2 mg of Human Chorionic Gonadotrophin for Injection: the pH of this solution is between 5.0 and 7.0.

Loss on drying
Not more than 5.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Bacterial endotoxins
Less than 0.03 EU/unit.

Uniformity of dosage units
It meets the requirement. Proceed as directed in the Mean of estimated contents of the units tested as the low-dose standard solution, SL. Store these solutions at 2 – 8°C.

Foreign insoluble matter
Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter
It meets the requirement.

Sterility
Perform the test according to the Membrane filtration method: it meets the requirement.

Assay
Proceed as directed in the Assay under Human Chorionic Gonadotrophin. The ratio of the assayed Units to the labeled Units should be calculated by the following equation.

The ratio of the assayed Units to the labeled Units = antilog M

Containers and storage
Containers—Hermetic containers. Storage—Light-resistant, and in a cold place.

Human Menopausal Gonadotrophin

Human Menopausal Gonadotrophin is a dried preparation of gonad-stimulating hormone obtained from the urine of postmenopausal healthy women, after processing for virus removal or inactivation. It has follicle-stimulating hormonal action and luteinizing hormonal action.

It contains not less than 40 follicle-stimulating hormone Units per mg.

Description
Human Menopausal Gonadotrophin occurs as a white to pale yellow powder. It is soluble in water.

Purity
Interstitial cell-stimulating hormone—Perform the test according to the following method: the ratio of the unit of interstitial cell-stimulating hormone (luteinizing hormone) to that of follicle-stimulating hormone is not more than 1. The luteinizing activity of the hormone is determined by the seminal vesicle weight assay or ovarian ascorbic acid depletion assay. The seminal vesicle weight assay may be used when the ratio of the unit of interstitial cell-stimulating hormone to that of follicle-stimulating hormone is not more than 1 and not less than 0.10.

1. Seminal vesicle weight assay
(i) Test animals—Select healthy male albino rats weighing about 45 to 65 g.
(ii) Standard solutions—Weigh accurately a suitable amount of Human Menopausal Gonadotrophin RS, and dissolve in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare three kinds of solutions, containing 10, 20 and 40 interstitial cell-stimulating hormone (luteinizing hormone) units per 1.0 mL, respectively. Inject these solutions into three groups consisting of five test animals each, and weigh their seminal vesicles as directed in (iv). According to the result of the test, designate the concentration of the reference standard, which will make the mass of the seminal vesicle 20 to 35 mg, as the high-dose standard solution, S_H. Dilute the S_H to 1.5 to 2.0 times the initial volume with the bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, and designate this solution as the low-dose standard solution, S_L. Store these solutions at 2 – 8°C.
(iii) Sample solutions—According to the labeled units, weigh accurately a suitable amount of Human Menopausal Gonadotrophin, and dissolve in the bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare the high-dose sample solution, S_H, and the low-dose sample solution, S_L, so that their concentrations are similar to those of the corresponding standard solutions, respectively. Store these solutions at 2 – 8°C.
(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously once every day 0.2 mL each of S_H, S_L, T_H and T_L to each animal in the respective groups for five days. On the sixth day, excise the seminal vesicles, remove extraneous tissue, remove fluid adhering to the vesicles and the contents of the vesicles by lightly pressing between filter papers, and weigh the vesi-
(v) Calculation—Proceed as directed in (v) in the Assay by changing the mass of ovaries to the mass of seminal vesicles to read.

2. Ovarian ascorbic acid depletion assay

(i) Test animals—Select healthy female albino rats weighing about 45 to 65 g.

(ii) Standard solutions—Dissolve Human Menopausal Gonadotrophin RS in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare four kinds of solutions, containing 2, 4, 8 and 16 interstitial cell-stimulating hormone (luteinizing hormone) units per 1.0 mL, respectively. Inject these solutions into four groups consisting of five test animals each, and determine the amount of ovarian ascorbic acid. Separately, inject bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to a control group of animals. According to the result of the test, designate the concentration of the reference standard, which will make the amount of ovarian ascorbic acid 0.8 to 0.85 times that in the control group, as the concentration for the low-dose standard solution, and 4 to 6 times that as the concentration for the high-dose standard solution. Dissolve Human Menopausal Gonadotrophin RS in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare the high-dose standard solution and low-dose standard solution to contain the concentrations described above, and designate them as SH and SL, respectively.

(iii) Sample solutions—According to the labeled units, weigh accurately a suitable amount of Human Menopausal Gonadotrophin, and dissolve in the bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare the high-dose sample solution and low-dose sample solution to contain units equal to those of the high-dose standard solution and low-dose standard solution, and designate them as TH and TL, respectively.

(iv) Procedure—Inject subcutaneously to each animal 80 units of serum gonadotrophin dissolved in 0.5 mL of isotonic sodium chloride solution. At 56 to 72 hours after the injection, inject subcutaneously to each animal 40 units of human chorionic gonadotrophin dissolved in 0.5 mL of isotonic sodium chloride solution. On 6 to 9 days after the last injection, divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject 1 mL each of SH, SL, TH and TL into the tail vein of each animal in groups A, B, C and D, respectively. At 2 to 4 hours after the injection, excise the both ovaries, remove the fat and other unwanted tissues attached to the ovaries, weigh, add a prescribed volume between 5 and 15 mL of metaphosphoric acid solution (1 in 40), homogenize with a homogenizer on ice, and centrifuge. To 0.5 to 1 mL (1 mL in principle. 0.5 mL may be used when the absorbance is not more than 0.1) of the supernatant liquid, add 1.5 mL of metaphosphoric acid solution (1 in 40) and 2.5 mL of 2,6-dichloroindophenol sodium-sodium acetate TS, mix the solution, and immediately determine the absorbance of the solution at 520 nm as directed under Ultraviolet-visible Spectrophotometry. Separately, weigh accurately 10.0 mg of Ascorbic Acid RS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 50 mL. Pipet a suitable volume of this solution, and add metaphosphoric acid solution (1 in 40) to make a solution so that each mL contains 2.0 to 10.0 μg of ascorbic acid (C₆H₈O₆; 176.12). To 2.5 mL of this solution, add 2.5 mL of 2,6-dichloroindophenol sodium-sodium acetate TS, mix the solution, immediately determine the absorbance in the same manner as mentioned above, and prepare the calibration curve. From the calibration curve of ascorbic acid, determine the amount (mg) of ascorbic acid in 100 g of ovary.

(v) Calculation—Proceed as directed in (v) in the Assay by changing the mass of ovaries to the amount of ascorbic acid to read.

**Bacterial endotoxins**

Dissolve Human Menopausal Gonadotrophin in water for bacterial endotoxins test to prepare a solution containing 75 follicle-stimulating hormone Units per mL, and perform the test: less than 0.66 EU/ follicle-stimulating hormone Unit.

**Water**

Not more than 5.0% (0.2 g, volumetric titration, direct titration).

**Specific activity**

Perform the test with Human Menopausal Gonadotrophin according to the following method, and calculate the specific activity using the amount (Unit) obtained in the Assay: it is not less than 50 follicle-stimulating hormone Units per 1 mg of protein.

(i) Sample solution—Weigh accurately about 10 mg of Human Menopausal Gonadotrophin, dissolve in water so that each mL contains exactly 200 μg, and use this solution as the sample solution.

(ii) Standard solutions—Weigh accurately about 10 mg of bovine serum albumin, and dissolve in water to make exactly 20 mL. To this solution add water to make four solutions containing exactly 300 μg, 200 μg, 100 μg and 50 μg of the albumin per mL, respectively, and use these solutions as the standard solutions.

(iii) Procedure—To glass test tubes, about 18 mm in inside diameter and about 130 mm in height, add separately exactly 0.5 mL each of the sample solution and the standard solutions. To these tubes add exactly 5 mL of alkaline copper TS, warm in a water bath at 30°C for 10 minutes, then add exactly 0.5 mL of diluted Folin’s TS (1 in 2), and warm in a water bath at 30°C for 20 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry, and determine the absorbances at 750 nm, using a liquid obtained with 0.5 mL of water in the same manner as above as a blank.

Prepare a calibration curve from the absorbances of the standard solutions, with absorbance on the vertical axis and concentration on the horizontal axis. Calculate the amount of protein in the sample solution from the absorbance of the sample solution using the curve, and calculate the protein content of the sample.

**Assay**

(i) Test animals—Select healthy female albino rats weighing about 45 to 65 g.

(ii) Standard solutions—Weigh accurately a suitable amount of Human Menopausal Gonadotrophin RS, dissolve in human chorionic gonadotrophin TS to make three solutions which contain 0.75, 1.5 and 3.0 follicle-stimulating hormone Units per 1.0 mL, respectively. Inject these solutions into three groups consisting of five test animals each, and weigh their ovaries, as directed in (iv). According to the result of the test, designate the concentration of the reference standard, which will make the mass of the ovary about 120 to 160 mg, as the high-dose standard solution, S₉. Dilute...
the SH to 1.5 to 2.0 times the initial volume with the human chorionic gonadotrophin TS, and designate the solution as the low-dose standard solution, SL.

(iii) Sample solutions—According to the labeled units, weigh accurately a suitable amount of Human Menopausal Gonadotrophin, dissolve in human chorionic gonadotrophin TS, and prepare the high-dose sample solution, TH, and the low-dose sample solution, TL, which have similar numbers of units to those of corresponding standard solutions in equal volume, respectively.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.2 mL of each of SH, SL, TH and TL into the animals in each group, once in the morning and afternoon on the third day, and two times in the morning and afternoon on the second day, and two times in the morning and afternoon on the third day. On the fifth day, excise the ovaries, remove the fat and extraneous tissue, remove fluid adhering to the ovaries by lightly pressing between filter papers, and immediately weigh the ovaries.

(v) Calculation—Designate the mass of ovaries by SH, SL, TH and TL, respectively. Sum up y₁, y₂, y₃ and y₄ on each set to obtain Y₁, Y₂, Y₃ and Y₄.

Units per mg of Human Menopausal Gonadotrophin

\[ M = \text{antilog } M \times \text{units per mL of } SH \times b/a \]

\[ I = \log (SH/SL) = \log (TH/TL) \]

\[ Y_1 = Y_2 + Y_3 + Y_4 \]

\[ Y_3 = Y_1 - Y_2 + Y_3 + Y_4 \]

\[ a: \text{Mass (mg) of sample} \]

\[ b: \text{Total volume (mL) of the high dose of the test solution prepared by diluting with bovine serum albumin-iso-ionic sodium chloride solution} \]

F computed by the following equation should be smaller than \( F_1 \) against n when \( s^2 \) is calculated. And compute L (P = 0.95) by the following equation: L should be not more than 0.3. If \( F \) exceeds \( F_1 \), or if \( L \) exceeds 0.3, repeat the test increasing the number of the test animals or arranging the assay method in a better way until \( F \) is smaller than \( F_1 \) or \( L \) is not more than 0.3.

\[ F = \frac{(Y_1 - Y_2 - Y_3 + Y_4)^2}{4f} \]

\[ f: \text{Number of test animals per group} \]

\[ s^2 = \frac{1}{n} \sum (Y_i - \bar{Y})^2 \]

\[ 2Y_3: \text{The sum of the squares of each } Y_1, Y_2, Y_3 \text{ and } Y_4 \]

\[ Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2 \]

\[ n = 4(f - 1) \]

\[ L = 2\{C - 1\}(CM^2 + \bar{T}^2) \]

\[ C = \frac{Y_3^2}{(Y_1^2 - 4f^2)^2} \]

\[ t^2: \text{Value shown in the following table against } n \text{ used to calculate } s^2 \]

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Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

**Serum Gonadotrophin**

血清性性腺刺激ホルモン

Serum Gonadotrophin is a dried preparation of gonad-stimulating hormone, obtained from pregnant mares' serum which has adequately inspected viruses, and subjected to a suitable process for removal or inactivation of viruses.

It contains not less than 2000 serum gonadotrophin Units per mg.

It contains not less than 80% and not more than 125% of the labeled serum gonadotrophin Units.

**Description** Serum Gonadotrophin occurs as a white powder.

It is freely soluble in water.

**Identification** Calculate b by the following equation, using \( Y_3 \) and \( Y_4 \) obtained in the Assay: b is not less than 120.

\[ b = E/I \]

\[ E = (Y_1 - Y_2)/f \]

\[ f: \text{Number of test animals per group} \]

\[ I = \log (TH/TL) \]

**Purity** Clarity and color of solution—Dissolve Serum Gonadotrophin in isotonic sodium chloride solution to prepare a solution containing 9000 units per mL according to the labeled Units: the solution is clear and colorless.

**Loss on drying** Less than 0.1 g, in vacuum, phosphorus (V) oxide, 4 hours.

**Bacterial endotoxins** Less than 0.1 EU/unit.

**Abnormal toxicity** Dissolve Serum Gonadotrophin in isotonic sodium chloride solution so that each 5 mL of the solution contains 4000 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g, and inject 0.5 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy mice aged about 5 weeks.
Observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

**Specific activity** When calculated from the results obtained by the Assay and the following test, Serum Gonadotrophin contains not less than 3000 serum gonadotrophin Units per mg of protein.

(i) Standard solutions—Dissolve about 3 mg of bovine serum albumin in water to make a solution containing 500 μg of the albumin in each mL. To this solution add water to make four standard solutions so that each mL contains exactly 200 μg, 150 μg, 100 μg and 50 μg of the albumin, respectively.

(ii) Sample solution—Dissolve about 1 mg of Serum Gonadotrophin in water to make a solution containing exactly 180 μg in each mL.

(iii) Sodium carbonate solution—Dissolve 2 g of sodium carbonate (standard reagent) in diluted sodium hydroxide TS (1 in 10) to make 100 mL.

(iv) Sodium tartrate solution—Dissolve about 1 g of sodium tartrate pentahydrate in the sodium tartrate solution prepared by diluting with bovine serum albumin isotonic sodium chloride solution.

(v) Copper (II) sulfate solution—Dissolve 0.5 g of copper (II) sulfate pentahydrate in the sodium tartrate solution to make 100 mL.

(vi) Alkaline copper solution—Mix 50 mL of the sodium carbonate solution and 1 mL of the copper (II) sulfate solution. Prepare before use. Use within the day of preparation.

(vii) Procedure—Pipet 0.5 mL each of the standard solutions and the sample solution in small test tubes, add 3 mL of the alkaline copper solution to them, and mix. Allow them to stand at the room temperature for not less than 10 minutes, add 0.3 mL of diluted Folin’s TS (1 in 2), mix immediately, and allow to stand for not less than 30 minutes. Determine the absorbances of these solutions so obtained at 750 nm as directed under Ultraviolet-visible Spectrophotometry using a solution, prepared in the same manner with 0.5 mL of water, as the blank. Plot the calibration curve from the absorbances obtained with the standard solutions, and determine the amount of protein in the sample solution from this curve.

Specific activity (unit/mg protein) = ([units per mg, obtained in the Assay]/(amount (%) of protein in the sample) × 100

**Assay**

(i) Test animals—Select healthy female albino rats weighing about 45 g.

(ii) Standard solution—Dissolve a quantity of Serum Gonadotrophin RS in bovine serum albumin-isotonic sodium chloride solution to prepare four kinds of solutions, containing 10, 20, 40 and 80 Units per 0.5 mL, respectively. Inject these solutions into four groups consisting of five test animals each, and weigh their ovaries, as directed in procedure of (iv). Inject bovine serum albumin-isotonic sodium chloride solution to another group, and use this group as the control group. According to the result of this test, designate the concentration of the reference standard which will increase the masses of the ovaries about 3 times the mass of the ovaries of the control group as a low-dose concentration of the standard solution, and the concentration 1.5 to 2.0 times the low-dose concentration as a high-dose concentration. Weigh accurately a suitable quantity of Serum Gonadotrophin RS, dissolve in bovine serum albumin-isotonic sodium chloride solution, and prepare a high-dose standard solution SH and a low-dose standard solution SL whose concentrations are equal to those determined by the above test.

(iii) Sample solution—According to the labeled units, weigh accurately a suitable quantity of Serum Gonadotrophin, dissolve in bovine serum albumin-isotonic sodium chloride solution, and prepare a high-dose sample solution TH and a low-dose sample solution Tl having Units equal to the standard solutions in equal volumes, respectively.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject once subcutaneously 0.5 mL of SH, S1, T1, and Tl in each group. On the sixth day, excise the ovaries, remove the fat and other unwonted tissues attached to the ovaries, and remove the adhering water by lightly pressing between filter paper, and immediately weigh the ovaries.

(v) Calculation—Designate the mass of ovaries by SH, S1, T1 and TL as y1, y2, y3 and y4, respectively. Sum up y1, y2, y3 and y4 on each set to obtain Y1, Y2, Y3 and Y4.

Units per mg of Serum Gonadotrophin = antilog M × units per mL of SH × b/a

M = IYs/y5
I = log (SH/S1) = log (T1/Tl)
Ys = − Y1 − Y2 + Y3 + Y4
Y5 = Y1 − Y2 + Y3 − Y4

a: Mass (mg) of sample
b: Total volume (mL) of the high dose of the test solution prepared by diluting with bovine serum albumin-isotonic sodium chloride solution

F' computed by the following equation should be smaller than F1 against n when $s^2$ is calculated. And compute L (P = 0.95) by the following equation: L should be not more than 0.3. If $F'$ exceeds $F_1$, or if L exceeds 0.3, repeat the test increasing the number of the test animals or arranging the assay method in a better way until $F'$ is smaller than $F_1$ or L is not more than 0.3.

$$F' = \left( Y_1 - Y_2 - Y_3 + Y_4 \right)^2/(4fs^2)$$

f: Number of test animals per group
$s^2$ = [Σs2 − (Y/f)]/n
$\Sigma y^2$: The sum of the squares of each y1, y2, y3 and y4
$Y = Y_1 + Y_2 + Y_3 + Y_4$
$n = 4(f - 1)$
$L = 2f(C - 1)(CM^2 + F')$
$C = Y_2^2/(Y_3^2 - 4fs^2t^2)$
$t^2$: Value shown in the following table against n used to calculate $s^2$
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Containers and storage  Containers—Tight containers.  Storage—Light-resistant, and in a cold place.

**Serum Gonadotrophin for Injection**

Serum Gonadotrophin for Injection is a preparation for injection which is dissolved before use.

It contains not less than 80% and not more than 125% of the labeled Serum Gonadotrophin Units.

**Method of preparation** Prepare as directed under Injections with Serum Gonadotrophin.

**Description** Serum Gonadotrophin for Injection occurs as white powder or masses.

**Identification** Proceed as directed in the Identification under Serum Gonadotrophin.

**pH** Not more than 2.54

**Loss on drying** Not more than 5.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Bacterial endotoxins** Less than 0.1 EU/unit.

**Assay** Proceed as directed in the Assay under Serum Gonadotrophin. The ratio of the Units assayed to the labeled Units should be calculated by the following equation.

$$\text{The ratio of the assayed Units to the labeled Units} = \text{antilog } M$$

Containers and storage  Containers—Hermetic containers.  Storage—Light-resistant, and in a cold place.

**Gramicidin**

グラミシジン

[1405-97-6]

Gramicidin is a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus brevis* Dubos.

It contains not less than 900 μg (potency) per mg, calculated on the dried basis. The potency of Gramicidin is expressed as mass (potency) of gramicidin.

**Description** Gramicidin occurs as a white to light yellowish white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water.

**Identification**

1. To 10 mg of Gramicidin add 2 mL of 6 mol/L hydrochloric acid TS, and heat in a water bath for 30 minutes with occasional stirring. After cooling, neutralize with 6 mol/L sodium hydroxide TS, add 1 mL of ninhydrin TS and 0.5 mL of pyridine, and heat for 2 minutes: a blue-purple to red-purple color develops.

2. Determine the absorption spectrum of a solution of Gramicidin in ethanol (95) (1 in 20,000), as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Gramicidin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Loss on drying** Not more than 3.0% (0.1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** Not more than 1.0% (1 g).

**Assay** Perform the test according to the Turbidimetric method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Enterococcus hirae* ATCC 10541

(ii) Agar medium for transferring test organism

<table>
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<th>Amount</th>
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<td>Glucose</td>
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<td>Casein peptone</td>
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<tr>
<td>Yeast extract</td>
<td>20.0 g</td>
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<tr>
<td>Potassium dihydrogen phosphate</td>
<td>2.0 g</td>
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<tr>
<td>Polysorbate 80</td>
<td>0.1 g</td>
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<tr>
<td>Agar</td>
<td>15.0 g</td>
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<tr>
<td>Water</td>
<td>1000 mL</td>
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</table>

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.7 to 6.8 after sterilization.

(iii) Liquid medium for suspending test organism—Use the culture medium (2).

(iv) Preparation of the test organism suspension—Puncture the test organism in the medium, prepared by dispensing 10 mL of the agar medium for transferring test organism in a test tube about 16 mm in inside diameter, incubate at 36.5 to 37.5°C for 20 to 24 hours. After sub-culturing at least three times, keep between 1 to 5°C. Transfer the organism so obtained in 10 mL of the liquid medium for suspending test organism, incubate at 36.5 to 37.5°C for 20 to 24 hours, and use this medium as the test organism stock suspension. Before use, add the test organism stock suspension to the liquid medium for suspending test organism so that...
the transmittance at 580 nm is 50 to 60%. Mix one volume of this suspension and 200 volume of the liquid medium for suspending test organism, and use this as the test organism suspension.

(v) Standard solution—Weigh accurately an amount of Gramicidin RS, previously dried under reduced pressure not exceeding 0.67 kPa at 60°C for 3 hours, equivalent to about 10 mg (potency), dissolve in ethanol (99.5) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add the following diluting solution to make a solution so that each mL contains 0.02 µg (potency), and use this solution as the standard solution.

Diluting solution: To 390 mL of propylene glycol add 210 mL of a mixture of ethanol (99.5) and acetone (9:1) and Sterile Purified Water to make 1000 mL.

(vi) Sample solution—Weigh accurately an amount of Gramicidin, equivalent to about 10 mg (potency), and dissolve in ethanol (99.5) to make exactly 100 mL. Take exactly a suitable amount of this solution, add the diluting solution obtained in (v) to make a solution so that each mL contains 0.02 µg (potency), and use this solution as the sample solution.

(vii) Procedure—Transfer 0.155 mL, 0.125 mL, 0.100 mL, 0.080 mL and 0.065 mL each of the standard solution, separately, in test tubes about 14 mm in inside diameter and about 15 cm in length, and make three sets for each. To each of the test tube add 10 mL of the test organism suspension, stopper the tube, incubate in a water bath at 36.5 to 37.5°C for 180 to 270 minutes, add 0.5 mL of a solution of formaldehyde (1 in 3), and determine their transmittances at 580 nm.

Containers and storage  Containers—Tight containers.

Griseofulvin

Griseofulvin occurs as white, crystals or crystalline powder.

It is soluble in N,N-dimethylformamide, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification  (1) Determine the absorption spectrum of a solution of Griseofulvin in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.242, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Griseofulvin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Griseofulvin as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.259, and compare the spectrum with the Reference Spectrum or the spectrum of Griseofulvin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.492> [α]D 979: +350° to +364° (0.25 g calculated on the dried basis, N,N-dimethylformamide, 25 mL, 100 mm).

Melting point <2.602> 218 – 222°C

Purity  (1) Acidity—Dissolve 0.25 g of Griseofulvin in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 1.0 mL of 0.02 mol/L sodium hydroxide VS: the color of the solution is red.

(2) Heavy metals <1.072>—Proceed with 1.0 g of Griseofulvin according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 25 ppm).

(3) Arsenic <1.172>—Prepare the test solution with 1.0 g of Griseofulvin according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—To 0.10 g of Griseofulvin add exactly 1 mL of the internal standard solution and acetone to make 10 mL, and use this solution as the sample solution. Separately, to 5.0 mg of Griseofulvin RS add exactly 1 mL of the internal standard solution and acetone to make 10 mL, and use this solution as the standard solution. Perform the test with 2 µL each of the sample solution and standard solution as directed under Gas Chromatography 2.022 according to the following conditions, determine each peak area by the automatic integration method, and calculate the ratio, Qs, of the peak area of dechlorogriseofulvin, having the relative retention time of about 0.6 with respect to griseofulvin, to that of the internal standard obtained from the sample solution, the ratio, Qg, of the peak area of dehydrogriseofulvin, having the relative retention time of about 1.2, to that of the internal standard obtained from the sample solution and the ratio, Qs, of the peak area of griseofulvin to that of the internal standard obtained from the standard solution: Qg/Qs is not more than 0.6, and Qg/Qs is not more than 0.15.

Internal standard solution—A solution of 9,10-diphenylanthracene in acetone (1 in 500).

Operating conditions—Detector: An hydrogen flame-ionization detector.

Column: A glass column 4 mm in inside diameter and 1 m in length, packed with silicic earth for gas chromatography coated with 25% phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography in the ratio of 1%
Griseofulvin Tablets

グリセオフルビン錠

Griseofulvin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of griseofulvin (C₁₇H₁₇ClO₆: 352.77).

Method of preparation

Prepare as directed under Tables, with Griseofulvin.

Identification

To a quantity of powdered Griseofulvin Tablets, equivalent to 15 mg (potency) of Griseofulvin according to the labeled amount, add 100 mL of ethanol (95%), shake vigorously, and filter. To 1 mL of the filtrate add ethanol (95%) to make 10 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 234 nm and 238 nm, between 290 nm and 294 nm, and between 323 nm and 328 nm.

Uniformity of dosage units

Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Griseofulvin Tablets, add V/5 mL of water, treat with ultrasonic waves to disintegrate the tablet, add N,N-dimethylformamide to make 5F/8 mL, shake vigorously for 20 minutes, add N,N-dimethylformamide to make exactly F/mL so that each mL contains 1.25 mg (potency) of Griseofulvin, and centrifuge. Pipet 8 mL of the supernatant liquid, add exactly 20 mL of the internal standard solution, add water to make 100 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed under the Assay.

Dissolution

When the test is performed at 100 revol-
Dissolution rate (%) with respect to the labeled amount of griseofulvin (C17H17ClO6)

\[ M_5 = \frac{M_s \times A_T / A_S \times V/V \times 1/C \times 45/2}{M_5} \]

where
- \( M_s \): Amount (mg [potency]) of Griseofulvin RS
- \( A_T \): Labeled amount [mg (potency)] of griseofulvin (C17H17ClO6) in 1 tablet

**Assay**

Weigh accurately not less than 20 Griseofulvin Tablets, and pulverize into a powder. Weigh accurately a portion of the powder, equivalent to about 0.5 g (potency) of Griseofulvin, add 50 mL of water, and treat with ultrasonic waves. Add 100 mL of \( N,N \)-dimethylformamide, shake vigorously for 20 minutes, and add \( N,N \)-dimethylformamide to make exactly 250 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 20 mL of the internal standard solution, add water to make 100 mL, filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \)m, discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL so that each mL contains about 6.9 \( \mu \)g (potency) of Griseofulvin according to the labeled amount, and use this solution as the sample solution. Separate, weigh accurately an amount of Griseofulvin RS, equivalent to about 28 mg (potency), and dissolve in ethanol (95) to make exactly 200 mL. Pipet 5 mL of this solution, add 5 mL of the dissolution medium and water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), at 295 nm of the sample solution and standard solution as directed under Ultra-violet-visible Spectrophotometry <2.24>, using water as the blank.

\[ M_5 = \frac{M_s \times A_T / A_S \times V/V \times 1/C \times 45/2}{M_5} \]

\[ M_5: \text{Amount (mg [potency]) of Griseofulvin RS} \]

\[ C: \text{Labeled amount [mg (potency)] of griseofulvin (C17H17ClO6) in 1 tablet} \]

**Operating conditions**

- A solution of butyl parahydroxybenzoate in acetonitrile (1 in 2000).

**System suitability**

- A solution of Guaifenesin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Identification**

(1) Determine the absorption spectrum of a solution of Guaifenesin (1 in 50,000) as directed under Ultra-violet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Guaifenesin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Guaifenesin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Guaifenesin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point**

\(<2.60^\circ\) 80 – 83°C

**pH**

\(<2.5^\circ\) Dissolve 1.0 g of Guaifenesin in 100 mL of water: the pH of the solution is between 5.0 and 7.0.

**Purity**

(1) Clarity and color of solution—Dissolve 0.20 g of Guaifenesin in 10 mL of water: the solution is clear and colorless.

(2) Chloride \(<1.0^\circ\)—Dissolve 0.7 g of Guaifenesin in 25 mL of water by warming. Cool, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.020%).

(3) Heavy metals \(<1.0^\circ\)—Dissolve 2.0 g of Guaifenesin

**Containers and storage**

Containers—Tight containers.

**Guaifenesin**

**Guaiacol Glyceryl Ether**

ゲアイフェネシン

\( \text{C}_{10}H_{14}O_4 \): 198.22

(2RS)-3-(2-Methoxyphenoxy)propane-1,2-diol [93-14-1]

Guaifenesin, when dried, contains not less than 98.0% and not more than 102.0% of \( \text{C}_{10}H_{14}O_4 \).

**Description**

Guaifenesin occurs as a white crystals or crystalline powder.

It is freely soluble in ethanol (95), and sparingly soluble in water.

A solution of ethanol (95) (1 in 20) shows no optical rotation.

**Identification**

(1) Determine the absorption spectrum of a solution of Guaifenesin (1 in 50,000) as directed under Ultra-violet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Guaifenesin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Guaifenesin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Guaifenesin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.
in 25 mL of water by warming. Cool, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Guaifenesin according to Method 3, and perform the test (not more than 2 ppm).

(5) Free guaiacol—To 1.0 g of Guaifenesin add exactly 25 mL of water, dissolve by warming, cool, and use this solution as the sample solution. Separately, dissolve 0.100 g of guaiacol in water to make exactly 1000 mL. Pipet 3 mL of this solution, add exactly 22 mL of water, and use this solution as the standard solution. To each of the sample solution and standard solution add 1.0 mL of potassium hexacyanoferrate (III) TS and 5.0 mL of a solution of 4-aminoantipyrine (1 in 200), and immediately after shaking for exactly 5 seconds add a solution of sodium hydrogen carbonate (1 in 1200) to make exactly 100 mL. Determine the absorbances of these solutions at 500 nm exactly 15 minutes after the addition of the 4-aminoantipyrine solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared in the same manner with 25 mL of water, as the blank: the absorbance of the solution obtained from the sample solution is not more than that from the standard solution.

(6) Related substances—Dissolve 1.0 g of Guaifenesin in 100 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, ethanol (95), and ammonia solution (28) (40:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 110°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.44>—Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44>—Not more than 0.1% (1 g).

Assay Weigh accurately about 60 mg of Guaifenesin and Guaifenesin RS, previously dried, and dissolve each then in water to make exactly 100 mL. Pipet 5 mL of these solutions, and add water to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, A1 and A2, of the sample solution and standard solution at 273 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
M_2 = \frac{M_S \times A_1}{A_2}
\]

Amount (mg) of C10H14O4 = \(M_2 \times M_S\)

Containers and storage Containers—Tight containers.
Guanethidine Sulfate

グアネチジン硫酸塩

C₁₀H₂₂N₄.H₂SO₄: 296.39
1-[2-(Hexahydroazocin-1(2H)-yl)ethyl]guanidine monosulfate

645-43-2

Guanethidine Sulfate, when dried, contains not less than 98.5% of C₁₀H₂₂N₄.H₂SO₄.

Description Guanethidine Sulfate occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor and a bitter taste.

It is very soluble in formic acid, freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Melting point: 251 – 256°C (an evacuated sealed capillary tube, with decomposition).

Identification (1) To 4 mL of a solution of Guanethidine Sulfate (1 in 4000) add 2 mL of 1-naphthol TS, 1 mL of diazotized sulfanilic acid, 2 g of sodium bromide and water to make 200 mL. Then, to this solution add 0.70 mL of 1/60 mol/L potassium bromate VS and 2 mL of zinc iodide-starch paste TS: a blue color develops.

(2) Methylisothiourea sulfide—Dissolve 2.0 g of Guanethidine Sulfate in 80 mL of sodium hydroxide TS, and allow to stand for 10 minutes. Add 60 mL of hydrochloric acid, 2 g of sodium bromide and water to make 200 mL. Then, to this solution add 0.70 mL of 1/60 mol/L potassium bromate VS and 2 mL of zinc iodide-starch paste TS: a blue color develops.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Guanethidine Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.47> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Guanethidine Sulfate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 29.11 mg of C₁₀H₂₂N₄.H₂SO₄

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Adsorbed Habu-venom Toxoid

沈殿はぶトキソイド

Adsorbed Habu-venom Toxoid is a liquid for injection containing habu toxoid prepared by treating toxic substances produced by habu (Trimeresurus flavoviridis) with formaldehyde by a method involving no appreciable loss of the immunogenicity and rendered insoluble by the addition of aluminum salt.

It conforms to the requirements of Adsorbed Habu-venom Toxoid in the Minimum Requirements for Biological Products.

Description Adsorbed Habu-venom Toxoid becomes a uniform whitish turbid liquid on shaking.

Freeze-dried Habu Antivenom, Equine

乾燥はぶウマ抗毒素

Freeze-dried Habu Antivenom, Equine, is a preparation for injection which is dissolved before use.

It contains Trimeresurus flavoviridis antivenin in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Habu Anti-venom, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Habu Antivenom, Equine, becomes colorless or light yellow-brown, clear liquid or a
Under Infrared Spectrophotometry

Both spectra exhibit similar intensities of absorption at the same wavelengths.

Under Ultraviolet-visible Spectrophotometry

Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry with 10 mL of this solution add 700 mL of methanol and 1.0 g of sodium lauryl sulfate.

Flow rate: Adjust the flow rate so that the retention time of haloperidol is about 9 minutes.

Time span of measurement: About 3 times as long as the retention time of haloperidol beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of haloperidol obtained with 10 μL of this solution is equivalent to 15 to 25% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of haloperidol are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times, the relative standard deviation of the peak area of haloperidol from the sample solution is not larger than 2.41%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.95 g of trisodium citrate dihydrate in 900 mL of water, adjust to pH 3.5 with dilute hydrochloric acid, and add water to make 1000 mL. To 300 mL of this solution add 700 mL of methanol and 1.0 g of sodium lauryl sulfate.

Adsorbed Hepatitis B Vaccine

Adsorbed Hepatitis B Vaccine is a liquid for injection prepared by adding an aluminum salt to a liquid containing a surface antigen of hepatitis B virus to make the HBs antigen insoluble.

It conforms to the requirements of Adsorbed Hepatitis B Vaccine in the Minimum Requirements for Biological Products.

Description Adsorbed Hepatitis B Vaccine becomes a homogeneous, whitish turbid liquid on shaking.

Haloperidol

Haloperidol occurs as white to pale yellow crystals or powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in 2-propanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Dissolve 30 mg of Haloperidol in 100 mL of 2-propanol. To 5 mL of the solution add 10 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2,24> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Haloperidol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2,60> 149 – 153°C.

Purity (1) Sulfate <1,14>—To 1.0 g of Haloperidol add 50 mL of water, shake, and filter. To 25 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Heavy metals <1,07>—Proceed with 1.0 g of Haloperidol according to method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 25 mg of Haloperidol in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2,01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than haloperidol obtained from the sample solution is not larger than the peak area of haroperidol from the standard solution, and the total area of these peaks is not larger than 2 times the peak area of haloperidol from the standard solution. For this calculation, use the peak areas for the related substances, having the relative retention time of about 0.5, about 1.2 and about 2.6 with respect to haloperidol, after multiplying by their relative response factors, 0.75, 1.47 and 0.76, respectively.

Related substances—Dissolve 25 mg of Haloperidol in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2,01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than haloperidol obtained from the sample solution is not larger than the peak area of haroperidol from the standard solution, and the total area of these peaks is not larger than 2 times the peak area of haloperidol from the standard solution. For this calculation, use the peak areas for the related substances, having the relative retention time of about 0.5, about 1.2 and about 2.6 with respect to haloperidol, after multiplying by their relative response factors, 0.75, 1.47 and 0.76, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.95 g of trisodium citrate dihydrate in 900 mL of water, adjust to pH 3.5 with dilute hydrochloric acid, and add water to make 1000 mL. To 300 mL of this solution add 700 mL of methanol and 1.0 g of sodium lauryl sulfate.

Flow rate: Adjust the flow rate so that the retention time of haloperidol is about 9 minutes.

Time span of measurement: About 3 times as long as the retention time of haloperidol beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of haloperidol obtained with 10 μL of this solution is equivalent to 15 to 25% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of haloperidol are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of haloperidol is not more than 2.0%.

Loss on drying <2,41> Not more than 0.5% (1 g, in vacuum, 60°C, phosphorus (V) oxide, 3 hours).

Residue on ignition <2,44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of haloperidol, previously dried, and dissolve in 40 mL of acetic acid (100), and titrate <2,50> with 0.1 mol/L perchloric acid VS (indicator: 1...
Haloperidol Fine Granules

Haloperidol Fine Granules contains not less than 95.0% and not more than 105.0% of the labeled amount of haloperidol (C21H23ClFNO2: 375.86).

Method of preparation  Prepare as directed under Granules, with Haloperidol.

Identification Pulverized Haloperidol Fine Granules. To a portion of the powder, equivalent to 6 mg of Haloperidol according to the labeled amount, add 70 mL of 2-propanol, and heat to boiling on a water bath while shaking. After cooling, add 2-propanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add 2 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24); it exhibits maxima between 219 nm and 223 nm and between 243 nm and 247 nm.

Dissolution  When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Haloperidol Fine Granules is not less than 70%.

Start the test with an accurately weighed amount of Haloperidol Fine Granules, equivalent to about 3 mg of haloperidol (C21H23ClFNO2) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of haloperidol for assay, previously dried in vacuum at 60°C for 3 hours using phosphorus (V) oxide as a desiccant, and dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 20 mL of the internal standard solution, extract for 30 minutes with the aid of ultrasonic waves with occasional shaking, and add the mobile phase to make 100 mL. Centrifuge after shaking for more 30 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of haloperidol for assay, previously dried in vacuum at 60°C for 3 hours on phosphorus (V) oxide, and dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 20 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.20), according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak areas of haloperidol to that of the internal standard.

\[
\text{Amount (mg) of haloperidol (C}_2\text{H}_3\text{ClFNO}_2) = M_5 \times Q_1/Q_2 \times 2/5
\]

\[
M_5: \text{Amount (mg) of haloperidol for assay}
\]

Internal standard solution—A solution of diphenyl in methanol (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.95 g of trisodium citrate dihydrate in 900 mL of water, adjust to pH 3.5 with dilute hydrochloric acid, and add water to make 1000 mL. To 250 mL of this solution add 750 mL of methanol and 1.0 g of sodium lauryl sulfate, and mix to dissolve.

Flow rate: Adjust the flow rate so that the retention time of haloperidol is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 µL of the internal standard solution under the above operating conditions, the relative standard deviation of the peak area of haloperidol is not more than 2.0%.

Particle size <6.00 It meets to the requirements of Fine granules.

Assay Pulverize Haloperidol Fine Granules. Weigh accurately a portion of the powder, equivalent to about 10 mg of haloperidol (C21H23ClFNO2), add 10 mL of water, disperse the particle with the aid of ultrasonic waves, add exactly 20 mL of the internal standard solution, extract for 30 minutes with the aid of ultrasonic waves with occasional shaking, and add the mobile phase to make 100 mL. Centrifuge after shaking for more 30 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of haloperidol for assay, previously dried in vacuum at 60°C for 3 hours on phosphorus (V) oxide, and dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 20 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.20) according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak areas of haloperidol to that of the internal standard.

\[
\text{Amount (mg) of haloperidol (C}_2\text{H}_3\text{ClFNO}_2) = M_2 \times Q_1/Q_2 \times 2/5
\]

\[
M_2: \text{Amount (mg) of haloperidol for assay}
\]

System suitability—

System performance: When the procedure is run with 10 µL of the internal standard solution under the above operating conditions, haloperidol and diphenyl are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of haloperidol to that of the internal standard is not more than 1.0%

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Haloperidol Tablets

Haloperidol Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of haloperidol (C₂₁H₂₃ClFNO₂·375.86).

Method of preparation Prepare as directed under Tablets, with Haloperidol.

Identification To pulverized Haloperidol Tablets, equivalent to 6 mg of Haloperidol according to the labeled amount, add 70 mL of 2-propanol, and heat on a water bath until to boiling while shaking. After cooling, add 2-propanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add 2 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24a> : it exhibits maxima between 219 nm and 223 nm and between 243 nm and 247 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Uniformity of dosage units test.

To 1 tablet of Haloperidol Tablets add 5 mL of the mobile phase, disperse the particle with the aid of ultrasonic waves, add 30 mL of the mobile phase, and extract for 30 minutes with the aid of ultrasonic waves with occasional shaking. Shake for more 30 minutes, and add mobile phase to make exactly 50 mL. Centrifuge the solution, pipet 5 mL of the supernatant liquid, equivalent to about 0.3 mg of haloperidol (C₂₁H₂₃ClFNO₂), add 2 mL of the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of haloperidol for assay, previously dried in vacuum at 60°C, and add exactly 20 mL of the internal standard solution, extract for 30 minutes with the aid of ultrasonic waves with occasional shaking, and add the mobile phase to make 100 mL. Centrifuge after shaking for more 30 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of haloperidol for assay, previously dried in vacuum at 60°C, and add exactly 20 mL of the internal standard solution and the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 µL of the mobile phase and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₄ and Q₅, of the peak area of haloperidol to that of the internal standard.

\[
M_S = \frac{M_S}{Q_S} = \frac{Q_T}{Q_5} \times 2/5
\]

Proceed as detected in the operating condition in the Assay.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, haloperidol and diphenyl are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of haloperidol to that of the internal standard is not more than 1.0%.

Dissolution Being specified separately.

Assay Weigh accurately, and powder not less than 20 Haloperidol Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of haloperidol (C₂₁H₂₃ClFNO₂), add 10 mL of water, disperse the particle with the aid of ultrasonic waves, add exactly 20 mL of the internal standard solution, extract for 30 minutes with the aid of ultrasonic waves with occasional shaking, and add the mobile phase to make 100 mL. Centrifuge after shaking for more 30 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of haloperidol for assay, previously dried in vacuum at 60°C for 3 hours on phosphorus (V) oxide, and dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 20 mL of the internal standard solution and the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 µL of the mobile phase and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₄ and Q₅, of the peak area of haloperidol to that of the internal standard.

\[
M_S = \frac{M_S}{Q_S} = \frac{Q_T}{Q_5} \times 2/5
\]

Internal standard solution—A solution of diphenyl in methanol (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.95 g of trisodium citrate dihydrate in 900 mL of water, adjust to pH 3.5 with dilute hydrochloric acid, and add water to make 1000 mL. To 250 mL of this solution add 750 mL of methanol and 1.0 g of sodium lauryl sulfate, and mix to dissolve.

Flow rate: Adjust the flow rate so that the retention time of haloperidol is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, haloperidol and diphenyl are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operat-
ing conditions, the relative standard deviation of the ratio of the peak area of haloperidol to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Tight containers.
   Storage—Light-resistant for the tablets without coating.

Halothane

Halothane contains not less than 0.008% and not more than 0.012% of Thymol as a stabilizer.

Description  Halothane is a clear, colorless, and mobile liquid.
   It is miscible with ethanol (95), with diethyl ether and with isooctane.
   It is slightly soluble in water.
   It is a volatile, nonflammable liquid, and setting fire to its heated vapor does not support combustion.
   It is affected by light.
   Refractive index \( n_20^D \): 1.369 – 1.371

Identification  Transfer about 3 \( \mu \)L of Halothane to a gas cell having light path 10 cm in length, and determine the infrared absorption spectrum as directed in the gas sampling method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity \( <2.56> \) \( \dalpha: 1.872 – 1.877 \)

Purity  (1) Acidity or alkalinity—Shake 60 mL of Halothane with 60 mL of freshly boiled and cooled water vigorously for 3 minutes. Separate the water layer, and use this as the sample solution. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide VS: a red-purple color develops. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.6 mL of 0.01 mol/L hydrochloric acid VS: a yellow color is produced.
   (2) Halide and halogen—To 5 mL of the sample solution obtained in (1) add 1 drop of nitric acid and 0.20 mL of silver nitrate TS: no turbidity is produced. To 10 mL of the sample solution obtained in (1) add 1 mL of potassium iodide TS and 2 drops of starch TS, and allow to stand for 5 minutes: no blue color develops.
   (3) Phosgene—Transfer 50 mL of Halothane to a dried 300-mL conical flask, suspend a strip of phosgene test paper vertically inside the flask with the lower end about 10 mm above the surface of the liquid, insert the stopper, and allow to stand at a dark place for 20 to 24 hours: the test paper shows no yellow color.
   (4) Residue on evaporation—Pipet 50 mL of Halothane, evaporate on a water bath, and dry the residue at 105°C for 2 hours: the mass of the residue is not more than 1.0 mg.
   (5) Volatile related substances—To 100 mL of Halothane add exactly 5.0 \( \mu \)L of the internal standard, and use this solution as the sample solution. Perform the test with 5 \( \mu \)L of the sample solution as directed under Gas Chromatography \(<2.02>\), and determine each peak area by the automatic integration method: the total area of the peaks other than those of halothane and the internal standard is not larger than the peak area of the internal standard.

Internal standard—1,1,2-Trichloro-1,2,2-trifluoroethane

Operating conditions—
   Detector: A hydrogen flame-ionization detector.
   Column: A column about 3 mm in inside diameter and 3 m in length, at the first 2 m from the injection port, having macrogol 400 coated in the ratio of 30% on siliceous earth for gas chromatography (180 to 250 \( \mu \)m in particle diameter), and at the remaining 1 m, having dinonyl phthalate coated in the ratio of 30% on siliceous earth for gas chromatography (180 to 250 \( \mu \)m in particle diameter).
   Column temperature: A constant temperature of about 50°C.
   Carrier gas: Nitrogen.
   Flow rate: Adjust the flow rate so that the retention time of the internal standard is 2 to 3 minutes.
   Selection of column: Mix 3 mL of Halothane and 1 mL of the internal standard. Proceed with 1 \( \mu \)L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and halothane in this order with the resolution between these peaks being not less than 10.
   Detection sensitivity: Adjust the detection sensitivity so that the peak height of the internal standard obtained from 5 \( \mu \)L of the sample solution composes 30 to 70% of the full scale.
   Time span of measurement: About 3 times as long as the retention time of halothane.

Distilling range \(<2.57>\) Not less than 95 vol distils within a 1°C range between 49°C and 51°C.

Thymol  To 0.50 mL of Halothane add 5.0 mL of isooctane and 5.0 mL of titanium (IV) oxide TS, shake vigorously for 30 seconds, and allow to stand: the separated upper layer has more color than the following control solution A, and has no more color than the following control solution B.

Control solution: Dissolve 0.225 g of thymol for assay in isooctane to make exactly 100 mL. To 10 mL each of this solution, accurately measured, add isooctane to make exactly 150 mL and 100 mL, respectively. Proceed with 0.50 mL each of these solutions in the same manner as Halothane, and use the separated upper layers so obtained as the control solution A and B, respectively.

Containers and storage  Containers—Tight containers.
   Storage—Light-resistant, and not exceeding 30°C.
Haloxazolam

ハロキサゾラム

C₁₇H₁₄BrFN₂O₂: 377.21
(11Br5S)-10-Bromo-11b-(2-fluorophenyl)-2,3,7,11b-
tetrahydro[1,3]oxazolo[3,2-d][1,4]benzodiazepin-6(5H)-
one
[59128-97-1]

Haloxazolam, when dried, contains not less than 99.0% of C₁₇H₁₄BrFN₂O₂.

Description Haloxazolam occurs as white crystals or crystalline powder. It is odorless and tasteless. It is freely soluble in acetic acid (100), sparingly soluble in acetonitrile, in methanol and in ethanol (99.5), slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 183°C (with decomposition).

Identification (1) Dissolve 10 mg of Haloxazolam in 10 mL of methanol, add 1 drop of hydrochloric acid: the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). To this solution add 1 mL of sodium hydroxide TS: the fluorescence disappears immediately.

(2) Prepare the test solution with 50 mg of Haloxazolam as directed under Oxygen Flask Combustion Method 1.086, using a mixture of 20 mL of dilute sodium hydroxide TS and 1 mL of hydrogen peroxide (30) as an absorbing liquid: the test solution responds to the Qualitative Tests 1.09 for bromide and for fluoride.

(3) Determine the absorption spectrum of a solution of Haloxazolam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Haloxazolam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance 2.24  Ɛ₅₀₉₅ (247 nm): 390 – 410 (10 mg, methanol, 1000 mL).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Haloxazolam in 20 mL of ethanol (99.5): the solution is clear and colorless.

(2) Soluble halides—To 1.0 g of Haloxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as directed under Chloride Limit Test 1.03). Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid VS.

(3) Heavy metals 1.07)—Proceed with 1.0 g of Haloxazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic 1.11D—To 1.0 g of Haloxazolam in a decomposition flask add 5 mL of nitric acid and 2 mL of sulfuric acid, place a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, heat, repeat this procedure twice, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution is colorless to pale yellow. After cooling, add 2 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution: the solution has no more color than the following control solution (not more than 2 ppm).

Control solution: Proceed in the same manner as above without using Haloxazolam, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and proceed in the same manner as the test solution.

(5) Related substances—Dissolve 0.10 g of Haloxazolam in 100 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the area of haloxazolam from the sample solution is not larger than the peak area of the haloxazolam from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 250 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 6.2 g of boric acid and 7.5 g of potassium chloride in 900 mL of water, adjust the pH with triethylamine to 8.5, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of haloxazolam is about 10 minutes.
Time span of measurement: About 3 times as long as the retention time of haloxazolam beginning after the solvent peak.

System suitability—
Test for required detection: To exactly 5 mL of the standard solution add acetonitrile to make exactly 50 mL. Confirm that the peak area of haloxazolam obtained from 10 µL of this solution is equivalent to 8 to 12% of that from 10 µL of the standard solution.

System performance: Dissolve 10 mg each of Haloxazolam and cloxazolam in 200 mL of acetonitrile. When the procedure is run with 10 µL of this solution under the above operating conditions, haloxazolam and cloxazolam are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operat-
Heparin Calcium

ヘパリンカルシウム

Heparin Calcium is the calcium salt of sulfated glycosaminoglycans composed of disaccharide units of α-glucosamine and uronic acid (β-iduronic acid or β-glucuronic acid) obtained from the intestinal mucosa of healthy edible swine.

It prolongs the clotting time of blood. It contains not less than 150 Heparin Units per mg. It, calculated on the dried basis, contains not less than 90% and not more than 110% of the labeled Units, and not less than 8.0% and not more than 12.0% of calcium (Ca: 40.08).

Description Heparin Calcium occurs as a white to grayish brown powder or grains. It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Dissolve 10 mg of Heparin Calcium in 5 mL of water, and add 0.1 mL of 1 mol/L hydrochloric acid TS and 5 mL of toluidine blue O solution (1 in 20,000): a purple to red-purple color develops.

(2) Dissolve 1 mg each of Heparin Calcium and Heparin Sodium RS for Physicochemical Test in 1 mL of water, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.0J according to the following conditions: the retention times for the major peaks from the sample solution and the standard solution are identical.

Operating conditions—
Detector, column, column temperature, mobile phase A, mobile phase B, flowing of the mobile phase and flow rate: Proceed as directed under the operating conditions in Purity (9).

System suitability—
System performance: Dissolve 1.0 mg of Heparin Sodium RS for physicochemical test in 0.60 mL of water. Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water. Dissolve 1.0 mg of dermatan sulfate in 2.0 mL of water. To 90 μL of the solution of Heparin Sodium RS add 30 μL each of the solutions of Over-sulfated Chondroitin Sulfate RS and dermatan sulfate, and mix. When the procedure is run with 20 μL of the mixture under the above operating conditions, dermatan sulfate, heparin and over-sulfated chondroitin sulfate are eluted in this order with the resolution between the peaks of dermatan sulfate and heparin being not less than 1.0 and that between the peaks of heparin and over-sulfated chondroitin sulfate being not less than 1.5.

(3) A solution of 50 mg of Heparin Calcium in 5 mL of water responds to the Qualitative Tests 1.09J for calcium salt.

pH 2.54—Dissolve 1.0 g of Heparin Calcium in 100 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Heparin Calcium in 20 mL of water: the solution is clear. Determine the absorbance of this solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry 2.24J: the absorbance is not more than 0.05.

(2) Chloride 1.07J—Perform the test with 0.5 g of Heparin Calcium. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Heavy metals 1.07J—Proceed with 0.5 g of Heparin Calcium according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(4) Barium—Dissolve 30 mg of Heparin Calcium in 3.0 mL of water, and use this solution as the sample solution. To 1.0 mL of the sample solution add 3 drops of dilute sulfuric acid, and allow to stand for 10 minutes: no turbidity is produced.

(5) Residual solvent—Being specified separately.

(6) Total nitrogen—Weigh accurately about 0.1 g of Heparin Calcium, previously dried, and perform the test as directed under Nitrogen Determination 1.08J: the amount of nitrogen (N: 14.01) is not more than 3.0%.

(7) Protein—To 1.0 mL of the sample solution obtained in (4) add 5 drops of a solution of trichloroacetic acid (1 in 5): neither a precipitate nor turbidity is produced.

(8) Over-sulfated chondroitin sulfate—Dissolve 20 mg of Heparin Calcium in 0.60 mL of a solution of sodium 3-trimethylsilylpropionate-d₄ for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). Determine the spectrum of this solution as directed under Nuclear Magnetic Resonance
Spectroscopy \( <2.21> \) \((1H)\) in accordance with the following conditions, using sodium 3-trimethylsilylpropionate-\( d_4 \) for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits no signal corresponding to \( N \)-acetyl proton of over-sulfated chondroitin sulfate at \( \delta 2.18 \pm 0.05 \) ppm, or the signal disappears when determining the spectrum of the sample solutions as directed under \( 1H \) with \( ^{13}C \)-decoupling.

**Operating conditions**—

- **Spectrometer:** FT-NMR, not less than 400 MHz.
- **Temperature:** 25°C.
- **Spinning:** off.
- **Number of data points:** 32,768.
- **Spectral range:** Signal of DHO \( \pm 6.0 \) ppm
- **Flip angle:** 90°
- **Delay time:** 20 seconds
- **Dummy scans:** 4
- **Number of scans:** S/N ratio of the signal of \( N \)-acetyl proton signal of heparin is not less than 1000.
- **Window function:** Exponential function (Line broadening factor = 0.2 Hz).

**System performance**—

- **System performance:** Dissolve 20 mg of Heparin Calcium in 0.40 mL of a solution of sodium 3-trimethylsilylpropionate-\( d_4 \) for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 1.0 mL of a solution of sodium 3-trimethylsilylpropionate-\( d_4 \) for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). To the solution of heparin calcium add 0.20 mL of the solution of Over-sulfated Chondroitin Sulfate RS. When determining the spectrum of this solution under the above operating conditions, it exhibits the signal of \( N \)-acetyl proton of heparin and the signal of \( N \)-acetyl proton of over-sulfated chondroitin sulfate at \( \delta 2.04 \pm 0.02 \) ppm and \( \delta 2.18 \pm 0.05 \) ppm, respectively.

(9) Related substances—Dissolve 2.0 mg of Heparin Calcium in 0.1 mL of water, and perform the test with exactly 20 \( \mu \)L of this solution as directed under Liquid Chromatography \( <2.01> \) according to the following conditions: it exhibits no peaks after the heparin peak.

**Operating conditions**—

- **Detector:** An ultraviolet absorption photometer (wavelength: 202 nm).
- **Column:** A stainless steel column 2.0 mm in inside diameter and 7.5 cm in length, packed with diethylaminoethyl group bound to synthetic polymer for liquid chromatography (10 \( \mu \)m in particle diameter).
- **Column temperature:** A constant temperature of about 35°C.

Mobile phase A: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10).

Mobile phase B: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate and 106.4 g of lithium perchlorate in 1000 mL of water, and adjust to a pH of 3.0 with diluted phosphoric acid (1 in 10).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 3</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>3 – 15</td>
<td>90 → 0</td>
<td>10 → 100</td>
</tr>
</tbody>
</table>

Flow rate: 0.2 mL per minute.

**Time span of measurement:** About 2 times as long as the retention time of heparin, beginning after the solvent peak.

**System suitability**—

Test for required detectability: Dissolve 10 mg of Heparin Sodium RS for physicochemical test in 0.40 mL of water, and use this solution as the Heparin Sodium standard stock solution. Separately, dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water, and use this solution as the over-sulfated chondroitin sulfate standard solution. To 60 \( \mu \)L of the Heparin Sodium standard stock solution add 3 \( \mu \)L of the over-sulfated chondroitin sulfate standard solution and 12 \( \mu \)L of water, and mix. When the procedure is run with 20 \( \mu \)L of the mixture under the above operating conditions, it exhibits an over-sulfated chondroitin sulfate peak.

**System performance:** To 120 \( \mu \)L of the Heparin Sodium standard stock solution add 30 \( \mu \)L of the over-sulfated chondroitin sulfate standard solution, mix and use this solution as the solution for system suitability test. When the procedure is run with 20 \( \mu \)L of the solution for system suitability test under the above operating conditions, heparin and over-sulfated chondroitin sulfate are eluted in this order with the resolution between these peaks being not less than 1.5.

**System repeatability:** When the test is repeated 6 times with 20 \( \mu \)L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of over-sulfated chondroitin sulfate is not more than 2.0%.

**Loss on drying \( <2.41> \)** Not more than 8% (50 mg, in vacuum, 60°C, 3 hours).

**Bacterial endotoxins \( <4.01> \)** Less than 0.0030 EU/heparin Unit.

**Assay (1) Heparin**

(i) **Substrate solution:** Dissolve 15 mg of \( N \)-benzoyl-l-isoleucyl-l-glutamyl(y-OR)-glycyl-l-arginyl-p-nitroanilide hydrochloride in 20 mL of water.

(ii) **Activated blood coagulation factor X solution:** Dissolve bovine activated blood coagulation factor X in water to make a solution containing 0.426 Unit per mL.

(iii) **Buffer solution:** Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 750 mL of water, adjust the pH to 8.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

(iv) **Reaction stop solution:** To 20 mL of acetic acid (100) add water to make 40 mL.

(v) **Heparin standard solutions:** Dissolve Heparin Sodium RS in isotonic sodium chloride solution to make a solution containing 10 Units per mL. To 100 \( \mu \)L of this solution add buffer solution to make exactly 5 mL, and use this solution as the standard stock solution. Prepare the heparin standard solutions (1), (2), (3), (4) and (5) by addition of anti-thrombin III TS, human normal plasma and the buffer solution to the standard solution as directed in the following
table.

<table>
<thead>
<tr>
<th>Heparin standard solution</th>
<th>Buffer solution (μL)</th>
<th>Anti-thrombin III TS (μL)</th>
<th>Human normal plasma (μL)</th>
<th>Standard solution (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Heparin concentration (Unit/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1)</td>
<td>0</td>
<td>800</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(2)</td>
<td>0.02</td>
<td>700</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(3)</td>
<td>0.04</td>
<td>600</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(4)</td>
<td>0.06</td>
<td>500</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(5)</td>
<td>0.08</td>
<td>400</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

(vii) Sample solution: Weigh accurately an adequate amount of Heparin Calcium, dissolve in isotonic sodium chloride solution so that each mL contains about 0.5 Units according to the labeled amount. To 100 μL of this solution add 100 μL of anti-thrombin III TS, 100 μL of human normal plasma and 700 μL of the buffer solution, and use this solution as the sample solution.

(viii) Procedure: Transfer 400 μL of the sample solution to a test tube, and warm at 37°C for 4 minutes. To this solution add 200 μL of the activated blood coagulation factor X solution, mix well, warm at 37°C for exactly 30 seconds, add 400 μL of the substrate solution, previously warmed at 37°C, and mix well. Warm this solution at 37°C for exactly 3 minutes, add 600 μL of the reaction stop solution, and mix immediately. Determine the absorbance at 405 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the blank solution prepared by addition of 600 μL of the reaction stop solution and 600 μL of water to 400 μL of the sample solution. Proceed the same way with the heparin standard solution (1), the heparin standard solution (2), the heparin standard solution (3), the heparin standard solution (4) and the heparin standard solution (5), and determine their absorbances.

(viii) Calculation: Plot the absorbances of the standard solutions and their heparin concentrations to prepare a calibration curve. Determine the heparin concentration, C, of the sample solution, and calculate heparin Units per mg of Heparin Calcium from the following formula.

\[
\text{Units per mg of Heparin Calcium} = C \times 10 \times \frac{b}{a}
\]

where:

- \(a\): Amount of sample (mg)
- \(b\): Total volume (mL) of isotonic sodium chloride solution used to dissolve the sample to make the solution containing about 0.5 Units per mL

(2) Calcium: Weigh accurately about 50 mg of Heparin Calcium, dissolve in 20 mL of water, add 2 mL of 8 mol/L sodium hydroxide TS, allow to stand for 3 to 5 minutes with occasional shaking, add 0.1 g of NN indicator, and immediately titrate <2.50> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 0.4008 mg of Ca

Heparin Sodium

ヘパリンナトリウム

Heparin Sodium is the sodium salt of sulfated glycosaminoglycans composed of disaccharide units of D-glucosamine and uronic acid (L-iduronic acid or D-glucuronic acid) obtained from the livers, the lungs and the intestinal mucosa of healthy edible animals. Heparin Sodium prolongs the clotting time of blood. It obtained from the livers and the lungs contains not less than 110 Heparin Units per mg, and that obtained from the intestinal mucosa contains not less than 130 Heparin Units per mg.

It calculated on the dried basis, contains not less than 90% and not more than 110% of the labeled Units.

Label the name of the organ used as the starting material.

Description Heparin Sodium occurs as a white to grayish brown powder or grains. It is odorless.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification Dissolve 1 mg each of Heparin Sodium and Heparin Sodium RS for Physicochemical Test in 1 mL of water, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the retention times for the major peaks from the sample solution and the standard solution are identical.

Operating conditions—
Detector, column, column temperature, mobile phase A, mobile phase B, flowing of the mobile phase and flow rate: Proceed as directed under the operating conditions in Purity (6).

System suitability—
System performance: Dissolve 1.0 mg of Heparin Sodium...
RS for physicochemical test in 0.60 mL of water. Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water. Dissolve 1.0 mg of dermatan sulfate in 2.0 mL of water. To 90 μL of the solution of Heparin Sodium RS add 30 μL each of the solutions of over-sulfated chondroitin sulfate and dermatan sulfate, and mix. When the procedure is run with 20 μL of the mixture under the above operating conditions, dermatan sulfate, heparin and over-sulfated chondroitin sulfate are eluted in this order with the resolution between the peaks of dermatan sulfate and heparin being not less than 1.0 and that between the peaks of heparin and over-sulfated chondroitin sulfate being not less than 1.5.

\[ \text{pH} < 2.5 \] The pH of a solution of 1.0 g of Heparin Sodium in 100 mL of water is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Heparin Sodium in 20 mL of water: the solution is clear and colorless to light yellow.

(2) Barium—Dissolve 0.03 g of Heparin Sodium in 3.0 mL of water, and use this solution as the sample solution. To 1.0 mL of the sample solution add 3 drops of dilute sulfuric acid, and allow to stand for 10 minutes: no turbidity is produced.

(3) Total nitrogen—Weigh accurately about 0.1 g of Heparin Sodium, previously dried at 60°C for 3 hours under reduced pressure, and perform the test as directed under Nitrogen Determination <1.085> to determine the amount of nitrogen (N: 14.01) not more than 3.0%.

(4) Protein—To 1.0 mL of the sample solution obtained in (2) add 5 drops of a solution of trichloroacetic acid (1 in 5): neither a precipitate nor turbidity is produced.

(5) Over-sulfated chondroitin sulfate—Dissolve 20 mg of Heparin Sodium in 0.60 mL of a solution of sodium 3-trimethylsilylpropionate-d₄ for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). To the solution of Heparin Sodium RS for physicochemical test add 0.2 mL of the solution of Over-sulfated Chondroitin Sulfate RS. When determining the spectrum of this solution under the above operating conditions, it exhibits the signal of N-acetyl proton of heparin and the signal of N-acetyl proton of over-sulfated chondroitin sulfate at δ 2.04 ± 0.02 ppm and δ 2.15 ± 0.02 ppm, respectively.

(6) Related substances—Dissolve 2.0 mg of Heparin Sodium in 0.1 mL of water and perform the test with exactly 20 μL of this solution as directed under Liquid Chromatography <2.01> according to the following conditions: it exhibits no peaks after the heparin peak.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 202 nm).
Column: A stainless steel column 2.0 mm in inside diameter and 7.5 cm in length, packed with diethylaminoethyl group bound to synthetic polymer for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase A: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10).
Mobile phase B: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate and 106.4 g of lithium perchlorate in 1000 mL of water, and adjust to a pH of 3.0 with diluted phosphoric acid (1 in 10).
Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

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</tr>
<tr>
<td>3 – 15</td>
<td>90 → 0</td>
<td>10 → 100</td>
</tr>
</tbody>
</table>

Flow rate: 0.2 mL per minute.

Time span of measurement: About 2 times as long as the retention time of heparin, beginning after the solvent peak.

System suitability—
Test for required detectability: Dissolve 10 mg of Heparin Sodium RS for physicochemical test in 0.40 mL of water, and use this solution as the Heparin Sodium standard stock solution. Separately, dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water, and use this solution as the over-sulfated chondroitin sulfate standard solution. To 60 μL of the Heparin Sodium standard stock solution add 3 μL of the over-sulfated chondroitin sulfate standard solution and 12 μL of water, and mix. When the procedure is run with 20 μL of the mixture under the above operating conditions, it exhibits a peak for over-sulfated chondroitin sulfate.

System performance: To 120 μL of the Heparin Sodium standard stock solution add 30 μL of the over-sulfated chondroitin sulfate standard solution, mix and use this solution as the solution for system suitability test. When the proce-
dure is run with 20 \mu L of the solution for system suitability test under the above operating conditions, heparin and oversulfated chondroitin sulfate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 \mu L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of over-sulfated chondroitin sulfate is not more than 2.0%.

(7) Galactosamine—Dissolve 2.4 mg of Heparin Sodium in 1.0 mL of water and hydrochloric acid (7:5), and use this solution as the Heparin Sodium stock solution. Dissolve 8.0 mg of D-glucosamine hydrochloride in water and hydrochloric acid (7:5) to make exactly 10 mL. Dissolve 8.0 mg of D-glucosamine hydrochloride in water and hydrochloric acid (7:5) to make exactly 10 mL. To 99 volumes of the solution of D-glucosamine add 1 volume of the solution of D-galactosamine, and use this solution as the standard stock solution. Transfer 500 \mu L each of Heparin Sodium stock solution and the standard stock solution to a glass-stoppered test tube, stopper tightly, and heat at 100°C for 6 hours. After cooling to room temperature, evaporate 100 \mu L of the reaction solutions to dryness. Add 50 \mu L of methanol to each of the residues and evaporate to dryness at room temperature. Dissolve each of the residues in 10 \mu L of water, add 40 \mu L of ethanol to each of the reaction solutions to dryness. Add 50 \mu L of methanol to each of the residues and evaporate to dryness at room temperature. Dissolve each of the residues in 10 \mu L of water, add 40 \mu L of ethanol to each of the residues, shake vigorously, and then centrifuge. After removing the upper layer, add 200 \mu L of ethyl acetate to the lower layer, shake vigorously, and then centrifuge. The lower layer is used as the standard solution for system suitability test. When the procedure is run with 5 \mu L of the solution for system suitability test under the above operating conditions, the ratio of the peak area of galactosamine to that of glucosamine is 0.7 – 2.0%.

System performance: When the procedure is run with 5 \mu L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratio of the peak area of galactosamine to that of glucosamine is not more than 4.0%.

**Loss on drying**<2.41> Not more than 10% (20 mg, in vacuum, 60°C, 3 hours).

**Residue on ignition**<2.44> Not more than 40% (after drying, 20 mg).

**Pyrogen**<4.04> Dissolve Heparin Sodium in isotonic sodium chloride solution so as to contain 1000 Units per mL according to the labeled Units. Inject into rabbits 2 mL of this solution per kg; it meets the requirements.

**Assay**

(i) Substrate solution: Dissolve 15 mg of N-benzoyl-L-isoleucyl-L-glutamyl(g-OR)-glycyl-L-arginylnitroanilide hydrochloride in 20 mL of water.

(ii) Anti-thrombin III solution: Dissolve human anti-thrombin III in water to make a solution containing 1 Unit per mL.

(iii) Activated blood coagulation factor X solution: Dissolve bovine activated blood coagulation factor X in water to make a solution containing 0.426 Units per mL.

(iv) Buffer solution: Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 750 mL of water, adjust the pH to 8.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

(v) Reaction stop solution: To 20 mL of acetic acid (100) add water to make 40 mL.

(vi) Heparin standard solutions: Dissolve Heparin Sodium RS in isotonic sodium chloride solution to make a solution containing 10 Units per mL, and use as the standard stock solution. To 100 \mu L of the standard stock solution add the buffer solution to make exactly 5 mL, and use this solution as the standard solution. Prepare the heparin standard solutions (1), (2), (3), (4) and (5) by addition of anti-thrombin III solution, human normal plasma and the buffer solution to the standard solution as directed in the following table.
Heparin Sodium Injection

ヘパリンナトリウム注射液

Heparin Sodium Injection is an aqueous solution for injection. It contains not less than 90% and not more than 110% of the labeled heparin Units. Label the name of organ used as the starting material of Heparin Sodium supplied for preparing Heparin Sodium Injection.

**Method of preparation** Dissolve Heparin Sodium in Isotonic Sodium Chloride Solution and prepare as directed under Injections.

**Description** Heparin Sodium Injection is a clear, colorless to light yellow liquid.

**pH** $<2.54 \approx 5.5 - 8.0$

**Purity** (1) Barium—Measure exactly a portion of Heparin Sodium Injection, equivalent to 3000 Units of Heparin Sodium according to the labeled Unit. Add water to make 3.0 mL and use this solution as the sample solution. To 1.0 mL of the sample solution add 3 drops of dilute sulfuric acid, and allow to stand for 10 minutes: no turbidity is produced.

(2) Protein—Proceed as directed in the Purity (4) under Heparin Sodium.

**Bacterial endotoxins** $<4.01 \approx$ Less than 0.0030 EU/unit.

**Extractable volume** $<6.05 \approx$ It meets the requirement.

**Foreign insoluble matter** $<6.06 \approx$ Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** $<6.07 \approx$ It meets the requirement.

**Sterility** $<4.06 \approx$ Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Proceed as directed in the Assay under Heparin Sodium, replacing the sample solution indicated in (vii) and the calculation in (ix) with the following:

Sample solution: Measure exactly an adequate portion of Heparin Sodium Injection according to the labeled Units, dilute it with isotonic sodium chloride solution so that each mL contains about 0.5 Units. To 100 $\mu$L of this solution add 100 $\mu$L of human normal plasma and 700 $\mu$L of the buffer solution, and use this solution as the sample solution.

Calculation: Plot the absorbances of the standard solutions on the vertical axis and their heparin concentrations on the horizontal axis to prepare a calibration curve. Determine the heparin concentration, $C$, of the sample solution from its absorbance by using the curve, and calculate heparin Units per mL of Heparin Sodium Injection from the following formula:

\[
\text{Units per mL of Heparin Sodium Injection} = C \times 10 \times \frac{b}{a}
\]

\(a\): Amount of sample (mL)
\(b\): Total volume (mL) of isotonic sodium chloride solution
used to dilute the sample to make the solution containing about 0.5 Units per mL.

<table>
<thead>
<tr>
<th>Heparin standard solution</th>
<th>Buffer solution ($\mu$L)</th>
<th>Anti-thrombin III solution ($\mu$L)</th>
<th>Human normal plasma ($\mu$L)</th>
<th>Standard solution ($\mu$L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Heparin concentration (Unit/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1)</td>
<td>0</td>
<td>800</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(2)</td>
<td>0.02</td>
<td>700</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(3)</td>
<td>0.04</td>
<td>600</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>(4)</td>
<td>0.06</td>
<td>500</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>(5)</td>
<td>0.08</td>
<td>400</td>
<td>100</td>
<td>400</td>
</tr>
</tbody>
</table>

Containers and storage Containers—Tight containers.

**Containers and storage** Containers—Hermetic containers. Storage—Light-resistant.

**Containers and storage** Containers—Tight containers.
L-Histidine

L-ヒスチジン

C₆H₉N₃O₂: 155.15
(2S)-2-Amino-3-(1H-imidazol-4-yl)propanoic acid [71-00-1]

L-Histidine contains not less than 99.0% and not more than 101.0% of C₆H₉N₃O₂, calculated on the dried basis.

**Description** L-Histidine occurs as white crystals or a white crystalline powder, having a slight bitter taste.

It is freely soluble in formic acid, and soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 6 mol/L hydrochloric acid TS.

**Identification** Determine the infrared absorption spectrum of L-Histidine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample with a little amount of water, evaporate the numbers. If any difference appears between the spectra, dissolve the sample with a little amount of water, evaporate the numbers.

**Optical rotation** <2.49> [α]D: +11.8° – +12.8° (5.5 g, calculated on the dried basis, 6 mol/L hydrochloric acid TS, 50 mL, 100 mm).

**pH** <2.54> The pH of a solution of 1.0 g of L-Histidine in 50 mL of water is between 7.0 and 8.5.

**Purity** (1) Clarity and color of solution—A solution of 0.40 g of L-Histidine in 20 mL of water is clear and colorless.

(2) Chloride <1.07>—Perform the test with 0.5 g of L-Histidine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.02%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Histidine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Histidine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Dissolve 1.0 g of L-Histidine in 30 mL of water by warming. To this solution add 2.4 mL of 0.01 mol/L hydrochloric acid, 2 mL of 6 mol/L acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 1.0 mL of Standard Lead Solution add 2 mL of 6 mol/L acetic acid and water to make 50 mL (not more than 10 ppm).

(6) Iron <1.10>—Prepare the test solution with 1.0 g of L-Histidine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Histidine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution.

Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of L-propanol and ammonia solution (28) (67:33) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) to the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.3% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.15 g of L-Histidine, dissolve in 2 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 15.52 mg of C₆H₉N₃O₂.

**Containers and storage** Containers—Tight containers.

L-Histidine Hydrochloride Hydrate

L-ヒスチジン塩酸塩水和物

C₆H₉N₃O₂·HCl·H₂O: 209.63
(2S)-2-Amino-3-(1H-imidazol-4-yl)propanoic acid monohydrochloride monohydrate [5934-29-2]

L-Histidine Hydrochloride Hydrate contains not less than 99.0% and not more than 101.0% of L-histidine hydrochloride (C₆H₉N₃O₂·HCl: 191.62), calculated on the anhydrous basis.

**Description** L-Histidine Hydrochloride Hydrate occurs as white crystals or a white crystalline powder. It has an acid taste at first, and a slight bitter taste later.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (99.5).

It dissolves in 6 mol/L hydrochloric acid TS.

**Identification** (1) Determine the infrared absorption spectrum of L-Histidine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of L-Histidine Hydrochloride Hydrate (1 in 10) responds to the Qualitative Tests <1.08> for chloride.

**Optical rotation** <2.49> [α]D: +9.2° – +10.6° (5.5 g, calculated on the anhydrous basis, 6 mol/L hydrochloric acid TS, 50 mL, 100 mm).

**pH** <2.54> The pH of a solution of 1.0 g of L-Histidine Hy-
**Homatropine Hydrobromide**

ホマトロピン臭化水素酸塩

\[
\text{C}_{16}\text{H}_{21}\text{NO}_3\cdot\text{HBr}: 356.25 \\
(1R,3r,5S)-8-\text{Methyl}-8-azabicyclo[3.2.1]oct-3-yl \\
(2RS)-2-\text{hydroxy}-2-\text{phenylacetate monohydrobromide} \\
[31.56-9]
\]

Homatropine Hydrobromide contains not less than 99.0% of C\textsubscript{16}H\textsubscript{21}NO\textsubscript{3}.HBr, calculated on the dried basis.

**Description** Homatropine Hydrobromide occurs as white crystals or crystalline powder. It is odorless. It is freely soluble in water, sparingly soluble in ethanol (95), slightly soluble in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is affected by light.

Melting point: about 214°C (with decomposition).

**Identification**
1. To 5 mL of a solution of Homatropine Hydrobromide (1 in 20) add 2 to 3 drops of iodine TS: a brown precipitate is produced.
2. Dissolve 0.05 g of Homatropine Hydrobromide in 5 mL of water, and add 3 mL of 2,4,6-trinitrophenol TS: a yellow precipitate is produced. Filter the precipitate, wash with five 10-mL portions of water, and dry at 105°C for 2 hours: it melts <2.60°C between 184°C and 187°C.
3. A solution of Homatropine Hydrobromide (1 in 20) responds to the Qualitative Tests <1.09> for bromide.

**Purity**
1. Acidity—Dissolve 1.0 g of Homatropine Hydrobromide in 20 mL of water, and add 0.40 mL of 0.01 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.
2. Atropine, hyoscyamine and scopolamine—To 10 mg of Homatropine Hydrobromide add 5 drops of nitric acid, evaporate on a water bath to dryness, and cool. Dissolve the residue in 1 mL of N,N-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: no red-purple color is produced.
3. Related substances—Dissolve 0.15 g of Homatropine Hydrobromide in 3 mL of water, and use this solution as the sample solution.
   (i) To 1 mL of the sample solution add 2 to 3 drops of tannic acid TS: no precipitate is produced.
   (ii) To 1 mL of the sample solution add 2 to 3 drops each of dilute hydrochloric acid and platinic chloride TS: no precipitate is produced.

**Loss on drying** <2.41> Not more than 1.5% (0.5 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.2% (0.2 g).

**Assay** Dissolve by warming about 0.4 g of Homatropine Hydrobromide in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration).

Each mL of 0.1 mol/L perchloric acid VS = 9.581 mg of C\textsubscript{16}H\textsubscript{21}N\textsubscript{2}O\textsubscript{3}HCl

**Water** <2.48> 7.2 – 10.0% (0.12 g, volumetric titration, direct titration, using a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method.).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Containers and storage** Containers—Tight containers.
mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 35.63 mg of C₁₉H₂₃ClN₂·2HCl·HBr

Containers and storage  Containers—Tight containers.
Storage—Light-resistant.

Homochlorcyclizine Hydrochloride

ホモクロルシクリジン塩酸塩

\[
\text{C}_{19}\text{H}_{23}\text{ClN}_2\cdot2\text{HCl} : 387.77
\]

1-\{(\text{RS})-(4\text{-Chlorophenyl})(\text{phenyl})\text{methyl}\}-4\text{-methylhexahydro-1H-1,4-diazepine dihydrochloride} [1982-36-1]

Homochlorcyclizine Hydrochloride, when dried, contains not less than 98.0% of C₁₉H₂₃ClN₂·2HCl.

Description  Homochlorcyclizine Hydrochloride occurs as white to pale brown, crystals or powder.
It is very soluble in water, freely soluble in acetic acid (100), slightly soluble in ethanol (99.5), and very slightly soluble in acetonitrile and in acetic anhydride.
It dissolves in 0.1 mol/L hydrochloric acid TS.
It is hygroscopic.
It is colored slightly by light.
A solution of Homochlorcyclizine Hydrochloride (1 in 10) shows no optical rotation.
Melting point: about 227°C (with decomposition).

Identification (1)  Determine the absorption spectrum of a solution of Homochlorcyclizine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultra-violet-visible Spectrophotometry <2.07>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave lengths.
(2)  Determine the infrared absorption spectrum of Homochlorcyclizine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
(3)  A solution of Homochlorcyclizine Hydrochloride (1 in 100) responds to the Qualitative Tests <1.05> for chloride.

Purity (1)  Heavy metals <1.07>—Proceed with 1.0 g of Homochlorcyclizine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(2)  Related substances—Dissolve 0.10 g of Homochlorcyclizine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas by the automatic integration method: the areas of the peaks other than homochlorcyclizine obtained from the sample solution are not larger than 1/2 times the peak area of homochlorcyclizine from the standard solution, and the total area of the peaks other than homochlorcyclizine is not larger than the peak area of homochlorcyclizine from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 223 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of water, acetonitrile and perchloric acid (134:66:1).
Flow rate: Adjust the flow rate so that the retention time of homochlorcyclizine is about 10 minutes.
Time span of measurement: About 2 times as long as the retention time of homochlorcyclizine.

System suitability—
Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 50 mL. Confirm that the peak area of homochlorcyclizine obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.
System performance: Dissolve 5 mg each of Homochlorcyclizine Hydrochloride and methyl parahydroxybenzoic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoic acid and homochlorcyclizine are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of homochlorcyclizine is not more than 1.0%.

Loss on drying <2.41>  Not more than 2.0% (1 g, 110°C, 4 hours).

Residue on ignition <2.44>  Not more than 0.2% (1 g).

Assay  Weigh accurately about 0.3 g of Homochlorcyclizine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 19.39 mg of C₁₉H₂₃ClN₂·2HCl

Containers and storage  Containers—Tight containers.
Storage—Light-resistant.
**Hydralazine Hydrochloride**

Hydralazine Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 275°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Hydralazine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**pH** <2.54> Dissolve 1.0 g of Hydralazine Hydrochloride in 50 mL of water: the pH of the solution is between 3.5 and 4.5.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Hydralazine Hydrochloride in 50 mL of water: the solution is clear, and colorless or pale yellow.

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.15 g of Hydralazine Hydrochloride, previously dried, transfer it to a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, cool to room temperature, and titrate <2.50> with 0.05 mol/L potassium iodate VS while shaking until the purple color of the chloroform layer disappears. The end point is reached when the red-purple color no more reappears in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS = 9.832 mg of C₈H₈N₄.HCl

**Containers and storage** Containers—Tight containers.

**Hydralazine Hydrochloride for Injection**

Hydralazine Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 99.0% and not more than 113.0% of the labeled amount of hydralazine hydrochloride (C₈H₈N₄.HCl: 196.64).

**Method of preparation** Prepare as directed under Injection, with Hydralazine Hydrochloride.

**Description** Hydralazine Hydrochloride for Injection occurs as a white to pale yellow powder or mass. It is odorless, and has a bitter taste.

**Identification** Determine the absorption spectrum of a solution of Hydralazine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm, and between 313 nm and 317 nm.

**pH** <2.54> Dissolve 1.0 g of Hydralazine Hydrochloride for Injection in 50 mL of water: the pH of this solution is between 3.5 and 4.5.

**Bacterial endotoxins** <4.01> Less than 5.0 EU/mg.

**Uniformity of dosage units** <6.02> It meets the requirement of the Mass variation test. (T: 106.0%)

**Foreign insoluble matter** <5.66> Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Weigh accurately the contents of not less than 10 samples of Hydralazine Hydrochloride for Injection. Weigh accurately about 0.15 g of the contents, transfer it to a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, cool to room temperature, and proceed as
Containers—Tight containers.

Containers and storage Containers—Hermetic containers.

Hydralazine Hydrochloride Powder

ヒドララジン塩酸塩

Hydralazine Hydrochloride Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of hydralazine hydrochloride (C₈H₈N₄.HCl: 196.64).

Method of preparation Prepare as directed under Granules or Powders, with Hydralazine Hydrochloride.

Identification Weigh a quantity of powdered Hydralazine Hydrochloride Powder, equivalent to about 0.15 g of hydralazine hydrochloride (C₈H₈N₄.HCl), transfer it to a glass-stoppered flask, and proceed as directed in the Assay under Hydralazine Hydrochloride.

Assay Weigh accurately a portion of Hydralazine Hydrochloride Powder, equivalent to about 0.15 g of hydralazine hydrochloride, transfer it to a glass-stoppered flask, add 100 mL of water, shake well, and filter, if necessary. Add water to 2 mL of the filtrate to make 50 mL and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm, and between 313 nm and 317 nm.

Each mL of 0.05 mol/L potassium iodate VS = 9.832 mg of C₈H₈N₄.HCl

Containers and storage Containers—Tight containers.

Hydralazine Hydrochloride Tablets

ヒドララジン塩酸塩錠

Hydralazine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of hydralazine hydrochloride (C₈H₈N₄.HCl: 196.64).

Method of preparation Prepare as directed under Tablets, with Hydralazine Hydrochloride.

Identification Weigh a quantity of powdered Hydralazine Hydrochloride Tablets, equivalent to 25 mg of Hydralazine Hydrochloride according to the labeled amount, add 100 mL of water, mix well, and filter if necessary. To 2 mL of this solution add water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm, and between 313 nm and 317 nm.

Uniformity of dosage units 6.02 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Hydralazine Hydrochloride Tablets add 25 mL of 0.1 mol/L hydrochloric acid TS, disperse the tablet into a small particles using ultrasonic waves, then shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 10 µg of hydralazine hydrochloride (C₈H₈N₄.HCl), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of hydralazine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 260 nm, A₁₁, and at 350 nm, A₁₂ and A₁₃, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24.

\[
M₂ = \frac{M_S \times (A_{T₁} - A_{T₂}) / (A_{S₁} - A_{S₂}) \times V' / V \times 1/50}{V} \]

M₂: Amount (mg) of hydralazine hydrochloride (C₈H₈N₄.HCl) for assay

Dissolution 6.10 When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Hydralazine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Hydralazine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 µm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V mL so that each mL contains about 11 µg of hydralazine hydrochloride (C₈H₈N₄.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of hydralazine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₂, of the sample solution and standard solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry 2.24.

\[
\text{Dissolution rate (%)} = \frac{M_S \times A_{T₁} / A_S \times V' / V \times 1/C \times 18}{A₁} \]

M₂: Amount (mg) of hydralazine hydrochloride for assay
C: Labeled amount (mg) of hydralazine hydrochloride (C₈H₈N₄.HCl) in 1 tablet

Assay Weigh accurately not less than 20 Hydralazine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of hydralazine hydrochloride (C₈H₈N₄.HCl), transfer it to a glass-stoppered flask, and proceed as directed in the Assay under Hydralazine Hydrochloride.

Each mL of 0.05 mol/L potassium iodate VS = 9.832 mg of C₈H₈N₄.HCl

Containers and storage Containers—Tight containers.
Hydrochloric Acid

塩酸

Hydrochloric Acid contains not less than 35.0% and not more than 38.0% of hydrogen chloride (HCl: 36.46).

Description Hydrochloric Acid is a colorless liquid having a pungent odor.

It is fuming but ceases to fume when it is diluted with 2 volumes of water.

Specific gravity $d_20^0$: about 1.18

Identification (1) Allow a glass stick wet with ammonia TS to come near the surface of Hydrochloric Acid: a remarkable white smoke evolves.

(2) A solution of Hydrochloric Acid (1 in 100) changes blue litmus paper to red, and responds to the Qualitative Tests $<2.44$ for chloride.

Purity (1) Sulfate $<1.14$—To 15 mL of Hydrochloric Acid add water to make 50 mL, and use this solution as the sample solution. To 3.0 mL of the sample solution add 5 mL of water and 5 drops of barium chloride TS, and allow to stand for 1 hour: no turbidity is produced.

(2) Sulfite—To 3.0 mL of the sample solution obtained in (1) add 5 mL of water and 1 drop of iodine TS: the color of iodine TS does not disappear.

(3) Bromide or iodide—Place 10 mL of the sample solution obtained in (1) in a glass-stoppered test tube, add 1 mL of chloroform and 1 drop of 0.002 mol/L potassium permanganate VS, and shake well: the chloroform layer remains colorless.

(4) Bromine or chlorine—Place 10 mL of the sample solution obtained in (1) in a glass-stoppered test tube, add 5 drops of potassium iodide TS and 1 mL of chloroform, and shake for 1 minute: the chloroform layer remains free from a purple color.

(5) Heavy metals $<1.07$—Evaporate 5 mL of Hydrochloric Acid on a water bath to dryness, and add 2 mL of dilute acetic acid and water to the residue to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 5 ppm).

(6) Arsenic $<1.17$—Prepare the test solution with 1.7 mL of Hydrochloric Acid according to Method 1, and perform the test (not more than 1 ppm).

(7) Mercury—Dilute 20 mL of Hydrochloric Acid with water to make exactly 100 mL, and use the solution as the sample solution. Perform the test with this sample solution as directed under Atomic Absorption Spectrophotometry $<2.23$ (cold vapor type). Place the sample solution in a sample bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the spectrophotometer, circulate air, and determine the absorbance $A_t$ of the solution obtained after the recorder reading has risen rapidly, and becomes constant at a wavelength of 253.7 nm. On the other hand, to 8 mL of Standard Mercury Solution add water to make exactly 100 mL, and determine the absorbance $A_s$ of the solution obtained by the same procedure as used for the sample solution: $A_t$ is smaller than $A_s$ (not more than 0.04 ppm).

Residue on ignition $<2.44$—Pipet 10 mL of Hydrochloric Acid, add 2 drops of sulfuric acid, evaporate to dryness, and ignite: not more than 1.0 mg of residue remains.

Assay Weigh accurately a glass-stoppered flask containing 20 mL of water, add about 3 mL of Hydrochloric Acid, and weigh accurately again. Dilute with 25 mL of water, and titrate $<2.50$ with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of methyl red TS).

Each mL of 1 mol/L sodium hydroxide VS $= 36.46$ mg of HCl

Containers and storage Containers—Tight containers.

Dilute Hydrochloric Acid

稀塩酸

Dilute Hydrochloric Acid contains not less than 9.5 w/v% and not more than 10.5 w/v% of hydrogen chloride (HCl: 36.46).

Description Dilute Hydrochloric Acid is a colorless liquid. It is odorless and has a strong acid taste.

Specific gravity $d_20^0$: about 1.05

Identification A solution of Dilute Hydrochloric Acid (1 in 30) changes blue litmus paper to red and responds to the Qualitative Tests $<1.09$ for chloride.

Purity (1) Sulfate—To 3.0 mL of Dilute Hydrochloric Acid add 5 mL of water and 5 drops of barium chloride TS, and allow to stand for 1 hour: no turbidity is produced.

(2) Sulfite—To 3.0 mL of Dilute Hydrochloric Acid add 5 mL of water and 1 drop of iodine TS: the color of iodine TS does not disappear.

(3) Bromide or iodide—Place 10 mL of Dilute Hydrochloric Acid in a glass-stoppered test tube, add 1 mL of chloroform and 1 drop of potassium permanganate VS, and shake well: the chloroform layer remains colorless.

(4) Bromine or chlorine—Place 10 mL of Dilute Hydrochloric Acid in a glass-stoppered test tube, add 5 drops of potassium iodide TS and 1 mL of chloroform, and shake for 1 minute: the chloroform layer remains free from a purple color.

(5) Heavy metals $<1.07$—Evaporate 9.5 mL of Dilute Hydrochloric Acid on a water bath to dryness, add 2 mL of dilute acetic acid and water to the residue to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 3 ppm).

(6) Arsenic $<1.17$—Prepare the test solution with 4.0 mL of Dilute Hydrochloric Acid according to Method 1, and perform the test (not more than 0.5 ppm).

(7) Mercury—Dilute 80 mL of Dilute Hydrochloric Acid with water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with this solution according to the Atomic Absorption Spectrophotometry $<2.23$ (cold vapor type). Place the sample solution in a sample bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle imme-
diately to the spectrophotometer, circulate air, and read the absorbance $A_T$ of the sample solution after the recorder reading has risen rapidly and become constant at a wavelength of 253.7 nm. On the other hand, to 8 mL of Standard Mercury Solution add water to make exactly 100 mL, and read the absorbance $A_S$ of the solution obtained by the same procedure as used for the sample solution: $A_T$ is smaller than $A_S$ (not more than 0.01 ppm).

**Residue on ignition** If 10 mL of Dilute Hydrochloric Acid, and dilute with 20 mL of water. Titrate with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of methyl red TS).

Each mL of 1 mol/L sodium hydroxide VS = 36.46 mg of HCl.

**Containers and storage** Containers—Tight containers.

### Hydrochloric Acid Lemonade

#### Method of preparation

- **Dilute Hydrochloric Acid** 5 mL
- **Simple Syrup** 80 mL
- **Purified Water or Purified Water in Containers** a sufficient quantity

To make 1000 mL

Prepare before use as directed under Lemonades, with the above ingredients.

**Description** Hydrochloric Acid Lemonade is a clear, colorless liquid. It has a sweet, cool, acid taste.

**Containers and storage** Containers—Tight containers.

### Hydrochlorothiazide

#### Method of preparation

C$_2$H$_8$ClN$_3$O$_4$S$_2$: 297.74

6-Chloro-3,4-dihydro-2H-1,2,4-benzo thiadiazine-7-sulfonamide 1,1-dioxide [58-93-5]

Hydrochlorothiazide, when dried, contains not less than 99.0% of C$_2$H$_8$ClN$_3$O$_4$S$_2$.

**Description** Hydrochlorothiazide occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetone, sparingly soluble in acetonitrile, very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: about 267°C (with decomposition).

**Identification (1)** To 5 mg of Hydrochlorothiazide add 5 mL of chloromotropic acid TS, and allow to stand for 5 minutes: a purple color develops.

(2) Fuse a mixture of 0.1 g of Hydrochlorothiazide and 0.5 g of sodium carbonate decahydrate cautiously: the gas evolved changes moistened red litmus paper to blue. After cooling, crush with a glass rod, add 10 mL of water, stir, and filter. To 4 mL of the filtrate add 2 drops of hydrogen peroxide (30), 5 mL of diluted hydrochloric acid (1 in 5) and 2 to 3 drops of barium chloride TS: a white precipitate is produced.

(3) To 4 mL of the filtrate obtained in (2) add 5 mL of dilute nitric acid and 3 drops of silver nitrate TS: a white precipitate is produced.

(4) Dissolve 12 mg of Hydrochlorothiazide in 100 mL of sodium hydroxide TS. Dilute 10 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Hydrochlorothiazide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)** Chloride $<$1.03$>$—Dissolve 1.0 g of Hydrochlorothiazide in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.036%).

(2) Sulfate $<$1.0$>$—Dissolve 1.0 g of Hydrochlorothiazide in 30 mL of acetone, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.005 mol/L sulfuric acid VS add 30 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals $<$1.0$>$—Proceed with 1.0 g of Hydrochlorothiazide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Primary aromatic amines—Dissolve 80 mg of Hydrochlorothiazide in acetone to make exactly 100 mL. Measure exactly 1 mL of the solution, add 3.0 mL of dilute hydrochloric acid, 3.0 mL of water and 0.15 mL of sodium nitrite TS, shake, and allow to stand for 1 minute. Shake this solution with 1.0 mL of ammonium amidosulfate TS, allow to stand for 3 minutes, then add 1.0 mL of N-(1-naphthyl)-N’-diethylthylethylenediamine oxalate TS, shake, and allow to stand for 5 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 1.0 mL of acetone in the same manner as the blank: the absorbance at 525 nm is not more than 0.10.

**Loss on drying** Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition** Not more than 0.1% (1 g).
Assay  Weigh accurately about 30 mg each of Hydrochlorothiazide and Hydrochlorothiazide RS, previously dried, and dissolve in 150 mL of the mobile phase, add exactly 10 mL each of the internal standard solution, then add the mobile phase to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q₂ and Q₅, of the peak area of hydrochlorothiazide to that of the internal standard.

\[ \text{Amount (mg) of } C₂H₃ClN₂O₆S₂ = M₅ \times \frac{Q₅}{Q₅} \]

**Internal standard solution**—A solution of 4-aminoacetophenone in acetonitrile (9 in 2000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of 0.1 mol/L sodium dihydrogen phosphate TS, pH 3.0 and acetonitrile (9:1).
Flow rate: Adjust the flow rate so that the retention time of hydrochlorothiazide is about 10 minutes.

**System suitability**—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, hydrochlorothiazide and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrochlorothiazide to that of the internal standard is not more than 1.0%.

**Containers and storage**  Containers—Well-closed containers.

**Hydrocortisone**

C₂₁H₃₀O₅ · 3H₂O₂: 362.46
11β,17,21-Trihydroxypregn-4-ene-3,20-dione [30-23-7]

Hydrocortisone, when dried, contains not less than 97.0% and not more than 102.0% of C₂₁H₃₀O₅.

**Description**  Hydrocortisone occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol, in ethanol (95) and in 1,4-dioxane, slightly soluble in chloroform, and very slightly soluble in diethyl ether and in water.

Melting point: 212 – 220°C (with decomposition).

**Identification**  (1) Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone: the solution shows a yellow-green fluorescence immediately, and the color of the solution changes gradually from orange to dark red. Dilute carefully the solution with 10 mL of water: the color changes through yellow to orange-yellow with green fluorescence, and a small amount of a flocculent precipitate is formed.

(2) Dissolve 0.01 g of Hydrocortisone in 1 mL of methanol, add 1 mL of Fehling’s TS, and heat: a red precipitate is formed.

(3) Determine the infrared absorption spectrum of Hydrocortisone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Hydrocortisone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone and Hydrocortisone RS in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

**Optical rotation** <2.49>  \([\alpha]^{25}_D: +150 – +156^\circ\) (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

**Purity**  Related substances—Dissolve 20 mg of Hydrocortisone in 10 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (17:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41>  Not more than 1.0% (0.5 g, 105°C, 3 hours).

**Residue on ignition** <2.44>  Not more than 0.1% (0.5 g).

**Assay**  Dissolve about 20 mg each of Hydrocortisone and Hydrocortisone RS, previously dried and accurately weighed, in 20 mL each of a mixture of chloroform and methanol (9:1), add 10 mL each of the internal standard solution, then add a mixture of chloroform and methanol (9:1) to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 µL each of these solutions as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q₂ and Q₅, of the peak area of hydrocortisone to that of the internal standard, respectively.

\[ \text{Amount (mg) of } C₂₁H₃₀O₅ = M₅ \times \frac{Q₅}{Q₅} \]

**Internal standard solution**—A solution of prednisone in a
Hydrocortisone Acetate

**Description**
Hydrocortisone Acetate occurs as white crystals or crystalline powder. It is odorless.

It is sparingly soluble in 1,4-dioxane, slightly soluble in methanol, in ethanol (95) and in chloroform, very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 220°C (with decomposition).

**Identification (1)**
Add 2 mL of sulfuric acid to 2 mL of Hydrocortisone Acetate: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown, flocculent precipitate is formed.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of chloroform, methanol and acetic acid (1000:20:1).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone is about 15 minutes.

**System suitability—**
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and hydrocortisone are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone to that of the internal standard is not more than 1.0%.

**Containers and storage**
Containers—Tight containers.

**Hydrocortisone Acetate**

**Description**

Hydrocortisone Acetate, when dried, contains not less than 97.0% and not more than 102.0% of C_{23}H_{32}O_6.

**Identification (1)**
Add 2 mL of sulfuric acid to 2 mL of Hydrocortisone Acetate: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown, flocculent precipitate is formed.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of chloroform, methanol and acetic acid (1000:20:1).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone is about 15 minutes.

**System suitability—**
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and hydrocortisone are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone to that of the internal standard is not more than 1.0%.

**Containers and storage**
Containers—Tight containers.

**Hydrocortisone Acetate**

**Description**

Hydrocortisone Acetate, when dried, contains not less than 97.0% and not more than 102.0% of C_{23}H_{32}O_6.
Mobile phase: A mixture of water and acetonitrile (13:7). Flow rate: Adjust the flow rate so that the retention time of hydrocortisone acetate is about 8 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, hydrocortisone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone acetate to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Tight containers. Storage—Light-resistant.

**Hydrocortisone and Diphenhydramine Ointment**

**Method of preparation**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone Acetate</td>
<td>5 g</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>5 g</td>
</tr>
<tr>
<td>White Petrolatum</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Ointments, with the above ingredients.

**Description** Hydrocortisone and Diphenhydramine Ointment is white to pale yellow in color.

**Identification (1)** To 1 g of Hydrocortisone and Diphenhydramine Ointment add 10 mL of ethanol (95%), heat on a water bath for 5 minutes with occasional shaking, cool, and filter. Take 5 mL of the filtrate, distill off the ethanol, and to the residue add 2 mL of sulfuric acid: the solution shows a yellow-green fluorescence immediately and the color of the solution gradually changes through yellow to yellow-brown. Add carefully 10 mL of water to this solution: the color changes to yellow with green fluorescence, and a light yellow, flocculent precipitate is formed (hydrocortisone acetate).

(2) To 1 mL of the filtrate obtained in (1) add 5 mL of potassium hydrogen phthalate buffer solution, pH 4.6, and 2 mL of bromophenol blue TS, and add further 5 mL of chloroform. Shake well, and allow to stand: a yellow color develops in the chloroform layer (diphenhydramine).

(3) To 1 g of Hydrocortisone and Diphenhydramine Ointment add 5 mL of methanol, warm, and shake. After cooling, separate the methanol layer, and use this layer as the sample solution. Dissolve 10 mg each of hydrocortisone acetate and diphenhydramine in 10 mL each of methanol, and use these solutions as standard solutions (1) and (2). Perform the test with the sample solution and standard solutions (1) and (2) as directed under Thin-layer Chromatography <2.07>. Spot 5 μL of each of these solutions on a plate of silica gel with a complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diethyl ether (4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): two spots from the sample solution show the same Rf value as the corresponding spots from standard solutions (1) and (2).

**Containers and storage** Containers—Tight containers.

**Hydrocortisone Butyrate**

ヒドロコルチゾン酪酸エステル

C₂₅H₃₆O₆: 432.55
11β,17,21-Trihydroxypregn-4-ene-3,20-dione 17-butyanoate [13609-67-1]

Hydrocortisone Butyrate, when dried, contains not less than 96.0% and not more than 104.0% of C₂₅H₃₆O₆.

**Description** Hydrocortisone Butyrate occurs as a white powder. It is odorless.

It is freely soluble in tetrahydrofuran, in chloroform and in 1,2-dichloroethane, soluble in methanol, sparingly soluble in ethanol (99.5), slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 200°C (with decomposition).

**Identification (1)** Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone Butyrate: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light (main wavelength: 254 nm). Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown, flocculent precipitate is formed.

(2) Dissolve 0.01 g of Hydrocortisone Butyrate in 1 mL of methanol by warming, add 1 mL of Fehling’s TS, and heat: an orange to red precipitate is formed.

(3) To 50 mg of Hydrocortisone Butyrate add 2 mL of potassium hydroxide-ethanol TS, and heat on a water bath for 5 minutes. Cool, add 2 mL of diluted sulfuric acid (2 in 7), and boil gently for 1 minute: the odor of ethyl butyrate is perceptible.

(4) Determine the infrared absorption spectrum of Hydrocortisone Butyrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [α]D: +48 – +52° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Hydrocortisone Butyrate according to method 2, and per-
form the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 25 mg of Hydrocortisone Butyrate in 5 mL of tetrahydrofuran, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, add tetrahydrofuran to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\)\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (470:30:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly alkaline blue tetrazonium TS on the plate: the spots other than the principal spot from the sample solution are not more than two in number, and not more intense than those from the standard solution in color.

**Loss on drying \(<2.41\)** Not more than 1.0\% (1 g, 105°C, 3 hours).

**Residue on ignition \(<2.44\)** Not more than 0.1\% (1 g).

**Assay** Weigh accurately about 50 mg of Hydrocortisone Butyrate, previously dried, and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2 mL of this solution, and add ethanol (99.5) to make exactly 50 mL. Determine the absorbance \(A\) of this solution at the wavelength of maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24\)\).

\[
\text{Amount (mg) of } C_{25}H_{36}O_6 = A/375 \times 25,000
\]

**Containers and storage** Containers—Tight containers.

---

### Hydrocortisone Sodium Phosphate

**ヒドロコルチゾンリン酸エステルナトリウム**

\[
\text{C}_{21}\text{H}_{29}\text{Na}_2\text{O}_8\text{P} \quad 486.40
\]

Disodium 11\(β\),17,21-trihydroxypregn-4-ene-3,20-dione 21-phosphate

[6000-74-4]

Hydrocortisone Sodium Phosphate contains not less than 96.0\% and not more than 102.0\% of \(C_{21}H_{29}Na_2O_8P\), calculated on the anhydrous basis.

**Description** Hydrocortisone Sodium Phosphate occurs as a white to light yellow powder. It is odorless.

It is freely soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol (95\%), and practically insoluble in diethyl ether.

It is hygroscopic.

**Identification** (1) To 2 mg of Hydrocortisone Sodium Phosphate add 2 mL of sulfuric acid: a yellowish green fluorescence is exhibited initially, then gradually changes through orange-yellow to dark red. Examine the solution under ultraviolet light (main wavelength: 254 nm): an intense, light green fluorescence is exhibited. To this solution add carefully 10 mL of water: the color changes from yellow to orange-yellow with a light green fluorescence and a yellow-brown, flocculent floating substance is formed.

(2) Determine the infrared absorption spectrum of Hydrocortisone Sodium Phosphate as directed in the paste method under Infrared Spectrophotometry \(<2.25\)\), and compare the spectrum with the Reference Spectrum or the spectrum of Hydrocortisone Sodium Phosphate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Sodium Phosphate and Hydrocortisone Sodium Phosphate RS in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

(3) Moisten 1.0 g of Hydrocortisone Sodium Phosphate with a small quantity of sulfuric acid, and incinerate by gradual heating. After cooling, dissolve the residue in 10 mL of dilute nitric acid, and heat in a water bath for 30 minutes. After cooling, filter if necessary. This solution responds to the Qualitative Tests \(<1.09\)\) for sodium salt and for phosphate.

**Optical rotation** \(<2.49\) \([\alpha]_D^25\% +123 - +131^\circ\) (1 g, calculated on the anhydrous basis, phosphate buffer solution, pH 7.0, 100 mL, 100 mm).

**PH** \(<2.54\) Dissolve 1.0 g of Hydrocortisone Sodium Phosphate in 100 mL of water: the pH of this solution is between 7.5 and 9.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Hydrocortisone Sodium Phosphate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride \(<1.03\)—Dissolve 0.30 g of Hydrocortisone Sodium Phosphate in 20 mL of water, and add 6 mL of dilute nitric acid and water to make 100 mL. To 5 mL of this solution add water to make 30 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.600%).

(3) Heavy metals \(<1.07\)—Proceed with 0.5 g of Hydrocortisone Sodium Phosphate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 40 ppm).

(4) Arsenic \(<1.11\)—Prepare the test solution with 1.0 g of Hydrocortisone Sodium Phosphate according to Method 3, and perform the test (not more than 2 ppm).

(5) Free phosphoric acid—Weigh accurately about 0.25 g of Hydrocortisone Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Standard Phosphoric Acid Solution into separate 25-mL volumetric flasks, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-aminoo-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20 ± 1°C for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry \(<2.24\)\), using a solution prepared with 5 mL of water in the same manner as the blank. Determine the absorbances, \(A_T\) and \(A_S\), at 740 nm of the sample solution and Standard Phosphoric Acid Solution: the amount of free
phosphoric acid is not more than 1.0%.

Content (%) of free phosphoric acid (H₃PO₄)
= 1/M × A₁/A₃ × 258.0

M: Amount (mg) of Hydrocortisone Sodium Phosphate, calculated on the anhydrous basis

(6) Free hydrocortisone—Dissolve 25 mg of Hydrocortisone Sodium Phosphate in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh 25 mg of Hydrocortisone RS, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A₁ and A₃, of hydrocortisone from each solution: A₁ is not larger than A₃.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (7 µm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS, pH 2.6 and methanol (1:1).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone phosphate is about 10 minutes.

**System suitability—**
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the internal standard and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of hydrocortisone phosphate to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
filtrate with 1 mL of dilute hydrochloric acid, filter if necessary, then adjust the solution to a pH of about 6 with dilute ammonia TS (1 in 10), and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

(4) Determine the infrared absorption spectrum of the dried matter obtained in (1) as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Hydrocortisone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Sodium Succinate and Hydrocortisone Succinate RS in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

(5) Hydrocortisone Sodium Succinate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> [α]D 20 +135 – +145° (0.1 g, calculated on the dried basis, ethanol (95), 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Hydrocortisone Sodium Succinate in 10 mL of water: the solution is clear and colorless.

(2) Other steroids—Dissolve 25 mg of Hydrocortisone Sodium Succinate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of hydrocortisone in methanol to make exactly 10 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution (1). Pipet 6 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 3 µL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (99.5) and formic acid (150:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution (1) is not more intense than the spot from the standard solution (2). Any spot other than the principal spot and the above spot obtained from the sample solution is not more intense than the spot from the sample solution corresponding to the spot from the standard solution (1). Any spot other than the principal spot and the above spot obtained from the sample solution is not more intense than the spot from the standard solution (2).

Loss on drying <2.41> Not more than 2.0% (0.5 g, 105°C, 3 hours).

Assay Weigh accurately about 10 mg of Hydrocortisone Sodium Succinate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of Hydrocortisone Succinate RS, previously dried at 105°C for 3 hours, proceed in the same manner as directed for the sample solution, and use this solution as the standard solution. Determine the absorbances, A1 and A2, of the sample solution and standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of C25H34O8 NaO4 = M2 × A1/A2 × 1.048
M2: Amount (mg) of Hydrocortisone Succinate RS

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Hydrocortisone Succinate

ヒドロコルチゾンコハク酸エステル

C25H34O8: 462.53
11β,17,21-Trihydroxypregn-4-ene-3,20-dione
21-(hydrogen succinate)
[2203-97-6]

Hydrocortisone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of C25H34O8.

Description Hydrocortisone Succinate occurs as a white crystalline powder.
It is very soluble in methanol, freely soluble in ethanol (99.5), sparingly soluble in ethanol (95), and practically insoluble in water.

Identification (1) To 3 mg of Hydrocortisone Succinate add 2 mL of sulfuric acid: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Hydrocortisone Succinate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Hydrocortisone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Succinate and Hydrocortisone Succinate RS in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

Optical rotation <2.49> [α]D 20 +147 – +153° (after drying, 0.1 g, ethanol (99.5), 10 mL, 100 mm).

Purity Related substances—Dissolve 25 mg of Hydrocortisone Succinate in exactly 10 mL of methanol, and use this solution as the sample solution. Separately, dissolve 25 mg of hydrocortisone in exactly 10 mL of methanol. Pipet 1 mL of this solution, dilute with methanol to exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 3 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (99.5) and formic acid...
Perform the test with 10 mL, and use these solutions as the sample solution and the internal standard solution, then add methanol to make 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of dried, and dissolve in methanol to make exactly 50 mL.

Residue on ignition Not more than 2.0% (0.5 g, 105°C, 3 hours).

Assay Weigh accurately about 50 mg each of Hydrocortisone Succinate and Hydrocortisone Succinate RS, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography.

System suitability—When the procedure is run with 10 μL of the standard solution under the above operating conditions, hydrocortisone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability—When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone succinate to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers. Storage—Light-resistant.

Hydrocortisone Hydrochloride Hydrate

ヒドロコタルニン塩酸塩水和物

C₁₂H₁₅NO₃.HCl.H₂O: 275.73
4-Methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinoline monohydrochloride monohydrate [5985-55-7, anhydride]

Hydrocortisone Hydrochloride Hydrate, when dried, contains not less than 98.0% of hydrocortisone-hydrochloride (C₁₂H₁₅NO₃.HCl: 257.72).

Description Hydrocortisone Hydrochloride Hydrate occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in ethanol (95) and in acetic acid (100), and slightly soluble in acetic anhydride.

Identification (1) Determine the absorption spectrum of a solution of Hydrocortisone Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Hydrocortisone Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Hydrocortisone Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests (1.09) (2) for chloride.

pH (2.54) Dissolve 1.0 g of Hydrocortisone Hydrochloride Hydrate in 20 mL of water: the pH of the solution is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Hydrocortisone Hydrochloride Hydrate in 10 mL of water: the solution is clear, and when perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry (2.24), using water as the blank, the absorbance at 400 nm is not more than 0.17.

(2) Heavy metals (1.07)—Proceeds with 1.0 g of Hydrocortisone Hydrochloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Hydrocortisone Hydrochloride Hydrate in 10 mL of diluted ethanol (99.5) (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (99.5) (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as
directed under Thin-layer Chromatography <2.05>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia water (28) (20:20:3:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.44> Not more than 7.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Hydrocotarnine Hydrochloride Hydrate, previously dried. Dissolve in 50 mL Assay water. Take 5 mL of the solution. Evaporate 1 mL of 0.01 mol/L perchloric acid VS. Perform a blank determination. Each mL of 0.1 mol/L perchloric acid VS = 25.77 mg of C12H15NO3.HCl

Containers and storage Containers—Tight containers.

Hydrogenated Oil

硬化油

Hydrogenated Oil is the fat obtained by hydrogenation of fish oil or of other oils originating from animal or vegetable.

Description Hydrogenated Oil occurs as a white mass or powder and has a characteristic odor and a mild taste. It is freely soluble in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water. The oil obtained by hydrogenation of castor oil is slightly soluble in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

Acid value <1.17> Not more than 2.0.

Purity (1) Moisture and coloration—Hydrogenated Oil (5.0 g), melted by heating on a water bath, forms a clear liquid, from which no water separates. In a 10-mm thick layer of the liquid, it is colorless or slightly yellow.

(2) Alkalinity—To 2.0 g of Hydrogenated Oil add 10 mL of water, melt by heating on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: no color develops.

(3) Chloride—To 1.5 g of Hydrogenated Oil add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 5): the turbidity of the solution does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, then add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50).

(4) Heavy metals—Heat 2.0 g of Hydrogenated Oil with 5 mL of dilute hydrochloric acid and 10 mL of water on a water bath for 5 minutes with occasional shaking. After cooling, filter, and make 5 mL of the filtrate weakly alkaline with ammonia TS, then add 3 drops of sodium sulfide TS: the solution remains unchanged.

(5) Nickel—Place 5.0 g of Hydrogenated Oil in a quartz or porcelain crucible, heat slightly with caution at the beginning, and, after carbonization, incinerate by strong heating (500 ± 20°C). Cool, add 1 mL of hydrochloric acid, evaporate on a water bath to dryness, dissolve the residue in 3 mL of dilute hydrochloric acid, and add 7 mL of water. Then add 1 mL of bromine TS and 1 mL of a solution of citric acid monohydrate (1 in 5), make alkaline with 5 mL of ammonia TS, and cool in running water. To this solution add 1 mL of dimethylglyoxime TS, add water to make 20 mL, and use this solution as the test solution. Allow to stand for 5 minutes: the solution has no more color than the following control solution.

Control solution: Evaporate 1 mL of hydrochloric acid on a water bath to dryness, add 1 mL of Standard Nickel Solution and 3 mL of dilute hydrochloric acid, and add 6 mL of water. Then proceed as directed in the test solution, add water to make 20 mL, and allow to stand for 5 minutes.

Residue on ignition <2.44> Not more than 0.1% (5 g).

Containers and storage Containers—Well-closed containers.

Hydrophilic Cream

親水クリーム

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Petrolatum</td>
<td>250 g</td>
</tr>
<tr>
<td>Stearyl Alcohol</td>
<td>200 g</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>120 g</td>
</tr>
<tr>
<td>Polyoxylethylene hydrogenated castor oil 60</td>
<td>40 g</td>
</tr>
<tr>
<td>Glycerin Monostearate</td>
<td>10 g</td>
</tr>
<tr>
<td>Methyl Parahydroxybenzoate</td>
<td>1 g</td>
</tr>
<tr>
<td>Propyl Parahydroxybenzoate</td>
<td>1 g</td>
</tr>
<tr>
<td>Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Melt White Petrolatum, Stearyl Alcohol, polyoxylethylene hydrogenated castor oil 60 and Glycerin Monostearate by heating on a water bath, stir, and keep temperature of the mixture at about 75°C. To Propylene Glycol add Methyl Parahydroxybenzoate and Propyl Parahydroxybenzoate, melt by warming if necessary, dissolve in Purified Water or Purified Water in Containers, and warm to about 75°C. Add this solution to the above mixture, stir to form emulsion, cool, and stir thoroughly until it congeals.

Description Hydrophilic Cream is white in color. It has a slight, characteristic odor.

Containers and storage Containers—Tight containers.
Hydroxocobalamin Acetate

ヒドロキシコバラミン酢酸塩

\[
\text{C}_{62}\text{H}_{89}\text{CoN}_{13}\text{O}_{15}\text{P.C}_{2}\text{H}_{4}\text{O}_{2}: 1406.41
\]

\[
\text{Coo-}[\{(5,6-\text{Dimethyl-1H-benzoimidazol-1-yl})-\text{Coβ}}
\]

hydroxocobamide monoacetate [13422-51-0, Hydroxocobalamin]

Hydroxocobalamin Acetate contains not less than 95.0% of \(\text{C}_{62}\text{H}_{89}\text{CoN}_{13}\text{O}_{15}\text{P.C}_{2}\text{H}_{4}\text{O}_{2}\), calculated on the dried basis.

**Description**

Hydroxocobalamin Acetate occurs as dark red crystals or powder. It is odorless. It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether. It is hygroscopic.

**Identification (1)**

Determine the absorption spectrum of a solution of Hydroxocobalamin Acetate in acetic acid-sodium acetate buffer solution, pH 4.5 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \(\leq 2.4\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Hydroxocobalamin Acetate with 50 mg of potassium hydrogen sulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, and add dropwise sodium hydroxide TS until the solution is not more intense than the spot from the standard solution.

(3) Add 0.5 mL of ethanol (99.5) and 1 mL of sulfuric acid to 20 mg of Hydroxocobalamin Acetate, and heat the mixture: the odor of ethyl acetate is perceptible.

Purity

Cyanocobalamin and colored impurities—Dissolve 50 mg of Hydroxocobalamin Acetate in exactly 5 mL each of acetic acid-sodium acetate buffer solution, pH 5.0, in two tubes. To one tube add 0.15 mL of potassium thiocyanate solution, allow to stand for 30 minutes, and use this solution as the sample solution (1). To the other tube add 0.10 mL of potassium cyanide TS, allow to stand for 30 minutes, and use this solution as the sample solution (2). Separately, dissolve 3.0 mg of Cyanocobalamin RS in exactly 10 mL of acetic acid-sodium acetate buffer solution, pH 5.0, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(\leq 2.0\).

Apply 20 \(\mu\)L each of the sample solution and standard solution 25 mm in length along the starting line, 10 mm apart from each other, on a plate of silica gel for thin-layer chromatography. Develop the plate for 18 hours with 2-butanol saturated with water, while supporting the plate at an angle of about 15° to a horizontal plane, and air-dry the plate: the spot from the sample solution (1) corresponding to that from the standard solution is not more intense than the spot from the standard solution, and the spots other than the principal spot from the sample solution (2) are not more intense than the spot from the standard solution.

**Assay**

Weigh accurately about 20 mg of Hydroxocobalamin Acetate, and dissolve in acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL. Pipet 2 mL of this solution into a 50-mL volumetric flask, add 1 mL of a solution of potassium cyanide (1 in 1000), and allow to stand for 30 minutes at ordinary temperature. Add acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Cyanocobalamin RS after determining the loss on drying in the same manner as for Cyanocobalamin, and dissolve in water to make exactly 50 mL. To 2 mL of this solution, exactly measured, add acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL, and use this solution as the sample solution. Determine the absorbances, \(A_1\) and \(A_2\), of the sample solution and standard solution at 361 nm as directed under Ultraviolet-visible Spectrophotometry \(\leq 2.4\).

\[
\text{Amount (mg) of } \text{C}_{62}\text{H}_{89}\text{CoN}_{13}\text{O}_{15}\text{P.C}_{2}\text{H}_{4}\text{O}_{2} = M_c \times \frac{A_1}{A_2} \times 1.038
\]

\(M_c\): Amount (mg) of Cyanocobalamin RS, calculated on the dried basis

Containers and storage

Containers—Tight containers. Storage—Light-resistant, and in a cold place.

**Hydroxypropylcellulose**

ヒドロキシプロピルセルロース

Hydroxypropylcellulose is a hydroxypropyl ether of cellulose.

Hydroxypropylcellulose, when dried, contains not less than 53.4% and not more than 77.5% of hydroxypropoxy group (-OC3H6OH: 75.09).

**Description**

Hydroxypropylcellulose occurs as a white to yellowish white powder.
It is practically insoluble in diethyl ether.
It forms a viscous liquid upon addition of water or ethanol (95).

**Identification (1)** To 1 g of Hydroxypropylcellulose add 100 mL of water, heat in a water bath at 70°C for 5 minutes with stirring, and cool while shaking. Allow to stand at room temperature until it becomes more homogeneous and viscous, and use this solution as the sample solution. To 2 mL of the sample solution add 1 mL of an anthrone TS gently: a white turbidity or precipitate disappears when cooled.

(2) Heat the sample solution obtained in (1): a white turbidity or precipitate is produced, and the turbidity or precipitate disappears when cooled.

(3) To 1 g of Hydroxypropylcellulose add 100 mL of ethanol (95), and allow to stand after stirring: a homogeneous and viscous liquid is produced.

**pH (2.54)** Dissolve 1.0 g of Hydroxypropylcellulose in 50 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.5.

**Purity (1)** Clarity of solution—Use an outer glass cylinder, 250 mm in height, 25 mm in internal diameter, 2 mm in thickness, with a high-quality glass plate 2 mm thick at the bottom, and inner glass cylinder, 300 mm in height, 15 mm in internal diameter, 2 mm in thickness, with a high-quality glass plate 2 mm thick at the bottom. In the outer cylinder place a solution prepared by adding 1.0 g of Hydroxypropylcellulose to 100 mL of water, heat while stirring in a water bath at 70°C, and then cool to room temperature. Place this cylinder on a sheet of white paper on which 15 parallel, black, 1-mm width lines are drawn at 1-mm intervals. Place the inner cylinder, and move it up and down while viewing downward through the bottom of the inner cylinder, and measure the minimum height of the solution between the bottom of the outer cylinder and the lower end of the inner cylinder at the time when the lines on the paper cannot be differentiated. The average value obtained from three repeated procedures is greater than that obtained from the following control solution treated in the same manner.

- Control solution: To 5.50 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid, 5 mL of ethanol (95) and water to make 50 mL. To this solution add 2 mL of barium chloride TS, mix, allow to stand for 10 minutes, and shake well before use.
- Chloride (1.05)—Add 1.0 g of Hydroxypropylcellulose to 30 mL of water, heat in a water bath with stirring for 30 minutes, and filter while being hot. Wash the residue with three 15-mL portions of hot water, combine the washings with the filtrate, and add water to make 100 mL after cooling. To 10 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142%).
- Sulfate (1.14)—To 40 mL of the sample solution obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).
- Heavy metals (1.07)—Proceed with 1.0 g of Hydroxypropylcellulose according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic (1.17)—Prepare the test solution with 1.0 g of Hydroxypropylcellulose according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying (2.4I)** Not more than 5.0% (1 g, 105°C, 4 hours).

**Residue on ignition (2.4G)** Not more than 0.5% (1 g).

**Assay (i)** Apparatus—Reaction flask: A 5-mL screw-cap pressure-tight glass bottle, having an inverted conical bottom inside, 20 mm in outside diameter, 50 mm in height up to the neck, 2 mL in capacity up to a height of about 30 mm, equipped with a pressure-tight septum of heat-resisting resin and also with an inside stopper or sealer of fluoroplastic.

- Heater: A square aluminum block 60 to 80 mm thick, having holes 20.6 mm in diameter and 32 mm in depth, capable of maintaining the inside temperature within ±1°C.

(ii) Procedure—Weigh accurately about 65 mg of Hydroxypropylcellulose, previously dried, transfer to the reaction flask, add 65 mg of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the flask tightly, and weigh accurately. Shake the flask for 30 seconds, heat at 150°C on the heater for 30 minutes with repeated shaking at 5-minute intervals, and continue heating for an additional 30 minutes. Allow the flask to cool, and again weigh accurately. If the mass loss is less than 10 mg, use the upper layer of the mixture as the sample solution. Separately, take 65 mg of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid in another reaction flask, stopper tightly, and weigh accurately. Add 50 μL of isopropyl iodide for assay, and again weigh accurately. Shake the reaction flask for 30 seconds, and use the upper layer of the content as the standard solution. Perform the test as directed under Gas Chromatography (2.0D) with 1 μL each of the sample solution and standard solution according to the following conditions, and calculate the ratios, \( Q_r \) and \( Q_s \), of the peak area of isopropyl iodide to that of the internal standard.

\[
\text{Amount (mg) of isopropyl iodide for assay} = M_r/M_f \times Q_r/Q_s \times 44.17
\]

\( M_f \): Amount (mg) of the sample
\( M_r \): Amount (mg) of the internal standard

**Operating conditions**—A solution of n-octane in o-xylene (4 in 100).

**Column:** A glass column about 3 mm in inside diameter and about 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μm in particle diameter, coated with methyl silicone polymer for gas chromatography at the ratio of 20%.

**Column temperature:** A constant temperature of about 100°C.

**Carrier gas:** Helium (for thermal-conductivity detector); Helium or Nitrogen (for hydrogen flame-ionization detector).

**Flow rate:** Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

**Selection of column:** Proceed with 1 μL of the standard solution under the above operating conditions. Use a column
Low Substituted Hydroxypropylcellulose

Low Substituted Hydroxypropylcellulose is a low substituted hydroxypropyl ether of cellulose. Low Substituted Hydroxypropylcellulose, when dried, contains not less than 5.0% and not more than 16.0% of hydroxypropoxy group (\(-\text{OC}_3\text{H}_7\text{O}-\): 75.09).

**Description** Low Substituted Hydroxypropylcellulose occurs as a white to yellowish white powder or granules. It is odorless or has a slight, characteristic odor. It is tasteless. It is practically insoluble in ethanol (95%) and in diethyl ether. It dissolves in a solution of sodium hydroxide (1 in 10), and produces a viscous solution. It swells in water, in sodium carbonate TS and in 2 mol/L hydrochloric acid TS.

**Identification**

(1) To 20 mg of Low Substituted Hydroxypropylcellulose add 2 mL of water, shake, and produce a turbid solution. Add 1 mL of anthrone TS gently: a blue to blue-green color develops at the zone of contact.

(2) To 0.1 g of Low Substituted Hydroxypropylcellulose add 10 mL of water, stir and produce a turbid solution. Add 1 g of sodium hydroxide, shake until it becomes homogeneous, and use this solution as the sample solution. To 0.1 mL of the sample solution add 9 mL of diluted sulfuric acid (9 in 10), shake well, heat in a water bath for exactly 3 minutes, immediately cool in an ice bath, add carefully 0.6 mL of ninhydrin TS, shake well, and allow to stand at 25°C: a red color develops at first, and it changes to purple within 100 minutes.

(3) To 5 mL of the sample solution obtained in (2) add 10 mL of a mixture of acetone and methanol (4:1), and shake: a white, flocculent precipitate is produced.

**pH** To 1.0 g of Low Substituted Hydroxypropylcellulose add 100 mL of freshly boiled and cooled water, and shake: the pH of the solution is between 5.0 and 7.5.

**Purity**

(1) Chloride—To 0.5 g of Low Substituted Hydroxypropylcellulose add 30 mL of hot water, stir well, heat on a water bath for 10 minutes, and filter the supernatant liquid by decantation while being hot. Wash the residue thoroughly with 50 mL of hot water, combine the washings with the filtrate, and add water to make 100 mL after cooling. To 5 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.35%).

(2) Heavy metals—Proceed with 2.0 g of Low Substituted Hydroxypropylcellulose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Low Substituted Hydroxypropylcellulose, according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying** Not more than 6.0% (1 g, 105°C, 1 hour).

**Residue on ignition** Not more than 1.0% (1 g).

**Assay**

(i) Apparatus—Reaction flask: A 5-mL screw-cap pressure-tight glass bottle, having an inverted conical bottom inside, 20 mm in outside diameter, 50 mm in height up to the neck, 2 mL in capacity up to a height of about 30 mm, equipped with a pressure-tight septum of heat-resisting resin and also with an inside stopper or sealer of fluoroplastic. Heater: A square-shaped aluminum block 60 to 80 mm thick, having holes 20.6 mm in diameter and 32 mm in depth, capable of maintaining the inside temperature within ± 1°C.

(ii) Procedure—Weigh accurately about 65 mg of Low Substituted Hydroxypropylcellulose, previously dried, transfer to the reaction flask, add 65 mg of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the flask tightly, and weigh accurately. Shake the flask for 30 seconds, heat at 150°C on the heater for 30 minutes with repeated shaking at 5-minute intervals, and continue heating for an additional 30 minutes. Allow the flask to cool, and again weigh accurately. If the mass loss is less than 10 mg, use the upper layer of the mixture as the sample solution. Separately, take 65 mg of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid in another reaction flask, stopper tightly, and weigh accurately. Add 15 μL of isopropyl iodide for assay, and again weigh accurately. Shake the reaction flask for 30 seconds, and use the upper layer of the content as the standard solution. Perform the test as directed under Gas Chromatography with 2 μL each of the sample solution and standard solution according to the following conditions, and calculate the ratios, Q1 and Q0, of the peak area of isopropyl iodide to that of the internal standard.

\[
\text{Amount (mg) of hydroxypropoxy group (C}_3\text{H}_7\text{O}_2) = \frac{M_s}{M_I} \times \frac{Q_I}{Q_s} \times 44.17
\]

\[M_s: \text{Amount (mg) of isopropyl iodide for assay}
\]

\[M_I: \text{amount (mg) of the sample}
\]

**Internal standard solution**—A solution of n-octane in o-xylene (1 in 50).

**Operating conditions**

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μm in particle diameter, coated with methyl silicone polymer for gas chromatography at the ratio of 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium (for thermal-conductivity detector); Helium or Nitrogen (for hydrogen flame-ionization detector).

Flow rate: Adjust the flow rate so that the retention time

\[\text{Flow rate} = \text{Flow rate} \times \frac{t_{R}}{t_{0}}\]
of the internal standard is about 10 minutes.
Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions. Use a column giving well-resolved peaks of isopropyl iodide and the internal standard in this order.

Containers and storage  Containers—Tight containers.

Hydroxyzine Hydrochloride

ヒドロキシジン塩酸塩

C₂₁H₂₇ClN₂O₂·2HCl: 447.83
2-(2-\([RS]\)-(4-Chlorophenyl)phenylmethyl)piperazin-1-yl)ethoxy)ethanol dihydrochloride

[2192-20-3]

Hydroxyzine Hydrochloride, when dried, contains not less than 98.5% of C₂₁H₂₇ClN₂O₂·2HCl.

Description  Hydroxyzine Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, freely soluble in methanol, in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 200°C (with decomposition).

Identification (1)  To 5 mL of a solution of Hydroxyzine Hydrochloride (1 in 100) add 2 to 3 drops of ammonium thiocyanate-cobalt (II) nitrate TS: a blue precipitate is formed.

(2)  Determine the absorption spectrum of a solution of Hydroxyzine Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3)  A solution of Hydroxyzine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

pH  <2.54>  Dissolve 1.0 g of Hydroxyzine Hydrochloride in 20 mL of water: the pH of this solution is between 1.3 and 2.5.

Purity (1)  Clarity and color of solution—Dissolve 1.0 g of Hydroxyzine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2)  Heavy metals <1.07>—Proceed with 1.0 g of Hydroxyzine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3)  Related substances—Dissolve 0.20 g of Hydroxyzine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and ammonia solution (28) (150:95:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots other than the principal spot from the standard solution are not more intense than the spot from the standard solution.

Loss on drying  <2.4>  Not more than 3.0% (1 g, 105°C, 2 hours).

Residue on ignition  <2.44>  Not more than 0.2% (1 g).

Assay  Weigh accurately about 0.1 g of Hydroxyzine Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 22.39 mg of C₂₁H₂₇ClN₂O₂·2HCl

Containers and storage  Containers—Tight containers.

Hydroxyzine Pamoate

ヒドロキシジンパモ酸塩

C₂₁H₂₇ClN₂O₂·C₂₃H₁₆O₆: 763.27
2-(2-\([RS]\)-(4-Chlorophenyl)phenylmethyl)piperazin-1-yl)(ethoxy)ethanol mono[4,4′-methylenedioxy(3-hydroxy-2-naphthoate)][(1/1)]

[10246-75-0]

Hydroxyzine Pamoate contains not less than 98.0% of C₂₁H₂₇ClN₂O₂·C₂₃H₁₆O₆, calculated on the anhydrous basis.

Description  Hydroxyzine Pamoate occurs as a light yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in N,N-dimethylformamide, slightly soluble in acetone, and practically insoluble in water, in methanol, in ethanol (95) and in diethyl ether.

Identification (1)  To 0.1 g of Hydroxyzine Pamoate add 25 mL of sodium hydroxide TS, and shake well. Extract with 20 mL of chloroform, and use the chloroform layer as the sample solution. Use the water layer for test (4). To 5 mL of the sample solution add 2 mL of ammonium thiocyanate-cobalt (II) nitrate TS, shake well, and allow to stand: a blue color is produced in the chloroform layer.

(2)  Evaporate 2 mL of the sample solution obtained in (1) on a water bath to dryness, and dissolve the residue in 0.1 mol/L hydrochloric acid TS to make 500 mL. Determine the
absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Hydroxyzine Pamoate as directed under Flame Coloration Test <1.04> (2): a green color appears.

(4) To 1 mL of the water layer obtained in (1), add 2 mL of 1 mol/L hydrochloric acid TS: a yellow precipitate is produced. Collect the precipitate, dissolve the precipitate in 5 mL of methanol, and add 1 drop of iron (III) chloride TS: a green color is produced.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Hydroxyzine Pamoate in 10 mL of N₂N-dimethylformamide: the solution is clear, and shows a slightly greenish, light yellow-brown color.

(2) Chloride <1.03>—To 0.3 g of Hydroxyzine Pamoate add 6 mL of dilute nitric acid and 10 mL of water, shake for 5 minutes, and filter. Wash the residue with two 10-mL portions of water, combine the washings with the filtrate, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.095%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Hydroxyzine Pamoate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 2.0 g of Hydroxyzine Pamoate according to Method 3, and perform the test (not more than 1 ppm).

(5) Related substances—Dissolve 0.40 g of Hydroxyzine Pamoate in 10 mL of a mixture of sodium hydroxide TS and acetone (1:1), and use the solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of sodium hydroxide TS and acetone (1:1) to make exactly 20 mL. Pipet 5 mL of this solution, add a mixture of sodium hydroxide TS and acetone (1:1) to make exactly 50 mL, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and ammonia TS (150:95:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots other than hydroxyzine and pamoic acid obtained from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not more than 3.0% (1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Weigh accurately about 0.6 g of Hydroxyzine Pamoate, add 25 mL of sodium hydroxide TS, shake well, extract with six 25-mL portions of chloroform. Filter each extract through 5 g of anhydrous sodium sulfate on a pledget of absorbent cotton. Combine the chloroform extracts, and evaporate the combined chloroform extracts on a water bath to about 30 mL. Add 30 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 38.16 mg of C₁₁H₁₃ClN₂O₂, C₁₂H₁₄O₆

Containers and storage Containers—Tight containers.

Hymecromone

ヒメクロモン

C₁₀H₈O₃: 176.17
7-Hydroxy-4-methylchromen-2-one [90-33-5]

Hymecromone, when dried, contains not less than 98.0% of C₁₀H₈O₃.

Description Hymecromone occurs as white crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in N₂N-dimethylformamide, sparingly soluble in ethanol (95), in ethanol (99.5) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 2 mg of Hymecromone in 5 mL of ammonia-ammonium chloride buffer solution, pH 11.0: the solution shows an intense blue-purple fluorescence.

(2) Dissolve 0.025 g of Hymecromone in 5 mL of diluted ethanol (95) (1 in 2), and add 1 drop of iron (III) chloride TS: initially a blackish brown color develops, and when allowed to stand the color changes to yellow-brown.

(3) Determine the absorption spectrum of a solution of Himecromone in ethanol (99.5) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Himecromone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.66> 187 – 191°C

Purity (1) Chloride <1.03>—Dissolve 0.8 g of Hymecromone in 40 mL of a mixture of acetone and water (2:1), and add 6 mL of dilute nitric acid and a mixture of acetone and water (2:1) to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and a mixture of acetone and water (2:1) to make 50 mL (not more than 0.011%).

(2) Sulfate <1.14>—Dissolve 0.8 g of Hymecromone in 40 mL of a mixture of acetone and water (2:1), and add 1 mL of dilute hydrochloric acid and a mixture of acetone and water (2:1) to make 50 mL. Perform the test using this solu-
tion as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and a mixture of acetone and water (2:1) to make 50 mL (not more than 0.024%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Hymecromone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Hymecromone according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 80 mg of Hymecromone in 10 mL of ethanol (95%), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 50 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (94:6), wash the plate with a mixture of chloroform and methanol (90:10) and dry. Store the plates in a place that is not harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbol (∙). Hypromellose shown in the table below, calculated on the dried basis.

The viscosity is shown in millipascal second (mPa·s) on the label, together with the substitution type.

<table>
<thead>
<tr>
<th>Substitution Type</th>
<th>Methoxy Group (%)</th>
<th>Hydroxypropoxy Group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>1828</td>
<td>16.5</td>
<td>20.0</td>
</tr>
<tr>
<td>2208</td>
<td>19.0</td>
<td>24.0</td>
</tr>
<tr>
<td>2906</td>
<td>27.0</td>
<td>30.0</td>
</tr>
<tr>
<td>2910</td>
<td>28.0</td>
<td>30.0</td>
</tr>
</tbody>
</table>

**Description** Hypromellose occurs as a white to yellowish white, powder or granules. It is practically insoluble in ethanol (99.5). It swells with water and becomes a clear or slightly turbid, viscous solution.

**Identification** (1) Disperse evenly 1.0 g of Hypromellose over the surface of 100 mL of water in a beaker, while gently tapping the top of the container, if necessary, and allow the beaker to stand: it aggregates on the surface of water.

(2) Add 1.0 g of Hypromellose to 100 mL of hot water, and stir: it becomes a suspension. Cool the suspension to 10°C, and stir: the resulting liquid is a clear or a slightly cloudy, viscous fluid.

(3) To 0.1 mL of the viscous fluid obtained in (2) add 9 mL of dilute sulfuric acid (9 in 10), stir, heat in a water bath for exactly 3 minutes, and immediately cool in ice water. Add carefully 0.6 mL of ninhydrin TS, stir, and allow to stand at 25°C: the solution shows a light red color first, then changes to purple color within 100 minutes.

(4) Pour and spread out 2 to 3 mL of the viscous fluid obtained in (2) onto a glass plate, and allow the water to evaporate: a transparent film results.

(5) Pipet 50 mL of water, add exactly 50 mL of the viscous fluid obtained in (2), and warm to rise the temperature at a rate of 2 to 5°C per minute while stirring: the temperature, when a white turbidity of the solution starts to increase, is not less than 50°C.

**Viscosity** <2.53> Method I: Apply to Hypromellose having a labeled viscosity of less than 600 mPa·s. Put exactly an amount of Hypromellose, equivalent to 4.000 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 200.0 g, stopper the bottle, stir by mechanical means at 350- to 450-revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath not exceeding 10°C for 20 to 40 minutes while stirring. Add cooled water, if necessary, to make 200.0 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

Method II: Apply to Hypromellose having a labeled viscosity of not less than 600 mPa·s. Put exactly an amount of Hypromellose, equivalent to 10.00 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 500.0 g,
stopper the bottle, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method II (2) under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

Operating conditions—
Apparatus: Brookfield type viscometer LV model

<table>
<thead>
<tr>
<th>Labeled viscosity (mPa·s)</th>
<th>Rotor No.</th>
<th>Rotation frequency /min</th>
<th>Conversion factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not less than 600 and less than 1400</td>
<td>3</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>≥ 1400</td>
<td>4</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>≥ 3500</td>
<td>4</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>≥ 9500</td>
<td>4</td>
<td>3</td>
<td>2000</td>
</tr>
</tbody>
</table>

Procedure of apparatus: Read value after 2 minutes of rotation, and stop the rotation for 2 minutes. Repeat this procedure more two times, and average three observed values.

pH <2.54> Allow the sample solution obtained in the Viscosity to stand at 20 ± 2°C for 5 minutes: the pH of the solution thus obtained is between 5.0 and 8.0.

Purity Heavy metals—Put 1.0 g of Hypromellose in a 100-mL Kjeldahl flask, add a sufficient amount of a mixture of nitric acid and sulfuric acid (5:4) to wet the sample, and heat gently. Repeat this procedure until to use totally 18 mL of the mixture of nitric acid and sulfuric acid. Then boil gently until the solution changes to black. After cooling, add 2 mL of nitric acid, and heat until the solution changes to black. Repeat this procedure until the solution no longer changes to black, and heat strongly until dense white fumes are evolved. After cooling, add 5 mL of water, boil gently until dense white fumes are evolved, then heat until the volume of the solution becomes to 2 to 3 mL. After cooling, if the solution reveals yellow color by addition of 5 mL of water, add 1 mL of hydrogen peroxide (30), and heat until the volume of the solution becomes to 2 to 3 mL. After cooling, dilute the solution with 2 to 3 mL of water, transfer to a Nessler tube, add water to make 25 mL, and use this solution as the test solution. Separately, put 2.0 mL of Standard Lead Solution in a 100-mL kjeldahl flask, add 18 mL of the mixture of nitric acid and sulfuric acid (5:4) and an amount of nitric acid equal to that used for preparation of the test solution, and heat until white fumes are evolved. After cooling, add 10 mL of water. In the case where hydrogen peroxide (30) is added for the preparation of the test solution, add the same amount of hydrogen peroxide (30), then proceed in the same manner for preparation of the test solution, and use so obtained solution as the control solution. Adjust the test solution and the control solution to pH 3.0 to 4.0 with ammonia solution (28), and add water to make 40 mL, respectively. To these solutions add 1.2 mL of thioacetamide-alkaline glycerin TS, 2 mL of acetate buffer solution, pH 3.5 and water to make 50 mL, separately. After allowing to stand for 5 minutes, observe vertically both tubes on a white background: the color obtained with the test solution is not more intense than that with the control solution (not more than 20 ppm).

Loss on drying <2.4> Not more than 5.0% (1 g, 105°C, 1 hour).

Residue on ignition <2.4> Not more than 1.5% (1 g).

Assay (i) Apparatus—Reaction bottle: A 5-mL pressure-tight glass vial, having 20 mm in outside diameter and 50 mm in height, the neck 20 mm in outside diameter and 13 mm in inside diameter, equipped with a septum of butyl-rubber processed the surface with fluoroplastics, which can be fixed tightly to vial with aluminum cap, or equivalent. Heater: A square-shaped aluminum block, having holes 20 mm in diameter and 32 mm in depth, adopted to the reaction bottle. Capable of stirring the content of the reaction bottle by means of magnetic stirrer or of reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Hypromellose, transfer to the reaction bottle, add 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Stir or shake for 60 minutes while heating so that the temperature of the bottle content is 130 ± 2°C. In the case when the stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the bottle to cool, and again weigh accurately. If the mass loss is less than 0.50% or there is no evidence of a leak, use the upper layer of the mixture as the sample solution. Separately, put 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid in a reaction bottle, stopper the bottle immediately, and weigh accurately. Add 45 μL of iodomethane for assay 15 and 22 μL of isopropyl iodide for assay through the septum using micro-syringe with weighing accurately every time, stir thoroughly, and use the upper layer of the mixture as the standard solution. Perform the test with 1 to 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.2> according to the following conditions, and calculate the ratios, and of the peak area of iodomethane and isopropyl iodide to that of the internal standard obtained from the sample solution, and of the peak area of iodomethane and isopropyl iodide to that of the internal standard from the standard solution.

\[
\text{Content (mg)} = \frac{Q_{15}/Q_{30}}{M_S} \times M \times 21.86
\]

\[
\text{Content (mg)} = \frac{Q_{22}/Q_{30}}{M_S} \times M \times 44.17
\]

\[
M_S: \text{Amount (mg) of iodomethane for assay}
\]

\[
M_S: \text{Amount (mg) of isopropyl iodide for assay}
\]

\[
M: \text{Amount (mg) of sample, calculated on the dried basis}
\]

Internal standard solution—A solution of n-octane in o-xylene (3 in 100).

Operating conditions—
Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column 3–4 mm in inside diameter and 1.8–3 m in length, packed with siliceous earth for gas chro-
matography, 125 to 150 μm in diameter, coated with methyl silicone polymer at the ratio of 10 – 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium for thermal conductivity detector, or Helium or Nitrogen for hydrogen, flame-ionization detector.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

System suitability—

System performance: When the procedure is run with 1 – 2 μL of the standard solution under the above operating conditions, iodomethane, isopropyl iodide and the internal standard are eluted in this order, with complete separation of these peaks.

*Containers and storage* Containers—Well-closed containers.

**Hypromellose Phthalate**

ヒプロメロースフタル酸エステル

[9050-31-1]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (* • ).

Hypromellose Phthalate is a monophthalic acid ester of hypromellose.

It contains methoxy group (−OCH3: 31.03), hydroxypropoxy group (−OCH2CHOHCH3: 75.09), and carboxybenzoyl group (−COC6H4COOH: 149.12).

It contains not less than 21.0% and not more than 35.0% of carboxybenzoyl group, calculated on the anhydrous basis.

*Its substitution type and its viscosity in millipascal second (mPa·s) are shown on the label.*

<table>
<thead>
<tr>
<th>Substitution</th>
<th>Carboxybenzoyl group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Min.</td>
</tr>
<tr>
<td>200731</td>
<td>27.0</td>
</tr>
<tr>
<td>220824</td>
<td>21.0</td>
</tr>
</tbody>
</table>

*Description* Hypromellose Phthalate occurs as white powder or granules.

It is practically insoluble in water, in acetonitrile and in ethanol (99.5).

It becomes a viscous liquid when a mixture of methanol and dichloromethane (1:1) or a mixture of ethanol (99.5) and acetone (1:1) is added.

It dissolves in sodium hydroxide TS.

*Identification* Determine the infrared absorption spectrum of Hypromellose Phthalate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Viscosity** <2.53> To 10 g of Hypromellose Phthalate, previ-ously dried at 105°C for 1 hour, add 90 g of a mixture of methanol and dichloromethane in equal mass ratio, and stir to dissolve. Determine the viscosity at 20 ± 0.1°C as directed in Method 1 under Viscosity Determination: the viscosity is not less than 80% and not more than 120% of the labeled unit.

**Purity** (1) Chloride <1.03>—Dissolve 1.0 g of Hypromellose Phthalate in 40 mL of 0.2 mol/L sodium hydroxide VS, add 1 drop of phenolphthalein TS, and add dilute nitric acid dropwise with vigorous stirring until the red color is discharged. Further add 20 mL of dilute nitric acid with stirring. Heat on a water bath with stirring until the gelatinous precipitate formed turns to granular particles. After cooling, centrifuge, and take off the supernatant liquid. Wash the precipitate with three 20-mL portions of water by centrifuging each time, combine the supernatant liquid and the washings, add water to make 200 mL, and filter. Perform the test with 50 mL of the filtrate. Control solution: To 0.50 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of 0.2 mol/L sodium hydroxide VS and 7 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.07%).

* (2) Heavy metals <1.07>—Proceed with 2.0 g of Hypromellose Phthalate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Phthalic acid—Weigh accurately about 0.2 g of Hypromellose Phthalate, add about 50 mL of acetonitrile to dissolve partially with the aid of ultrasonic waves, add 10 mL of water, and dissolve further with the ultrasonic waves. After cooling, add acetonitrile to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 12.5 mg of phthalic acid, dissolve in about 125 mL of acetonitrile by mixing, add 25 mL of water, then add acetonitrile to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas of phthalic acid, A1 and A8, of both solutions: amount of phthalic acid (C8H6O4: 166.13) is not more than 1.0%.

Amount (%) of phthalic acid = M5/M1 × A1/A8 × 40

M6: Amount (mg) of phthalic acid
M7: Amount (mg) of sample, calculated on the anhydrous basis

**Operating conditions**—


Column: A stainless steel column about 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of 0.1% trifluoroacetic acid and acetonitrile (9:1).

Flow rate: About 2.0 mL per minute.

**System suitability—**

*System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of phthalic acid are not less than 2500 and...
not more than 1.5, respectively.

System repeatability: When repeat the test 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phthalic acid is not more than 1.0%.

Water Not more than 5.0% (1 g, volumetric titration direct titration, using a mixture of ethanol (99.5) and dichloromethane (3:2) instead of methanol for Karl Fischer method).

Residue on ignition Not more than 0.2% (1 g).

Assay Weigh accurately about 1 g of Hypromellose Phthalate, dissolve in 50 mL of a mixture of ethanol (95), acetone and water (2:2:1), and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Amount (%) of phthalic acid obtained in the Purity (3)

\[
P = \frac{\text{Amount (mol/L) of 0.1 mol/L sodium hydroxide VS consumed}}{\text{Amount (g) of sample, calculated on the anhydrous basis}}
\]

Containers and storage Containers—Tight containers.

Ibudilast

イブジラスト

C_{14}H_{18}N_{2}O: 230.31

1-[2-(1-Methylethyl)pyrazolo[1,5-a]pyridin-3-yl]-2-methylpropan-1-one

[50847-11-5]

Ibudilast, when dried, contains not less than 98.5% and not more than 101.0% of C_{14}H_{18}N_{2}O.

Description Ibudilast occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (99.5) and in acetic anhydride, and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Ibudilast in methanol (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ibudilast as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point 54 – 58°C.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Ibudilast according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Ibudilast in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than ibudilast obtained from the sample solution is not larger than the peak area of ibudilast from the standard solution, and the total area of the peaks other than ibudilast is not larger than 3 times the peak area of ibudilast from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 292 nm).

Column: A stainless steel column 2.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of hexane and ethyl acetate (50:1).

Flow rate: Adjust the flow rate so that the retention time of ibudilast is about 9 minutes.

Time span of measurement: About 4 times as long as the retention time of ibudilast, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ibudilast obtained with 10 μL of this solution is equivalent to 40 to 60% of that with 10 μL of the standard solution.

System performance: To 5 mL of the sample solution add the mobile phase to make 50 mL. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ibudilast are not less than 3500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ibudilast is not more than 3.0%.

Loss on drying Not more than 0.3% (1 g, in vacuum, 4 hours).

Residue on ignition Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ibudilast, previously dried, dissolve in 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 23.03 mg of C_{14}H_{18}N_{2}O

C_{14}H_{18}N_{2}O: 230.31

1-[2-(1-Methylethyl)pyrazolo[1,5-a]pyridin-3-yl]-2-methylpropan-1-one

[50847-11-5]
Ibuprofen

イブプロフェン

\[
C_{13}H_{18}O_2: 206.28
\]
\[(2RS)-2-[4-(2-Methylpropyl)phenyl]propanoic acid [15687-27-1]\]

Ibuprofen, when dried, contains not less than 98.5\% of \(C_{13}H_{18}O_2\).

**Description**  Ibuprofen occurs as a white crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

**Identification (1)** Determine the absorption spectrum of a solution of Ibuprofen in dilute sodium hydroxide TS (3 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ibuprofen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.60>  75 – 77°C.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of Ibuprofen according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ibuprofen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.50 g of Ibuprofen in exactly 5 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100:15:5:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41>  Not more than 0.5\% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 4 hours).

**Residue on ignition** <2.44>  Not more than 0.1\% (1 g).

**Assay** Weigh accurately 0.5 g of Ibuprofen, previously dried, dissolve in 50 mL of ethanol (95), and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 20.63 mg of \(C_{13}H_{18}O_2\).

**Containers and storage** Well-closed containers.

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Ichthammol

イクタモール

Ichthammol, calculated on the dried basis, contains not less than 2.5\% of ammonia (\(\text{NH}_3: 17.030\), not more than 8.0\% of ammonium sulfate \([\text{NH}_4\text{H}_2\text{SO}_4]: 132.14\), and not less than 10.0\% of total sulfur (as S: 32.07).

**Description** Ichthammol is a red-brown to blackish brown, viscous fluid. It has a characteristic odor.

It is miscible with water, and is partially soluble in ethanol (95) and in diethyl ether.

**Identification (1)** To 4 mL of a solution of Ichthammol (3 in 10) add 8 mL of hydrochloric acid: a yellow-brown to blackish brown, oily or resinous mass is produced. Cool the mass with ice to solidify, and discard the water layer. Wash the residue with diethyl ether: a part of the mass dissolves but it does not dissolve completely even when it is washed until almost no color develops in the washing. Perform the following tests with this residue.

(i) To 0.1 g of the residue add 1 mL of a mixture of diethyl ether and ethanol (95) (1:1): it dissolves.

(ii) To 0.1 g of the residue add 2 mL of water: it dissolves. To 1 mL of this solution add 0.4 mL of hydrochloric acid: a yellow-brown to blackish brown oily or resinous substance is produced.

(iii) To 1 mL of the solution obtained in (ii) add 0.3 g of sodium chloride: a yellow-brown or blackish brown oily or resinous substance is produced.

(2) Boil 2 mL of a solution of Ichthammol (1 in 10) with 2 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

**Loss on drying** <2.41>  Not more than 50\% (0.5 g, 105°C, 6 hours).

**Residue on ignition** <2.44>  Not more than 0.5\% (1 g).

**Assay (1)** Ammonia—Weigh accurately 5 g of Ichthammol, transfer to a Kjeldahl flask, and add 60 mL of water, 1 mL of 1-octanol and 4.5 mL of a solution of sodium hydroxide (2 in 5). Connect the flask to a distilling tube with a spray trap and a condenser, and immerse the lower outlet of the condenser in the receiver containing exactly 30 mL of 0.25 mol/L sulfuric acid VS. Distil slowly, collect about 50 mL of the distillate, and titrate with 0.5 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination, and make
any necessary correction.

Each mL of 0.25 mol/L sulfuric acid VS
= 8.515 mg of NH₃

(2) Ammonium sulfate—Weigh accurately about 1 g of Ichthammol, add 25 mL of ethanol (95), stir thoroughly, and filter. Wash with a mixture of diethyl ether and ethanol (95) (1:1) until the washings are clear and colorless. Dry the filter paper and the residue in air, dissolve the residue in 200 mL of hot water acidified slightly with hydrochloric acid, and filter. Boil the filtrate, add 30 mL of barium chloride TS slowly, heat for 30 minutes on a water bath, and filter. Wash the precipitate with water, dry, and ignite to constant mass. Weigh the residue as barium sulfate (BaSO₄: 233.39).

Amount (mg) of ammonium sulfate [(NH₄)₂SO₄] = amount (mg) of barium sulfate (BaSO₄) \times 0.566

(3) Total sulfur—Weigh accurately about 0.6 g of Ichthammol, transfer to a 200-mL Kjeldahl flask, and add 30 mL of water and 5 g of potassium chlorate, then add slowly 30 mL of nitric acid, and evaporate the mixture to about 5 mL. Transfer the residue to a 300-mL beaker with the aid of 25 mL of hydrochloric acid, and evaporate again to 5 mL. Add 100 mL of water, boil, filter, and wash with water. Heat the combined filtrate and washings to boil, add gradually 30 mL of barium chloride TS, heat the mixture on a water bath for 30 minutes, and filter. Wash the precipitate with water, dry, and ignite to constant mass. Weigh the residue as barium sulfate (BaSO₄).

Amount (mg) of total sulfur (S) = amount (mg) of barium sulfate (BaSO₄) \times 0.13739

Containers and storage Containers—Tight containers.

Idarubicin Hydrochloride

イダルビシン塩酸塩

C₂₆H₂₇NO₉.HCl: 533.95
(2S,4S)-2-Acetyl-4-(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyloxy)-2,5,12-trihydroxy-1,2,3,4-tetrahydrotetracene-6,11-dione monohydrochloride [57852-57-0]

Idarubicin Hydrochloride contains not less than 960 µg (potency) and not more than 1030 µg (potency) per mg, calculated on the anhydrous basis. The potency of Idarubicin Hydrochloride is expressed as mass (potency) of idarubicin hydrochloride (C₂₆H₂₇NO₉.HCl).

Description Idarubicin Hydrochloride occurs as a yellow-red powder.

It is sparingly soluble in methanol, slightly soluble in water and in ethanol (95), and practically insoluble in acetonitrile and in diethyl ether.

Identification (1) Determine the absorption spectra of a solution of Idarubicin Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Idarubicin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectra of Idarubicin Hydrochloride and Idarubicin Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 2 mg of Idarubicin Hydrochloride in 3 mL of water, and add 1 mL of dilute nitric acid and 3 drops of silver nitrate TS: a white turbidity is produced.

Absorbance <2.24> \( E_{1%1cm} \) (482 nm): 204 - 210 (20 mg calculated on the anhydrous basis, methanol, 1000 mL).

Optical rotation <2.49> \([\alpha]_{D}^20\): +191 - +197° (20 mg calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 50 mg of Idarubicin Hydrochloride in 10 mL of water is between 5.0 and 6.5.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals—Being specified separately.

(3) Related substances—Being specified separately.

(4) Residual solvent—Being specified separately.

Water <2.49> Not more than 5.0% (0.1 g, coulometric titration).

Residue on ignition Being specified separately.

Bacterial endotoxins <4.01> Less than 8.9 EU/mg (potency).

Assay Weigh accurately an amount of Idarubicin Hydrochloride and Idarubicin Hydrochloride RS, equivalent to about 10 mg (potency), dissolve each in the mobile phase containing no sodium lauryl sulfate to make exactly 50 mL, and use these solutions as the standard solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of idarubicin of these solutions.

Amount [µg (potency)] of C₂₆H₂₇NO₉.HCl = \( M_S \times A_T/A_S \times 1000 \)

M₅: Amount [mg (potency)] of Idarubicin Hydrochloride RS

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 µm in particle diameter).
Column temperature: A constant temperature of about
Idarubicin Hydrochloride for Injection / Official Monographs

**Idarubicin Hydrochloride for Injection**

注射用イダルビシン塩酸塩

Idarubicin Hydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of idarubicin hydrochloride (C$_{26}$H$_{27}$NO$_{9}$.HCl: 533.95).

**Method of preparation** Prepare as directed under Injections, with Idarubicin Hydrochloride.

**Description** Idarubicin Hydrochloride for Injection occurs as yellow-red masses.

**Identification**

1. Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 2 mg (potency) of Idarubicin Hydrochloride according to the labeled amount, in 5 mL of sodium hydroxide TS: the solution shows a blue-purple color.

2. Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 1 mg (potency) of Idarubicin Hydrochloride according to the labeled amount, in 1 mL of water, and add methanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (<2.24>): it exhibits maxima between 250 nm and 254 nm, between 285 nm and 289 nm, between 480 nm and 484 nm, and between 510 nm and 520 nm.

**pH**<2.54> The pH of a solution prepared by dissolving an amount of Idarubicin Hydrochloride for Injection, equivalent to 5 mg (potency) of Idarubicin Hydrochloride according to the labeled amount, in 5 mL of water is between 5.0 and 7.0.

**Purity** Clarity and color of solution—Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 5 mg (potency) of Idarubicin Hydrochloride according to the labeled amount, in 5 mL of water: the solution is clear and yellow-red.

**Sterility**<4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Weigh accurately the mass of Idarubicin Hydrochloride for Injection, add 5 mL of methanol for Karl Fischer method using a syringe, dissolve with thorough shaking, and perform the test with 4 mL of this solution as directed in the Volumetric titration (direct titration). Use 4 mL of methanol for Karl Fischer method as the blank. Determine the mass of the content from the difference between the mass of Idarubicin Hydrochloride for Injection obtained above and the mass of its bottle and rubber stopper, which are weighed accurately after washing with water then with ethanol (95), drying at 105°C for 1 hour and allowing to cool to room temperature in a desiccator (not more than 4.0%).

**Bacterial endotoxins**<4.01> Less than 8.9 EU/mg (potency).

**Uniformity of dosage units**<6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 Idarubicin Hydrochloride for Injection add the mobile phase prepared without addition of sodium lauryl sulfate to make exactly V mL so that each mL contains 0.2 mg (potency) of idarubicin hydrochloride (C$_{26}$H$_{27}$NO$_{9}$.HCl), and use this solution as the sample solution. Separately, weigh accurately an amount of Idarubicin Hydrochloride RS, equivalent to about 10 mg (potency), dissolve in the mobile phase without containing sodium lauryl sulfate to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Idarubicin Hydrochloride.

Amount [mg (potency)] of idarubicin hydrochloride (C$_{26}$H$_{27}$NO$_{9}$.HCl) = $M_S \times A_1/A_3 \times V/50$

$M_S$: Amount [mg (potency)] of Idarubicin Hydrochloride RS

**Foreign insoluble matter**<6.06> Perform the test according to the Method 2: it meets the requirement.

**Insoluble particulate matter**<6.07> It meets the requirement.

**Water**<6.02> Weigh accurately the mass of 1 Idarubicin Hydrochloride for Injection, add 5 mL of methanol for Karl Fischer method using a syringe, dissolve with thorough shaking, and perform the test with 4 mL of this solution as directed in the Volumetric titration (direct titration). Use 4 mL of methanol for Karl Fischer method as the blank. Determine the mass of the content from the difference between the mass of 1 Idarubicin Hydrochloride for Injection obtained above and the mass of its bottle and rubber stopper, which are weighed accurately after washing with water then with ethanol (95), drying at 105°C for 1 hour and allowing to cool to room temperature in a desiccator (not more than 4.0%).

**Bacterial endotoxins**<4.01> Less than 8.9 EU/mg (potency).

**Uniformity of dosage units**<6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 Idarubicin Hydrochloride for Injection add the mobile phase prepared without addition of sodium lauryl sulfate to make exactly V mL so that each mL contains 0.2 mg (potency) of idarubicin hydrochloride (C$_{26}$H$_{27}$NO$_{9}$.HCl), and use this solution as the sample solution. Separately, weigh accurately an amount of Idarubicin Hydrochloride RS, equivalent to about 10 mg (potency), dissolve in the mobile phase without containing sodium lauryl sulfate to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Idarubicin Hydrochloride.

Amount [mg (potency)] of idarubicin hydrochloride (C$_{26}$H$_{27}$NO$_{9}$.HCl) = $M_S \times A_1/A_3 \times V/50$

$M_S$: Amount [mg (potency)] of Idarubicin Hydrochloride RS
Idoxuridine

\[
C_9H_9IN_2O_5: \ 354.10
\]

5-Iodo-2′-deoxyuridine

[54-42-2]

Idoxuridine, when dried, contains not less than 98.0% of \(C_9H_9IN_2O_5\).

**Description**  Idoxuridine occurs as colorless, crystals or a white, crystalline powder. It is odorless.

It is freely soluble in dimethylamidine, slightly soluble in water, very slightly soluble in ethanol (95%), and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: about 176°C (with decomposition).

**Identification** (1) Dissolve 0.01 g of Idoxuridine in 5 mL of water by warming, add 5 mL of diphenylamine-acetic acid TS, and heat for 5 minutes: a blue color develops.

(2) Heat 0.1 g of Idoxuridine: a purple gas evolves.

(3) Dissolve 2 mg of Idoxuridine in 50 mL of 0.01 mol/L sodium hydroxide. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Idoxuridine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** \(<2.49\>\ [\alpha]_D^20: +28 – +31° (after drying, 0.2 g, sodium hydroxide TS, 20 mL, 100 mm).

**Purity** (1) Clarity and color of solution—Dissolve 0.20 g of Idoxuridine in 5 mL of a solution of sodium hydroxide (1 in 200): the solution is clear and colorless.

(2) Heavy metals \(<1.07\>\)—Proceed with 2.0 g of Idoxuridine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Idoxuridine in exactly 10 mL of a mixture of dilute ethanol and ammonia solution (28:99:1), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography \(<2.03\>\). Spot 50 \(\mu\)L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and dilute 2-propanol (2 in 3) (4:1) to a distance of about 10 cm, and air-dry the plate. Then develop two-dimensionally at right angles to the first, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

(4) Iodine and iodide—Dissolve 0.10 g of Idoxuridine in 20 mL of water and 5 mL of sodium hydroxide TS, and add immediately 5 mL of dilute sulfuric acid under ice-cooling. Allow to stand for 10 minutes with occasional shaking, and filter. Transfer the filtrate into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodate (1 in 100), shake for 30 seconds, and allow to stand: the chloroform layer has no more color than the following control solution.

Control solution: Weigh accurately 0.111 g of potassium iodide, and dissolve in water to make 1000 mL. To exactly 1 mL of this solution add 19 mL of water, 5 mL of sodium hydroxide TS and 5 mL of dilute sulfuric acid, mix, and filter. Transfer the filtrate to a Nessler tube, and proceed in the same manner.

**Loss on drying** \(<2.47\>\ Not more than 0.5% (2 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** \(<2.44\>\ Not more than 0.3% (1 g).

**Assay** Weigh accurately about 0.7 g of Idoxuridine, previously dried, dissolve in 80 mL of \(N,N\)-dimethylformamide, and titrate \(<2.50\>\) with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through yellow-green to blue (indicator: 5 drops of thymol blue-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS

\=
35.41 mg of \(C_9H_9IN_2O_5\)

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Idoxuridine Ophthalmic Solution**

イドクスウリジン点眼液

Idoxuridine Ophthalmic Solution contains not less than 90.0% and not more than 110.0% of the labeled amount of idoxuridine (\(C_9H_9IN_2O_5\): 354.10).

**Method of preparation** Prepare as directed under Ophthalmic Preparations, with Idoxuridine.

**Description** Idoxuridine Ophthalmic Solution is a clear, colorless liquid.

**Identification** (1) To a volume of Idoxuridine Ophthalmic Solution, equivalent to 5 mg of Idoxuridine according to the labeled amount, add 5 mL of diphenylamine-acetic acid TS, and heat for 20 minutes: a light blue color develops.

(2) Place a volume of Idoxuridine Ophthalmic Solution, equivalent to 5 mg of Idoxuridine according to the labeled amount, in a porcelain crucible, add 0.1 g of anhydrous sodium carbonate, heat slowly, evaporate to dryness and ignite until the residue is incinerated. Dissolve the residue in 5 mL of water, acidify with hydrochloric acid, and add 2 to 3 drops of sodium nitrite TS: a yellow-brown color develops. Then add 2 to 3 drops of starch TS: a deep blue color develops.
Ifenprodil Tartrate / Official Monographs

(3) To a volume of Idoxuridine Ophthalmic Solution, equivalent to 2 mg of Idoxuridine according to the labeled amount, add 0.01 mol/L sodium hydroxide TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.04>; it exhibits a maximum between 277 nm and 281 nm.

\[ \text{pH} < 2.5 \times 10^{-4}, 4.5 - 7.0 \]

**Purity** 5-Iodouracil and 2'-deoxyuridine—To a volume of Idoxuridine Ophthalmic Solution, equivalent to 4.0 mg of Idoxuridine according to the labeled amount, add water to make exactly 5 mL, and use this solution as the sample solution. Separately, dissolve 12.0 mg of 5-iodouracil for liquid chromatography and 4.0 mg of 2'-deoxyuridine for liquid chromatography in water to make exactly 200 mL. Measure exactly 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu \)L of each of the sample solution and standard solution as directed under Liquid Chromatography <2.04> according to the following conditions, and determine the peak areas of 5-iodouracil and 2'-deoxyuridine: the peak areas of 5-iodouracil and 2'-deoxyuridine of the sample solution are not larger than the peak areas of 5-iodouracil and 2'-deoxyuridine of the standard solution.

**Operating conditions**
- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 \( \mu \)m in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of water and methanol (24:1).
- Flow rate: Adjust the flow rate so that the retention time of 2'-deoxyuridine is about 6 minutes.

**System suitability**
- System performance: When the procedure is run with 10 \( \mu \)L of the standard solution under the above operating conditions, idoxuridine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.
- System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of idoxuridine to that of the internal standard is not more than 1.0%.

**Foreign insoluble matter** <6.11> It meets the requirement.

**Insoluble particulate matter** <6.08> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Measure exactly a volume of Idoxuridine Ophthalmic Solution, equivalent to 3 mg of idoxuridine \((C_9H_{11}IN_2O_5)\), add exactly 2 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the sample solution. Separately weigh accurately about 10 mg of Idoxuridine RS, previously dried at 60°C for 3 hours, dissolve in water to make exactly 10 mL. Measure exactly 3 mL of this solution, add exactly 2 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the standard solution. Perform the test with 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_z \) and \( Q_z \), of the peak area of idoxuridine to that of the internal standard, respectively.

\[ \text{Amount (mg) of idoxuridine (} C_9H_{11}IN_2O_5 \text{)} = M_S \times Q_T / Q_S \times 3/10 \]

\( M_S \): Amount (mg) of Idoxuridine RS

**Internal standard solution**—A solution of sulfathiazole in the mobile phase (1 in 4000).

**Operating conditions**
- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 \( \mu \)m in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of water and methanol (87:13).
- Flow rate: Adjust the flow rate so that the retention time of idoxuridine is about 9 minutes.

**System suitability**
- System performance: When the procedure is run with 10 \( \mu \)L of the standard solution under the above operating conditions, idoxuridine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.
- System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of idoxuridine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, in a cold place, and avoid freezing.

Ifenprodil Tartrate

イフェンプロジル酒石酸塩

\[
\text{Ifenprodil Tartrate contains not less than 98.5\% of (} C_{21}H_{27}NO_2)\text{.C}_4\text{H}_6\text{O}_6\text{, calculated on the anhydrous basis.}
\]

**Description** Ifenprodil Tartrate occurs as a white crystalline powder. It is odorless.

It is freely soluble in acetic acid (100), soluble in ethanol (95), slightly soluble in water and in methanol, and practically insoluble in diethyl ether.

Optical rotation \([\alpha]_D^{20} + 11 - + 15^\circ\) (1 g, calculated on the anhydrous basis, ethanol (95), 20 mL, 100 mm).
Melting point: about 148°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ifenprodil Tartrate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.44>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ifenprodil Tartrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.4 g of Ifenprodil Tartrate in 40 mL of water by warming. After cooling, add 0.5 mL of ammonia TS to this solution, extract with two 40-mL portions of chloroform, and collect the water layer. Evaporate 30 mL of the water layer on a water bath to dryness, and after cooling, dissolve the residue in 6 mL of water: the solution responds to the Qualitative Tests <1.09> for tartrate.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Ifenprodil Tartrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.30 g of Ifenprodil Tartrate in 10 mL of diluted ethanol (95) (3 in 4), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (95) (3 in 4) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.09>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thinlayer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, 1-butanol and ammonia solution (28:140:40:20:1) to a distance of about 10 cm, and air-dry the plate. Spray hydrogen hexachloroplatinate (IV)-potassium iodide TS evenly on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Ifenprodil Tartrate, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 40.05 mg of (C21H27NO2)2.C4H6O6

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Imidapril Hydrochloride

イミダプリル塩酸塩

C20H27N3O6.HCl: 441.91

(4S)-3-[(2S)-2-[(15)-1-Ethoxycarbonyl-3-phenylpropylamino]propanoyl]-1-methyl-2-oxoimidazolidine-4-carboxylic acid monohydrochloride

[89396-94-1]

Imidapril Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of C20H27N3O6.HCl.

Description Imidapril Hydrochloride occurs as a white crystals.

It is freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5).

Dissolve 1.0 g of Imidapril Hydrochloride in 100 mL of water: the pH of the solution is about 2.

Melting point: about 203°C (with decomposition).

Identification (1) To 3 mL of a solution of Imidapril Hydrochloride (1 in 50) add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Imidapril Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Imidapril Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> [α]D: −65.0 – −69.0° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Imidapril Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 25 mg of Imidapril Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.45 with respect to imidapril, obtained from the sample solution, is not larger than 2/5 times the peak area of imidapril from the standard solution, and the area of each peak other than the peaks of imidapril and other than those mentioned above from the sample solution is not larger than 1/5 times the...
peak area of imidapril from the standard solution. Furthermore, the total area of the peaks other than imidapril from the sample solution is not larger than 1/2 times the peak area of imidapril from the standard solution.

**Operating conditions**

- **Detector:** An ultraviolet absorption photometer (wavelength: 215 nm)
- **Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel (5 μm in particle diameter).
- **Column temperature:** A constant temperature of about 40°C.
- **Mobile phase:** Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.7 with phosphoric acid. To 600 mL of this solution add 400 mL of methanol.
- **Flow rate:** Adjust the flow rate so that the retention time of imidapril is about 8 minutes.
- **Time span of measurement:** About 2 times as long as the retention time of imidapril, beginning after the solvent peak.

**System suitability**

- Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of imidapril obtained from 20 μL of this solution is equivalent to 7 to 13% of that of imidapril from 20 μL of the standard solution.
- **System performance:** When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of imidapril are not less than 5000 and not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of this solution, add diluted methanol (2 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography. Perform the test with exactly 20 μL each of these solutions as directed under Thin-layer Chromatography. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.45 with respect to imidapril, obtained from the sample solution, is not larger than the peak area of imidapril from the standard solution, the area of the peak having the relative retention time of about 0.8 with respect to imidapril from the sample solution is not larger than 7/10 times the peak area of imidapril from the standard solution, and the area of each peak other than the peak of imidapril and other than those mentioned above from the sample solution is not larger than 3/10 times the peak area of imidapril from the standard solution. Furthermore, the total area of the peaks other than the peak of imidapril from the sample solution is not larger than 1.5 times the peak area of imidapril from the standard solution.

**Purity**

Related substances—To a quantity of powdered Imidapril Hydrochloride Tablets, equivalent to 25 mg of Imidapril Hydrochloride according to the labeled amount, add 40 mL of diluted methanol (2 in 5), shake vigorously for 10 minutes, add diluted ethanol (2 in 5) to make 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same Rf value as the spot from the standard solution.

**Containers and storage**

Containers—Well-closed containers.

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**Imidapril Hydrochloride Tablets**

imidapril塩酸塩

Imidapril Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of Imidapril Hydrochloride (C₂₀H₂₇N₃O₆·HCl: 441.91).

**Method of preparation**

Prepare as directed under Tablets, with Imidapril Hydrochloride.

**Identification**

Weigh accurately an amount of powdered Imidapril Hydrochloride Tablets, equivalent to 25 mg of Imidapril Hydrochloride according to the labeled amount, add 5 mL of ethanol (99.5), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 25 mg of imidapril hydrochloride in 5 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, ethyl acetate, water, ethanol (99.5) and acetic acid (100) (16:16:7:2:2) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same Rf value as the spot from the standard solution.

**Operating conditions**

- **Detector, column, column temperature, mobile phase and flow rate:** Proceed as directed in the operating conditions in the Assay.
- **Time span of measurement:** About 2 times as long as the retention time of imidapril, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 2 mL of the standard solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, ethyl acetate, water, ethanol (99.5) and acetic acid (100) (16:16:7:2:2) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same Rf value as the spot from the standard solution.
more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imidapril is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Imidapril Hydrochloride Tablets add 2V/5 mL of water, shake vigorously for 10 minutes, add diluted methanol (2 in 3) to make exactly V mL so that each mL contains about 0.1 mg of imidapril hydrochloride (C₂₀H₂₇N₃O₆.HCl), filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the standard solution. Separately, weigh accurately about 10 mg of imidapril for assay, previously dried at 105°C for 3 hours, dissolve in diluted methanol (2 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of imidapril in each solution.

Amount (mg) of imidapril hydrochloride (C₂₀H₂₇N₃O₆.HCl)
\[ M₅ \times A₁/A₅ \times V/100 \]

M₅: Amount (mg) of imidapril hydrochloride for assay

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of imidapril are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imidapril is not more than 1.0%.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Imidapril Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Imidapril Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 2.8 μg of imidapril hydrochloride (C₂₀H₂₇N₃O₆.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of imidapril hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of imidapril in each solution.

Dissolution rate (%) with respect to the labeled amount of imidapril hydrochloride (C₂₀H₂₇N₃O₆.HCl)
\[ M₅ \times A₁/A₅ \times V/V \times V/C \times 9 \]

M₅: Amount (mg) of imidapril hydrochloride for assay

C: Labeled amount (mg) of imidapril hydrochloride (C₂₀H₂₇N₃O₆.HCl) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of imidapril are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imidapril is not more than 2.0%.

Assay Weigh accurately not less than 20 Imidapril Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of imidapril hydrochloride (C₂₀H₂₇N₃O₆.HCl), add 30 mL of diluted methanol (2 in 5) and exactly 5 mL of the internal standard solution, shake vigorously for 10 minutes, add diluted methanol (2 in 5) to make 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add diluted methanol (2 in 5) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of imidapril hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in exactly 5 mL of the internal standard solution, add diluted methanol (2 in 5) to make 50 mL. Pipet 5 mL of this solution, add diluted methanol (2 in 5) to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₅, of the peak area of imidapril to that of the internal standard.

Amount (mg) of imidapril hydrochloride (C₂₀H₂₇N₃O₆.HCl)
\[ M₅ \times Q₁/Q₅ \]

M₅: Amount (mg) of imidapril hydrochloride for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (2 in 5) (1 in 500).

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.7 with phosphoric acid. To 600 mL of this solution add 400 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of imidapril is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, imidapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of imidapril to that of the internal standard is not more than 1.0%.

Containers and storage — Containers—Tight containers.

Imipenem Hydrate

イミペンネ氂和物

C₃₇H₆₃N₃O₇S.H₂O: 317.56  
(5R,6S)-3-2-(Formimidoylaminio)ethylsulfanyl]-6-[(1R)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate  
[74431-23-5]

Imipenem Hydrate contains not less than 980 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis. The potency of Imipenem Hydrate is expressed as mass (potency) of imipenem (C₁₂H₁₇N₃O₄S: 299.35).

Description — Imipenem Hydrate occurs as white to light yellow crystalline powder.

   It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) — Determine the absorption spectrum of a solution of Imipenem Hydrate in 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Imipenem RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) — Determine the infrared absorption spectrum of Imipenem Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Imipenem RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D: +89° to +94° (50 mg calculated on the anhydrous basis, 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0, 10 mL, 100 mm).

pH <2.54> — The pH of a solution obtained by dissolving 1.0 g of Imipenem Hydrate in 200 mL of water is between 4.5 and 7.0.

Purity (1) — Heavy metals <1.07> — Proceed with 1.0 g of Imipenem Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) — Arsenic <1.11> — Put 2.0 g of Imipenem Hydrate in a crucible, add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat carefully until white fumes evolve. After cooling, add 2 mL of nitric acid, heat, and repeat this procedure once more. Then add 2 mL of hydrogen peroxide (30), heat, and repeat this procedure several times until the color of the solution changes to colorless to pale yellow. After cooling, heat again until white fumes evolve. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 1 ppm).

(3) — Related substances — Dissolve 50 mg of Imipenem Hydrate in 50 mL of 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of thienamycin, having the relative retention time of about 0.8 with respect to imipenem, obtained from the sample solution is not larger than 1.4 times the peak area of imipenem from the standard solution, the area of the peak other than imipenem and thienamycin is not larger than 1/3 times the peak area of imipenem from the standard solution, and the total area of the peaks other than imipenem and thienamycin is not larger than the peak area of imipenem from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of imipenem.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, add 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 50 mL. Confirm that the peak area of imipenem from 10 μL of this solution is equivalent to 7 to 13% of that from the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imipenem is not more than 2.0%.

Water <2.48> — Not less than 5.0% and not more than 8.0% (20 mg, coulometric titration, water evaporation temperature: 140°C).

Residue on ignition <2.44> — Not more than 0.2% (1 g).

Assay — Weigh accurately an amount of Imipenem Hydrate and Imipenem RS, equivalent to about 50 mg (potency), dis-
solve each in 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution, within 30 minutes after preparation of these solutions, as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of imipenem of these solutions.

\[
\text{Amount [µg (potency)] of imipenem (C₁₂H₁₇N₃O₄S) = M_S \times A_T/A_S \times 1000}
\]

\( M_S \): Amount [mg (potency)] of Imipenem RS

**Operating conditions—**

- Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
- Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 and acetonitrile (100:1).
- Flow rate: Adjust the flow rate so that the retention time of imipenem is about 6 minutes.

**System suitability—**

- System performance: Dissolve 50 mg of Imipenem and 75 mg of resorcinol in 50 mL of 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0. When the procedure is run with 10 μL of this solution under the above operating conditions, imipenem and resorcinol are eluted in this order with the resolution between these peaks being not less than 4.
- System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imipenem is not more than 0.80%.

**Containers and storage** Containers—Hermetic containers.

### Imipenem and Cilastatin Sodium for Injection

**注射用イミペネム・シラスタチンナトリウム**

Imipenem and Cilastatin Sodium for Injection is a preparation for injection which is dissolved or suspended before use.

It contains not less than 93.0% and not more than 115.0% of the labeled amount of imipenem (C₁₂H₁₇N₃O₄S: 299.35) and an amount of cilastatin sodium (C₁₆H₂₅N₂NaO₅S: 380.43), equivalent to not less than 93.0% and not more than 115.0% of the labeled amount of cilastatin (C₁₆H₃₂N₂O₅S: 358.45).

**Method of preparation** Prepare as directed under Injections, with Imipenem Hydrate and Cilastatin Sodium.

**Description** Imipenem and Cilastatin Sodium for Injection occurs as a white to light yellowish white powder.

**Identification** (1) To 1 mL of a solution of Imipenem and Cilastatin Sodium for Injection (1 in 100) add 1 mL of ninhydrin TS, heat in a water bath for 5 minutes: a purple color appears (cilastatin).

(2) To 2 mL of a solution of Imipenem and Cilastatin Sodium for Injection (1 in 100) add 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 296 nm and 300 nm (imipenem).

**pH** <2.54> The pH of a solution prepared by dissolving an amount of Imipenem and Cilastatin Sodium for Injection, equivalent to 0.5 g (potency) of Imipenem Hydrate according to the labeled amount, in 100 mL of isotonic sodium chloride solution is between 6.5 and 8.0. The pH of the Injection intended for intramuscular use is between 6.0 and 7.5.

**Purity** Clarity and color of solution—Dissolve an amount of Imipenem and Cilastatin Sodium for Injection, equivalent to 0.5 g (potency) of Imipenem Hydrate according to the labeled amount, in 100 mL of isotonic sodium chloride solution: the solution is clear and colorless or pale yellow.

**Loss on drying** <2.41> Not more than 3.0% (1 g, in vacuum, 60°C, 3 hours).

**Bacterial endotoxins** <4.01> Less than 0.25 EU/mg (potency).

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test. Calculate the acceptance value by using the average of the limits specified in the potency definition for \( T \).

Dissolve the total amount of the content of 1 Imipenem and Cilastatin Sodium for Injection in isotonic sodium chloride solution to make 100 mL. Measure exactly \( V \) mL of this solution, equivalent to about 25 mg (potency) of Imipenem Hydrate, add 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 50 mL, and use this solution as the sample solution. Proceed hereafter as directed in the Assay.

\[
\text{Amount [mg (potency)] of imipenem (C₁₂H₁₇N₃O₄S) = M_S \times A_T/A_S \times 100/V}
\]

\( M_S \): Amount [mg (potency)] of Imipenem RS

\[
\text{Amount (mg) of cilastatin (C₁₆H₂₅N₂NaO₅S) = M_S \times A_T/A_S \times 100/V \times 0.955}
\]

\( M_S \): Amount (mg) of cilastatin ammonium for assay, calculated on the anhydrous basis and corrected on the amount of the residual solvent

**Foreign insoluble matter** <6.06> Perform the test according to the Method 2: the Injection which is dissolved before use meets the requirement.

**Insoluble particulate matter** <6.07> Perform the test according to the Method 1: the Injection which is dissolved before use meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.
Imipramine Hydrochloride
イミプラミン塩酸塩

C_{18}H_{24}N_{2}.HCl; 316.87
3-(10,11-Dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethylpropylamine monohydrochloride [113-52-0]

Imipramine Hydrochloride, when dried, contains not less than 98.5% of C_{18}H_{24}N_{2}.HCl.

Description
Imipramine Hydrochloride occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

The pH of the aqueous solution (1 in 10) is between 4.2 and 5.2.

It is gradually colored by light.

Identification
(1) Dissolve 5 mg of Imipramine Hydrochloride in 2 mL of nitric acid: a deep blue color develops.

(2) Dissolve 5 mg of Imipramine Hydrochloride in 250 mL of 0.01 mol/L hydrochloric acid TS. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Imipramine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Dissolve 0.05 g of Imipramine Hydrochloride in 5 mL of water, add 1 mL of ammonia TS, allow to stand for 5 minutes, filter, and acidify the filtrate with dilute nitric acid: it responds to the Qualitative Tests <1.09> (2) for chloride.

Melting point <2.60> 170 – 174°C (with decomposition).

Purity
(1) Clarity and color of solution—Dissolve 1.0 g of Imipramine Hydrochloride in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: Take exactly 1.0 mL of Cobalt (II) Chloride CS, 2.4 mL of Iron (III) Chloride CS, 0.4 mL of Copper (II) Sulfate CS and 6.2 mL of diluted hydrochloric acid (1 in 40), and mix them. Pipet 0.5 mL of this solution, and add exactly 9.5 mL of water.

(2) Iminodibenzyl—Dissolve 50 mg of Imipramine Hydrochloride in 10 mL of a mixture of hydrochloric acid and ethanol (95) (1:1) in a 25-mL brown volumetric flask. Cool the flask in ice water, add 5 mL of an ethanol (95) solution of furfural (1 in 250) and 5 mL of hydrochloric acid, and allow to stand at 25°C for 3 hours. Add a mixture of hydrochloric acid and ethanol (95) (1:1) to make 25 mL, and determine the absorbance of this solution at 565 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.16.

(3) Related substances—Dissolve 0.20 g of Imipramine Hydrochloride in 10 mL of ethanol (95), and use this solu-
tion as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 50 mL. Pipet 5 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \leq 0.03 \). Spot 5 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100), hydrochloric acid and water (11:7:1:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spots from the standard solution.

**Loss on drying** \( \leq 2.4\% \) Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** \( \leq 2.4\% \) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Imipramine Hydrochloride, previously dried, and dissolve in 20 mL of water. Add 5 mL of sodium hydroxide TS, and extract with three 20-mL portions of chloroform. Filter each extract through a membrane filter with a pore size not exceeding 0.8 \( \mu m \). Take 20 Imipramine Hydrochloride Tablets, add exactly 40 mL of 0.01 mol/L hydrochloric acid TS, disperse the tablet into a small particles using ultrasonic waves, then shake well. Centrifuge the solution, pipet 1 mL of the supernatant liquid, add water to make exactly 100 mL so that each mL contains about 20 \( \mu g \) of imipramine hydrochloride \( (C_{19}H_{24}N_2.HCl) \), and use this solution as the sample solution.

Separately, weigh accurately about 25 mg of Imipramine Hydrochloride RS, previously dried at 105°C for 2 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances at 251 nm, \( A_{T1} \), and \( A_{S1} \), and at 330 nm, \( A_{T2} \) and \( A_{S2} \), of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.24 \). Amount (mg) of imipramine hydrochloride \( (C_{19}H_{24}N_2.HCl) \)

\[
M_S = \frac{M_i \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times V/V \times 4/125}{M_S} \]

\[ M_S: \text{Amount (mg) of Imipramine Hydrochloride RS} \]

**Dissolution** \( \leq 6.10 \) When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Imipramine Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Imipramine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \( \mu m \). Discard the first 10 mL of the filtrate, pipet 1 mL of the subsequent filtrate, add the dissolution medium to make exactly 100 mL so that each mL of the filtrate contains about 10 \( \mu g \) of imipramine hydrochloride \( (C_{19}H_{24}N_2.HCl) \) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Imipramine Hydrochloride RS, previously dried at 105°C for 2 hours, dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and the standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.24 \).

Dissolution rate (%) with respect to the labeled amount of imipramine hydrochloride \( (C_{19}H_{24}N_2.HCl) \)

\[
C: \text{Labeled amount (mg) of imipramine hydrochloride (} C_{19}H_{24}N_2.HCl) \text{ in 1 tablet} \]

\[
M_S = \frac{M_i \times V/V \times 4/125}{M_S} \]

\[ M_S: \text{Amount (mg) of Imipramine Hydrochloride RS} \]

**Uniformity of dosage units** \( \leq 0.02 \) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Imipramine Hydrochloride Tablets add exactly 40 mL of 0.01 mol/L hydrochloric acid TS, disperse the tablet into a small particles using ultrasonic waves, then shake well. Centrifuging the solution, pipet a volume of the supernatant liquid, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Imipramine Hydrochloride RS, previously dried at 105°C for 2 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances at 251 nm, \( A_{T1} \), and \( A_{S1} \), and at 330 nm, \( A_{T2} \) and \( A_{S2} \), of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.24 \).
Indapamide

Indapamide contains not less than 98.5% and not more than 101.5% of C16H16ClN3O3S, calculated on the dried basis.

Description

Indapamide occurs as a white crystalline powder. It is freely soluble in ethanol (99.5), and practically insoluble in water.

A solution of Indapamide in ethanol (99.5) (1 in 10) shows no optical rotation.

Identification

(1) Determine the absorption spectrum of a solution of Indapamide in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Indapamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Indapamide as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum or the spectrum of Indapamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Indapamide as directed under Flame Coloration Test \(<1.04>\) (2): a green color appears.

Melting point

\(<2.60>\) 167 – 171°C.

Purity

(1) Chloride \(<1.03>\) – To 1.5 g of Indapamide add 50 mL of water, shake for 15 minutes, allow to stand in an ice bath for 30 minutes, and filter. To 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.01%).

(2) Heavy metals \(<1.07>\) – Proceed with 2.0 g of Indapamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances – Conduct this procedure using light-resistant vessels. Dissolve 0.10 g of Indapamide in 5 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (99.5) to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03>\). Spot 10 μL each of the sample solution, standard solution (1) and standard solution (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately develop the plate with a mixture of ethyl acetate, cyclohexane and acetic acid (100:80:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution (1), and the total amount of these related substances, calculated by comparison with the spots from the standard solutions (1) and (2), is not more than 2.0%.

(4) Residual solvent – Be specified separately.

Loss on drying \(<2.47>\) – Not more than 3.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 110°C, 2 hours).

Residue on ignition \(<2.44>\) – Not more than 0.1% (1 g).

Assay

Weigh accurately about 20 mg each of Indapamide and Indapamide RS (separately, determine the loss on drying \(<2.47>\) in the same condition as Indapamide), and dissolve each in a mixture of water and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 10 mL each of these solutions, add exactly 2 mL of the internal standard solution and a mixture of water and ethanol (99.5) (1:1) to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07>\) according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of indapamide to that of the internal standard.

\[
M_S = \frac{M_S \times Q_S}{Q_S}
\]

\(M_S\): Amount (mg) of Indapamide RS, calculated on the dried basis.
Indapamide Tablets

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Indapamide Tablets contain not less than 93.0% and not more than 103.0% of the labeled amount of indapamide (C₁₆H₁₆ClN₃O₃S: 365.83).

Method of preparation Prepare as directed under Tablets, with Indapamide.

Identification To an amount of powdered Indapamide Tablets, equivalent to 10 mg of Indapamide according to the labeled amount, add 5 mL of ethyl acetate, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Indapamide RS in 5 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, cyclohexane and acetic acid (100:80:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots of indapamide are not more than 1.5, respectively.

Uniformity of dosage units Dissolve 50 μL of the internal standard solution, add 1 mL of water and ethanol (99.5) (1:1) to make V mL so that each mL contains about 0.1 mg of indapamide (C₁₆H₁₆ClN₃O₃S), shake to disintegrate, treat with ultrasonic waves for 10 minutes, shake again for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of indapamide (C₁₆H₁₆ClN₃O₃S)

\[ M_S = \frac{V}{V/100} \]

\[ M_S: \text{Amount (mg) of Indapamide RS, calculated on the dried basis} \]

Dissolution When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rates in 45 minutes of 1-mg tablet and in 90 minutes of 2-mg tablet are not less than 70%, respectively.

Start the test with 1 tablet of Indapamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 1.1 μg of indapamide (C₁₆H₁₆ClN₃O₃S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Indapamide RS (separately, determine the loss on drying in the same condition as Indapamide), and dissolve in ethanol (99.5) to make exactly 10 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography in the following conditions, and determine the peak areas, \( A_T \) and \( A_{S} \), of indapamide in each solution.

Dissolution rate (%) with respect to the labeled amount of indapamide (C₁₆H₁₆ClN₃O₃S)

\[ \text{Dissolution rate} = \frac{A_T}{A_S} \times \frac{V}{V/100} \times 100 \]

\[ M_S: \text{Amount (mg) of Indapamide RS, calculated on the dried basis} \]

C: Labeled amount (mg) of indapamide (C₁₆H₁₆ClN₃O₃S) in 1 tablet

Operating conditions— Proceed as directed in the operating conditions in the Assay under Indapamide.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of indapamide are not less than 3500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of indapamide is not more than 1.5%.

Assay To 20 Indapamide Tablets add 80 mL of a mixture of water and ethanol (99.5) (1:1), shake well to disintegrate, and treat with ultrasonic waves for 10 minutes. Shake the solution for 10 minutes, and add a mixture of water and
ethanol (99.5) (1:1) to make exactly 100 mL. Pipet a volume of indapamide (C_{16}H_{16}ClN_{3}O_{3}S), equivalent to about 2 mg, and add exactly 2 mL of the internal standard solution and a mixture of water and ethanol (99.5) (1:1) to make 20 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Indapamide RS (separately, determine the loss on drying <2.41% in the same condition as Indapamide), and dissolve in a mixture of water and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution and a mixture of water and ethanol (99.5) (1:1) to make 20 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Indapamide.

\[
\text{Amount (mg) of indapamide (C}_{16}\text{H}_{16}\text{ClN}_{3}\text{O}_{3}\text{S}) = M_S \times Q_1/Q_2 \times 1/10
\]

M_S: Amount [mg (potency)] of Indapamide RS, calculated on the dried basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in a mixture of water and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet a volume of this solution, add exactly 2 mL of the internal standard solution and a mixture of water and ethanol (99.5) (1:1) to make 20 mL, and use this solution as the standard solution.

Containers—Tight containers.

## Indenolol Hydrochloride

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\[
\text{C}_{15}\text{H}_{21}\text{NO}_{2}\text{.HCl} : 283.79
\]

(2RS)-1-(3H-Inden-4-yl)-3-(1-methylethyl)aminopropan-2-ol monohydrochloride

(2RS)-1-(3H-Inden-7-yl)-3-(1-methylethyl)aminopropan-2-ol monohydrochloride

When dried, it contains not less than 98.5% of C_{15}H_{21}NO_{2}.HCl.

**Description**  Indenolol Hydrochloride occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in water and in acetic acid (100), soluble in ethanol (95) and in chloroform, slightly soluble in acetic anhydride, very slightly soluble in ethyl acetate, and practically insoluble in diethyl ether.

The pH of a solution of Indenolol Hydrochloride (1 in 10) is between 3.5 and 5.5.

It is colored by light.

**Identification** (1) Dissolve 0.1 g of Indenolol Hydrochloride in 1 to 2 drops of dilute hydrochloric acid and 5 mL of water, and add 1 mL of Reinecke salt TS: a red-purple precipitate is formed.

(2) Determine the absorption spectrum of a solution of Indenolol Hydrochloride (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Indenolol Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Indenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Indenolol Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

Absorbance <2.24> \( E_{1\%}^{1\text{cm}} (250 \text{ nm}) : 330 - 340 \) (after drying, 10 mg, water, 1000 mL).

**Melting point** <2.6b> 140 – 143°C.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Indenolol Hydrochloride in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Indenolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Indenolol Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Indenolol Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, ethanol (99.5) and ammonia solution (28:70:15:2) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Isomer ratio** Dissolve 5 mg of Indenolol Hydrochloride in 1.0 mL of a mixture of ethyl acetate and dehydrated trifluoroacetic acid (9:1), and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the areas of two adjacent peaks, A_s and A_p, having the retention times of about 16 minutes, where A_s is the peak area of shorter retention time and A_p is the peak area of longer retention time: the
ratio $A_s/(A_d + A_s)$ is between 0.6 and 0.7.

**Operating conditions—**

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 2 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm in particle diameter) coated with 65% phenyl-methyl silicon polymer for gas chromatography at the ratio of 2%.

Column temperature: A constant temperature between 150°C and 170°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of the peak showing earlier elution of the two peaks of indenolol hydrochloride is about 16 minutes.

Selection of column: Proceed with 2 μL of the sample solution under the above operating conditions, and calculate the resolution. Use a column with the resolution between the two peaks being not less than 1.1.

**Assay**

Weigh accurately about 0.5 g of Indenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), and titrate chloride, previously dried, dissolved in 50 mL of a mixture of ammonium acetate (1 in 650). To 1 mL of the solution add a solution of ammonium acetate (1 in 650) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2.2.4), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

1. Ignite 1 g of Indigocarmine to carbonize. After cooling, add 20 mL of water to the residue, shake, and filter the mixture: the filtrate responds to the Qualitative Tests (1.09) for sodium salt and for sulfate.

**pH**

$<2.54$ Dissolve 0.1 g of Indigocarmine in 20 mL of water: the pH of the solution is between 5.0 and 6.0.

**Purity (1)**

Water-insoluble substances—To 1.00 g of Indigocarmine add 200 mL of water, shake, and filter through a tared glass filter (G4). Wash the residue with water until the blue color of the filtrate becomes practically colorless, and dry the residue at 105°C for 4 hours: the mass of the residue does not exceed 0.5 mg.

2. Arsenic (1.11)—Place 0.8 g of Indigocarmine in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and ignite gently. Repeat the addition of 2 to 3 mL of nitric acid occasionally, and continue to heat until a colorless to light yellow solution is obtained. After cooling, add 15 mL of a saturated ammonium oxalate solution, heat the solution until dense white fumes are evolved, and concentrate to 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with 5 mL of this solution as the test solution (not more than 5 ppm).

**Loss on drying** $<2.41$ Not more than 10.0% (1 g, 105°C, 2 hours).

**Residue on ignition** $<2.44$ Not less than 28.0% and not more than 38.0% (after drying, 1 g).

**Assay**

Weigh accurately about 0.5 g of Indigocarmine, previously dried, add 15 g of sodium hydroxide tartrate monohydrate, and dissolve in 200 mL of water, boil with bubbling of a stream of carbon dioxide, and titrate $<2.50$, while being hot, with 0.1 mol/L titanium (III) chloride VS until the color of the solution changes from blue through yellow to orange.

Each mL of 0.1 mol/L titanium (III) chloride VS
$= 23.32$ mg of C$_{16}$H$_{9}$N$_{2}$Na$_{2}$O$_{8}$S$_{2}$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

**Indigocarmine Injection**

インジゴカルミン注射液

Indigocarmine Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of indigocarmine (C$_{16}$H$_{9}$N$_{2}$Na$_{2}$O$_{8}$S$_{2}$: 466.35).
Method of preparation Prepare as directed under Injection, with Indigocarmine.

Description Indigocarmine Injection is a dark blue liquid.

pH: 3.0 – 5.0

Identification (1) To a volume of Indigocarmine Injection, equivalent to 20 mg of Indigocarmine according to the labeled amount, add 1 mL of nitric acid: the dark blue color of the liquid disappears, and a yellow-brown color develops.

(2) To a volume of Indigocarmine Injection, equivalent to 20 mg of Indigocarmine according to the labeled amount, add 1 mL of bromine TS: the dark blue color disappears, and a yellow-brown color develops.

(3) To a volume of Indigocarmine Injection, equivalent to 20 mg of Indigocarmine according to the labeled amount, add 1 mL of chlorine TS: the dark blue color disappears, and a yellow-brown color develops.

(4) To a volume of Indigocarmine Injection, equivalent to 10 mg of Indigocarmine according to the labeled amount, add ammonium acetate solution (1 in 650) to make 1000 mL, and determine the absorbance of the solution as directed under Ultraviolet-visible Spectrophotometry \( <2.24 > \): it exhibits a maximum between 610 nm and 614 nm.

Bacterial endotoxins \( <4.06 > \) Less than 7.5 EU/mg.

Extractable volume \( <6.05 > \) It meets the requirement.

Foreign insoluble matter \( <6.06 > \) Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter \( <6.07 > \) Perform the test according to Method 2: it meets the requirement.

Sterility \( <4.06 > \) Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Indigocarmine Injection, equivalent to about 0.2 g of indigocarmine \( (C_{16}H_{8}N_{2}Na_{2}O_{8}S_{2}) \), add 6 g of sodium hydrogen tartrate monohydrate, and dissolve in water to make 200 mL. Then boil under a carbon dioxide stream, and proceed as directed in the Assay under Indigocarmine.

Each mL of 0.1 mol/L titanium (III) chloride VS = 23.32 mg of \( C_{16}H_{8}N_{2}Na_{2}O_{8}S_{2} \)

Containers and storage Containers—Hermetic containers, and colored containers may be used. Storage—Light-resistant.

Indium \( (^{111}\text{In}) \) Chloride Injection

塩化インジウム \( (^{111}\text{In}) \) 注射液

Indium \( (^{111}\text{In}) \) Chloride Injection is an aqueous solution for injection.

It contains indium-111 \( (^{111}\text{In}) \) in the form of indium chloride.

It conforms to the requirements of Indium \( (^{111}\text{In}) \) Chloride Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Indium \( (^{111}\text{In}) \) Chloride Injection is a clear, colorless liquid.
to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spots 25 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dehydrated diethyl ether and acetic acid (100) (100:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.7 g of Indometacin, previously dried, dissolve in 60 mL of methanol, add 30 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a back titration.

Each mL of 0.1 mol/L sodium hydroxide VS = 35.78 mg of C_{19}H_{16}ClNO_{4}

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Indometacin Capsules**

インドメタシンカプセル

Indometacin Capsules contain not less than 90.0% and not more than 110.0% of the labeled amount of indometacin (C_{19}H_{16}ClNO_{4}; 357.79).

**Method of preparation** Prepare as directed under Capsules, with Indometacin.

**Identification** Powder the contents of Indometacin Capsules. To a quantity of the powder, equivalent to 0.1 g of Indometacin according to the labeled amount, add 20 mL of chloroform, shake well, and centrifuge. Filter the supernatant liquid, and evaporate the filtrate to dryness. After cooling, dissolve the residue in 20 mL of methanol. To 10 mL of this solution add methanol to make 50 mL, then to 2 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits a maximum between 317 nm and 321 nm.

**Purity** Related substances—Powder the content of Indometacin Capsules. To a quantity of the powder, equivalent to 0.10 g of Indometacin according to the labeled amount, add exactly 10 mL of methanol, shake well, filter, and use the filtrate as the sample solution. Dissolve 25 mg of Indometacin RS in methanol to make exactly 50 mL. Pipet 1 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Proceed as directed in the Purity (4) under Indometacin.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the content of 1 capsule of Indometacin Capsules, and dissolve in methanol to make exactly V mL so that each mL contains about 1 mg of indometacin (C_{19}H_{16}ClNO_{4}). Filter the solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Indometacin RS, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 3 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of indometacin (C_{19}H_{16}ClNO_{4}) = \frac{M_S \times Q_1}{Q_2 \times V/25}

M_S: Amount (mg) of Indometacin RS

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 1000).

**Dissolution** <6.10> When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of a mixture of water and phosphate buffer solution, pH 7.2 (4:1) as the dissolution medium, the dissolution rate in 20 minutes of Indometacin Capsules is not less than 75%.

Start the test with 1 capsule of Indometacin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the dissolution medium to make exactly V mL so that each mL contains about 28 μg of indometacin (C_{19}H_{16}ClNO_{4}), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Indometacin RS, previously dried at 105°C for 4 hours, dissolve in the dissolution medium to make exactly 1000 mL, and use this as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 320 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of indometacin (C_{19}H_{16}ClNO_{4}) = \frac{M_S \times A_T}{A_S} \times \frac{1}{C} \times 90

M_S: Amount (mg) of Indometacin RS

C: Labeled amount (mg) of indometacin (C_{19}H_{16}ClNO_{4}) in 1 capsule

**Assay** Weigh accurately the contents of not less than 20 Indometacin Capsules. Powder the combined contents, and weigh accurately a portion of the powder, equivalent to about 50 mg of indometacin (C_{19}H_{16}ClNO_{4}). Dissolve in 40 mL of methanol, and add methanol to make exactly 50 mL. Filter this solution, discarding the first 10-mL portion of the filtrate. Pipet the subsequent 5 mL of the filtrate, add exactly 3 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Indometacin RS, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of the solution, add exactly 3 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution
as the sample solution. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and calculate the ratios, \(Q_1\) and \(Q_3\), of the peak area of indometacin to that of the internal standard, respectively.

\[
\text{Amount (mg) of indometacin (C}_{19}\text{H}_{16}\text{ClNO}_4) = M_s \times Q_1/Q_3
\]

\(M_s: \text{Amount (mg) of Indometacin RS}\)

**Internal standard solution**—A solution of butyl parahydroxybenzoate in methanol (1 in 1000).

**Operating conditions**—

- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 µm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of methanol and dilute phosphoric acid (1 in 1000) (7:3).
- Flow rate: Adjust the flow rate so that the retention time of indometacin is about 8 minutes.
- System suitability—

  System performance: Dissolve 50 mg of 4-chlorobenzoic acid, 30 mg of butyl parahydroxybenzoate and 50 mg of indometacin in 50 mL of methanol. To 5 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 20 µL of this solution under the above operating conditions, 4-chlorobenzoic acid, butyl parahydroxybenzoate and indometacin are eluted in this order, with the resolution between the peaks of 4-chlorobenzoic acid and butyl parahydroxybenzoate being not less than 2.0, and between the peaks of butyl parahydroxybenzoate and indometacin being not less than 5.

  System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of indometacin to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

### Indometacin Suppositories

インドメタシン坐剤

Indometacin Suppositories contain not less than 90.0% and not more than 110.0% of the labeled amount of indometacin (C<sub>19</sub>H<sub>16</sub>ClNO<sub>4</sub>; 357.79).

**Method of preparation**—Prepare as directed under Suppositories, with Indometacin.

**Identification**—Dissolve a quantity of Indometacin Suppositories, equivalent to 50 mg of Indometacin according to the labeled amount, in 20 mL of methanol by warming, add methanol to make 50 mL, and filter if necessary. To 2 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\); it exhibits a maximum between 317 nm and 321 nm.

**Uniformity of dosage units**—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 suppository of Indometacin Suppositories add 80 mL of a mixture of methanol and acetic acid (100) (200:1), dissolve by warming, add a mixture of methanol and acetic acid (100) (200:1) to make exactly 100 mL. Pipet V mL of this solution, equivalent to about 2 mg of indometacin (C<sub>19</sub>H<sub>16</sub>ClNO<sub>4</sub>), add a mixture of methanol and acetic acid (100) (200:1) to make exactly 30 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Indometacin RS, previously dried at 105°C for 4 hours, and dissolve in a mixture of methanol and acetic acid (100) (200:1) to make exactly 100 mL. Pipet 4 mL of this solution, add a mixture of methanol and acetic acid (100) (200:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\); and determine the absorbances, \(A_T\) and \(A_S\), at 320 nm.

\[
\text{Amount (mg) of indometacin (C}_{19}\text{H}_{16}\text{ClNO}_4) = M_s \times A_T/A_S \times 2/V
\]

\(M_s: \text{Amount (mg) of Indometacin RS}\)

**Assay**—Weigh accurately not less than 20 Indometacin Suppositories, cut into small pieces carefully, and mix well. Weigh accurately a portion of the mass, equivalent to about 50 mg of indometacin (C<sub>19</sub>H<sub>16</sub>ClNO<sub>4</sub>), add 40 mL of tetrahydrofuran, warm at 40°C, dissolve by shaking, cool, and add tetrahydrofuran to make exactly 50 mL. Filter the solution, discard the first 10 mL of the filtrate, and then filter 5 mL of the filtrate, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 100 mL. Allow the solution to stand for 30 minutes, filter through a membrane filter (0.5 µm pore size), discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Indometacin RS, previously dried at 105°C for 4 hours, and dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of the solution, proceed in the same manner as the sample solution, and use as the standard solution. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and calculate the ratios, \(Q_1\) and \(Q_3\), of the peak area of indometacin to that of the internal standard, respectively.

\[
\text{Amount (mg) of indometacin (C}_{19}\text{H}_{16}\text{ClNO}_4) = M_s \times Q_1/Q_3
\]

\(M_s: \text{Amount (mg) of Indometacin RS}\)

**Internal standard solution**—A solution of butyl parahydroxybenzoate in methanol (1 in 1000).

**Operating conditions**—

- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 µm in particle diameter).
- Column temperature: A constant temperature of about 40°C.
25°C.

Mobile phase: A mixture of methanol and dilute phosphoric acid (1 in 1000) (7:3).
Flow rate: Adjust the flow rate so that the retention time of indometacin is about 8 minutes.

System suitability—

System performance: Dissolve 50 mg of 4-chlorobenzoic acid, 30 mg of butyl parahydroxybenzoate and 50 mg of indometacin in 50 mL of methanol. To 5 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 20μL of this solution under the above operating conditions, 4-chlorobenzoic acid, butyl parahydroxybenzoate and indometacin are eluted in this order with the resolution between the peaks of 4-chlorobenzoic acid and butyl parahydroxybenzoate being not less than 2.0 and between the peaks of parahydroxybenzoate and indometacin being not less than 5.

System repeatability: When the test is repeated 6 times with 20μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of indometacin to that of the internal standard is not more than 1.0%

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant, and in a cold place.

Influenza HA Vaccine インフルエンザ HA ワクチン

Influenza HA Vaccine is a liquid for injection containing hemagglutinin of influenza virus.

It conforms to the requirements of Influenza HA Vaccine in the Minimum Requirements for Biological Products.

Description Influenza HA Vaccine is a clear liquid or a slightly whitish turbid liquid.

Insulin Human (Genetical Recombination) ヒトインスリン（遺伝子組換え）

C_{257}H_{383}N_{65}O_{77}S_{6}: 5807.57
[11061-68-0]

Insulin Human (Genetical Recombination) is a human insulin prepared by genetical recombinant technology.

It has an activity to reduce the blood sugar concentration. It contains not less than 27.5 Insulin Units per mg, calculated on the dried basis.

Description Insulin Human (Genetical Recombination) occurs as a white powder.

It is practically insoluble in water and in ethanol (95).

It dissolves in 0.01 mol/L hydrochloric acid TS and in sodium hydroxide TS with decomposition.

It is hygroscopic.

Identification Weigh accurately a suitable amount of Insulin Human (Genetical Recombination), and dissolve in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains 2.0 mg. Transfer 500μL of this solution into a clean test tube, add 2.0 mL of HEPES buffer solution, pH 7.5 and 400μL of V8-protease TS, react at 25°C for 6 hours, then add 2.9 mL of ammonium sulfate buffer solution to stop the reaction, and use this solution as the sample solution. Separately, proceed with Human Insulin RS in the same manner as above, and use this solution as the standard solution. Perform the test with exactly 50μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the chromatograms obtained from these solutions: the peak appears just after the peak of the solvent and the succeeding three peaks with apparently higher peak height show the same retention time and similar peak height each other on both chromatograms.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3μm in particle diameter).
Column temperature: A constant temperature of about 40°C.

Change the mixing ratio of the solutions A and B linearly from 9:1 to 3:7 in 60 minutes after sample injection, further change to 0:10 linearly in 5 minutes, and then flow the solution B only for 5 minutes.
Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 50μL of the standard solution under the above operating conditions, the symmetry factor of the two larger peaks which appear next to the first peak just after the solvent peak are not more than 1.5, and the resolution between these peaks is not less than 3.4.

Purity (I) Related substances—Perform this procedure rapidly. Dissolve 7.5 mg of Insulin Human (Genetical Recombination) in 2 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test with 20μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak area of human insulin, A_{t}, the peak area of the desamide substance at the relative retention time of 1.3 to the human insulin, A_{d}, and the total area of the peaks other than the solvent peak, A_{t}: the amounts of the desamide substance and related substances other than the desamide substance are each not more than 2.0%. Previously, perform the test with 0.01 mol/L hydrochloric acid TS in the same manner to confirm the solvent peak.

Amount (% of the desamide substance = A_{d}/A_{t} × 100
Amount (%) of related substances other than the desamide substance \( = \left[ \frac{(A_{2} - (A_{1} + A_{3})/A_{1}) \times 100}{M} \right] \)

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 241 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Solution A—A mixture of phosphoric acid-sodium sulfate buffer solution, pH 2.3 and acetonitrile for liquid chromatography (41:9). Solution B—A mixture of phosphoric acid-sodium sulfate buffer solution, pH 2.3 and acetonitrile for liquid chromatography (1:1).
Flow a mixture of the solution A and the solution B (78:22) for 36 minutes before and after the sample injection, then change the mixing ratio to 33:67 linearly in 25 minutes, and maintain this ratio for 6 minutes. Then flow the first mixture (78:22) for the next 15 minutes. Adjust the mixing ratio of the first mixture so that the retention time of human insulin is about 25 minutes.
Flow rate: 1.0 mL per minute.
Time span of measurement: For about 75 minutes after the sample is injected.

System suitability—
Test for required detection: Confirm that the peak height of the desamide substance obtained from 20 \( \mu \)L of human insulin desamide substance-containing TS is between 30% and 70% of the full scale.
System performance: When the procedure is run with 20 \( \mu \)L of human insulin desamide substance-containing TS under the above operating conditions, human insulin and human insulin desamide substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of human insulin is not more than 1.8.

(2) High-molecular proteins—Dissolve 4 mg of Insulin Human (Genetical Recombination) in 1 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 100 \( \mu \)L of this solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate each peak area: the total of areas of the peaks having smaller retention time than human insulin is not more than 1.0% of the total area of all peaks.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 276 nm).
Column: A stainless steel column 7.5 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography.
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of a solution of L-arginine (1 in 1000), acetonitrile, and acetic acid (100) (13:4:3).
Flow rate: Adjust the flow rate so that the retention time of human insulin is about 20 minutes.
Time span of measurement: Until the peak of human insulin monomer has appeared.

System suitability—
Test for required detection: Confirm that the peak height of the dimer obtained from 100 \( \mu \)L of human insulin dimer containing TS is between 10% and 50% of the full scale.
System performance: When the procedure is run with 100 \( \mu \)L of human insulin dimer containing TS under the above operating conditions, polymer, dimer, and monomer are eluted in this order, and the ratio, \( H_{1}/H_{2} \), of the peak height of the dimer \( H_{1} \) to the height of the bottom between the peaks of the dimer and the monomer \( H_{2} \) is not less than 2.0.

(3) Product related impurities—Being specified separately.
(4) Process related impurities—Being specified separately.

Zinc content Weigh accurately about 50 mg of Insulin Human (Genetical Recombination), and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL. If necessary, dilute with 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains between 0.4 \( \mu \)g and 1.6 \( \mu \)g of zinc (Zn: 65.38), and use this solution as the sample solution. Separately, take exactly a suitable amount of Standard Zinc Solution for Atomic Absorption Spectrophotometry, dilute with 0.01 mol/L hydrochloric acid TS to make solutions containing 0.40 \( \mu \)g, 0.80 \( \mu \)g, 1.20 \( \mu \)g and 1.60 \( \mu \)g of zinc (Zn: 65.38) in each mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <2.23>, and determine the amount of zinc (Zn: 65.38) in the sample solution by using a calibration curve obtained from the absorbances of the standard solutions: not more than 1.0%, calculated on the dried basis.
Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Zinc hollow cathode lamp.
Wavelength: 213.9 nm.

Loss on drying <2.41> Not more than 10.0% (0.2 g, 105°C, 24 hours).

Bacterial endotoxins <4.01> Less than 10 EU/mg.

Assay Perform this procedure quickly. Weigh accurately about 7.5 mg of Insulin Human (Genetical Recombination), dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately a suitable amount of Human Insulin RS, dissolve in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains about 40 Insulin Units, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.01> according to the following conditions, and determine the peak areas of human insulin, \( A_{SD} \) and \( A_{SI} \), and the peak areas of the desamide substance at the relative retention time of 1.3 to the human insulin, \( A_{TD} \) and \( A_{TD} \), respectively, of these solutions.

Amount (Insulin Unit/mg) of human insulin
\[
(C_{21}H_{17}N_{5}O_{5})_{2}\]

\[
= (M_{F} \times F) / D \times (A_{TD} + A_{SD}) / (A_{SI} + A_{SD}) \times 5 / M_{T}
\]

\( M_{T} \): Amount (mg) of the sample calculated on the dried basis
\( M_{F} \): Amount (mg) of Human Insulin RS

\( D \): Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve the reference standard
\( F \): Label unit (Insulin Unit/mg)

Time span of measurement: Until the peak of human insulin monomer has appeared.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 214 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of phosphoric acid-sodium sulfate buffer solution, pH 2.3 and acetonitrile for liquid chromatography (3:1). Adjust the mixing ratio of the component of the mobile phase so that the retention time of human insulin is between 10 minutes and 17 minutes.
Flow rate: 1.0 mL per minute.
System suitability—
System performance: When the procedure is run with 20 μL of human insulin desamide substance-containing TS under the above operating conditions, human insulin and human insulin desamide substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of human insulin is not more than 1.8.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of human insulin is not more than 1.6%.
Containers and storage Containers—Tight containers.
Storage—Light-resistant, and at −20°C or below.

Iodamide
ヨーダミド

C₁₂H₁₁I₃N₂O₄: 627.94
3-Acetylaminoo-5-acetylaminomethyl-2,4,6-triiodobenzoic acid [440-58-4]

Iodamide, calculated on the dried basis, contains not less than 98.5% of C₁₂H₁₁I₃N₂O₄.

Description Iodamide occurs as a white, crystalline powder. It is odorless.

It is slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

It gradually changes in color by light.

Identification (1) To 10 mg of Iodamide add 5 mL of hydrochloric acid, and heat in a water bath for 5 minutes: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Heat 0.1 g of Iodamide over a flame: a purple gas evolves.

(3) Determine the infrared absorption spectrum of Iodamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.257, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 1 g of Iodamide in 100 mL of water by heating, and concentrate the solution to about 30 mL by gentle boiling. After cooling, collect the formed crystals by filtration, dry, and repeat the test on the dried crystals.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Iodamide in 10 mL of diluted sodium hydroxide TS (1 in 5): the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.20 g of Iodamide in 5 mL of water and 1 mL of sodium hydroxide TS, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake well, and allow to stand for 2 minutes. To this solution add 5 mL of ammonium amidosulfate TS, shake thoroughly, allow to stand for 1 minute, add 0.4 mL of a solution of 1-naphthol in ethanol (95) (1 in 10), 15 mL of sodium hydroxide TS and water to make exactly 50 mL, and determine the absorbance at 485 nm as directed under Ultraviolet-visible Spectrophotometry 2.24a, using a solution, prepared in the same manner, as the blank: the absorbance of the solution is not more than 0.12.

(3) Soluble halide—Dissolve 2.5 g of Iodamide in 20 mL of water and 2.5 mL of ammonia TS, then add 20 mL of dilute nitric acid and water to make 100 mL. Allow to stand for 15 minutes with occasional shaking, and filter. Discard the first 10 mL of the filtrate, transfer 25 mL of the subsequent filtrate to a Nessler tube, and add ethanol (95) to make 50 mL. Use this solution as the test solution, and proceed as directed under Chloride Limit Test <1.03>. Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid VS and 6 mL of dilute nitric acid, and dilute with water to 25 mL, then with ethanol (95) to 50 mL.

(4) Iodine—Dissolve 0.20 g of Iodamide in 2 mL of sodium hydroxide TS, add 2.5 mL of 0.5 mol/L sulfuric acid TS, allow to stand for 10 minutes with occasional shaking, then add 5 mL of chloroform, shake vigorously and allow to stand: the chloroform layer remains colorless.

(5) Heavy metals <1.07>—Proceed with 2.0 g of Iodamide according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of Iodamide according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.47> Not more than 3.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Iodamide in a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, connect the flask with a reflux condenser, boil for 30 minutes, cool, and filter. Wash the flask and filter paper with 50 mL of water, and combine the washings with the filtrate. Add 5 mL of acetic acid (100), and titrate <2.59> with 0.1 mol/L silver nitrate VS until the color of the precipitate changes from yellow to green (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS).

Each mL of 0.1 mol/L silver nitrate VS = 20.93 mg of C₁₂H₁₁I₃N₂O₄
Iodinated (131I) Human Serum Albumin Injection

ヨウ化人血清アルブミン (131I) 注射液

Iodinated (131I) Human Serum Albumin Injection is an aqueous solution for injection containing normal human serum albumin iodinated by iodine-131 (131I).

It conforms to the requirements of Iodinated (131I) Human Serum Albumin Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Iodinated (131I) Human Serum Albumin Injection is a clear, colorless or light yellow liquid.

Iodine

ヨウ素

I: 126.90

Iodine contains not less than 99.5% of I.

Description Iodine occurs as grayish black plates or heavy, granular crystals, having a metallic luster and a characteristic odor.

It is freely soluble in diethyl ether, soluble in ethanol (95), sparingly soluble in chloroform, and very slightly soluble in water.

It dissolves in potassium iodide TS.

It sublimes at room temperature.

Identification (1) A solution of Iodine in ethanol (95) (1 in 50) shows a red-brown color.

(2) A solution of Iodine in chloroform (1 in 1000) shows a red-purple to purple color.

(3) Add 0.5 mL of starch TS to 10 mL of a saturated solution of Iodine: a dark blue color is produced. When the mixture is boiled, the color disappears, and it reappears on cooling.

Purity (1) Non-volatile residue—Sublimes 2.0 g of Iodine on a water bath, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

(2) Chloride or bromide—Mix 1.0 g of finely powdered Iodine with 20 mL of water, and filter the mixture. To 10 mL of the filtrate add dropwise diluted sulfuric acid solution (1 in 5) until the yellow color disappears. Add 1 mL of ammonia TS, followed by 1 mL of silver nitrate TS in small portions, and add water to make 20 mL. Shake well, filter, and after discarding the first 2 mL of the filtrate, take 10 mL of the subsequent filtrate. To the filtrate add 2.0 mL of nitric acid and water to make 20 mL: the solution so obtained has no more turbidity than the following control solution.

Control solution: To 0.20 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of water, 2.5 mL of ammonia TS, 1 mL of silver nitrate TS, 2.0 mL of nitric acid and water to make 20 mL.

Assay Place 1 g of potassium iodide and 1 mL of water in a glass-stoppered flask, weigh accurately, add about 0.3 g of iodine to the flask, and weigh accurately again. Dissolve the iodine by gentle shaking, add 20 mL of water and 1 mL of dilute hydrochloric acid, and titrate less than 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS = 12.69 mg of I

Containers and storage Containers—Tight containers.

Iodine Tincture

ヨードチンキ

Iodine Tincture contains not less than 5.7 w/v% and not more than 6.3 w/v% of iodine (I: 126.90), and not less than 3.8 w/v% and not more than 4.2 w/v% of potassium iodide (KI: 166.00).

Method of preparation

<table>
<thead>
<tr>
<th>Iodine</th>
<th>60 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Iodide</td>
<td>40 g</td>
</tr>
<tr>
<td>70 vol% Ethanol</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Spirits, with the above ingredients. It may be prepared with an appropriate quantity of Ethanol or Ethanol for Disinfectant and Purified Water or Purified Water in Containers in place of 70 vol% Ethanol.

Description Iodine Tincture is a dark red-brown liquid, and has a characteristic odor. Specific gravity d20°: about 0.97

Identification (1) To a mixture of 1 mL of starch TS and 9 mL of water add 1 drop of Iodine Tincture: a dark blue-purple color develops.

(2) Evaporate 3 mL of Iodine Tincture to dryness on a water bath, and heat gently over a free flame: a white residue is formed which responds to the Qualitative Tests <1.0% for potassium salt and iodide.

Alcohol number <1.0% Not less than 6.6 (Method 2). Perform the pretreatment (ii) in the Method 1.

Assay (1) Iodine—Pipe 5 mL of Iodine Tincture, add 0.5 g of potassium iodide, 20 mL of water and 1 mL of dilute hydrochloric acid, and titrate less than 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS = 12.69 mg of I

(2) Potassium iodide—Pipe 5 mL of Iodine Tincture into an iodine flask, add 20 mL of water, 50 mL of hydrochloric acid and 5 mL of chloroform. Cool to room temperature, and titrate less than 0.05 mol/L potassium iodate VS until the red-purple color disappears from the chloroform layer, with agitating the mixture vigorously and continuous-
Dilute Iodine Tincture

Dilute Iodine Tincture contains not less than 2.8 w/v% and not more than 3.2 w/v% of iodine (I: 126.90), and not less than 1.9 w/v% and not more than 2.1 w/v% of potassium iodide (KI: 166.00).

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine</td>
<td>30 g</td>
<td></td>
</tr>
<tr>
<td>Potassium Iodide</td>
<td>20 g</td>
<td></td>
</tr>
<tr>
<td>70 vol% Ethanol</td>
<td>a sufficient quantity</td>
<td>To make 1000 mL</td>
</tr>
</tbody>
</table>

Prepare as directed under Spirits, with the above ingredients. It may be prepared with an appropriate quantity of ethanol or ethanol for disinfection and purified water or purified water in containers in place of 70 vol% ethanol. It may also be prepared by adding 70 vol% ethanol to 500 mL of iodine tincture to make 1000 mL.

Description Dilute Iodine Tincture is a dark red-brown liquid, and has a characteristic odor.

Specific gravity $d_{20}^{20}$: about 0.93

Identification (1) To a mixture of 1 mL of starch TS and 9 mL of water add 1 drop of dilute iodine tincture: a dark blue-purple color develops.

(2) Evaporate 3 mL of dilute iodine tincture to dryness on a water bath, and heat gently over a free flame: a white residue is formed which responds to the qualitative tests $<1.00>$ for potassium salt and iodide.

Alcohol number $<1.01>$ Not less than 6.7 (Method 2). Perform the pretreatment (ii) in the Method 1.

Assay (1) Iodine—Pipet exactly 10 mL of dilute iodine tincture, add 0.5 g of potassium iodide, 20 mL of water and 1 mL of dilute hydrochloric acid, and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS

= $12.69$ mg of I

(2) Potassium iodide—Pipet exactly 10 mL of dilute iodine tincture into an iodine flask, add 20 mL of water, 50 mL of hydrochloric acid and 5 mL of chloroform. Cool to room temperature, and titrate with 0.05 mol/L potassium iodate VS until the red-purple color in the chloroform layer disappears while agitating vigorously and continuously.

After the chloroform layer has been decolorized, allow the mixture to stand for 5 minutes. If the color reappears, the mixture should be titrated with 0.05 mol/L potassium iodate VS. Calculate the amount (mg) of potassium iodide from the volume (a mL) of 0.05 mol/L potassium iodate VS consumed as above and the volume (b mL) of 0.1 mol/L sodium thiosulfate VS used in the titration under the Assay (1).

Amount (mg) of potassium iodide (KI)

= $16.60 \times [a - (b/2)]$

Containers and storage Containers—Tight containers.

Compound Iodine Glycerin

Compound Iodine Glycerin contains not less than 1.1 w/v% and not more than 1.3 w/v% of iodine (I: 126.90), not less than 2.2 w/v% and not more than 2.6 w/v% of potassium iodide (KI: 166.00), not less than 2.7 w/v% and not more than 3.3 w/v% of total iodine (as I), and not less than 0.43 w/v% and not more than 0.53 w/v% of phenol (C₆H₅O: 94.11).

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine</td>
<td>12 g</td>
<td></td>
</tr>
<tr>
<td>Potassium Iodide</td>
<td>24 g</td>
<td></td>
</tr>
<tr>
<td>Glycerin</td>
<td>900 mL</td>
<td></td>
</tr>
<tr>
<td>Mentha Water</td>
<td>45 mL</td>
<td></td>
</tr>
<tr>
<td>Liquefied Phenol</td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td>Water in Containers</td>
<td>a sufficient quantity</td>
<td>To make 1000 mL</td>
</tr>
</tbody>
</table>

Dissolve potassium iodide and iodine in about 25 mL of purified water or purified water in containers. After adding glycerin, add mentha water, liquefied phenol and sufficient purified water or purified water in containers to make 1000 mL, mixing thoroughly. It may be prepared with an appropriate quantity of concentrated glycerin and purified water or purified water in containers in place of glycerin, and with an appropriate quantity of phenol and purified water or purified water in containers in place of liquefied phenol.

Description Compound Iodine Glycerin is a red-brown, viscous liquid. It has a characteristic odor.

Specific gravity $d_{20}^{20}$: about 1.23

Identification (1) The colored solution obtained in the assay (1) acquires a red color. Determine the absorption spectrum of this solution as directed under ultraviolet-visible spectrophotometry $<2.24>$: it exhibits a maximum between 510 nm and 514 nm (iodine).

(2) The colored solution obtained in the assay (2) acquires a red color. Determine the absorption spectrum of this solution as directed under ultraviolet-visible spectrophotometry $<2.24>$: it exhibits a maximum between 510 nm and 514 nm (potassium iodide).

(3) The colored solution obtained in the assay (4) has a yellow color. Determine the absorption spectrum of this so-
solution as directed under Ultraviolet-visible Spectrophotometry (2.24); it exhibits a maximum between 401 nm and 405 nm (phenol).

4. Take 1 mL of Compound Iodine Glycerin in a glass-stoppered test tube, add 10 mL of ethanol (95), and mix. Then add 2 mL of sodium hydroxide TS, add 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color develops (glycerin).

**Assay** (1) Iodine—Measure the specific gravity of Compound Iodine Glycerin according to Method 2. Weigh exactly about 7 mL of it, add ethanol (95) to make exactly 200 mL, and use this solution as the sample solution. On the other hand, weigh accurately about 80 mg of iodine for assay and about 0.17 g of potassium iodide for assay, previously dried at 105°C for 4 hours, dissolve in ethanol (95) to make exactly 200 mL, and use this solution as the standard solution.

Pipet 3 mL each of the sample solution and the standard solution into 50-mL separators, to each add exactly 10 mL of a mixture of chloroform and hexane (2:1) and 15 mL of water successively, and shake immediately and vigorously. Separate the chloroform-hexane layers [use the water layers in (2)], and filter through a pledget of cotton. Determine the absorbances of the filtrates, \( A_T \) and \( A_S \), at 512 nm as directed under Ultraviolet-visible Spectrophotometry (2.24), using a mixture of chloroform and hexane (2:1) as the blank.

\[
\text{Amount (mg) of iodine (I)} = M_S \times A_T/A_S
\]

\( M_S \): Amount (mg) of iodine for assay

**Potassium iodide**—Separate the water layers of the sample solution and the standard solution obtained in (1), pipet 10 mL of each of the water layers, and to each add 1 mL of dilute hydrochloric acid (1 in 2), 1 mL of sodium nitrite TS, and shake immediately and vigorously. Shake immediately and vigorously, separate the chloroform-hexane layers, and filter through a pledget of cotton. Determine the absorbances of the filtrates, \( A_T \) and \( A_S \), of both solutions at 512 nm as directed under Ultraviolet-visible Spectrophotometry (2.24), using a mixture of chloroform and hexane (2:1) as the blank.

\[
\text{Amount (mg) of potassium iodide (KI)} = M_S \times A_T/A_S
\]

\( M_S \): Amount (mg) of potassium iodide for assay

**Total iodine**—Measure the specific gravity of Compound Iodine Glycerin according to Method 2. Weigh exactly about 5 mL of it, add water to make exactly 50 mL. Pipet 5 mL of this solution into a 50-mL flask, and add 0.5 g of zinc powder and 5 mL of acetic acid (100). Shake until the color of iodine disappears, and heat under a reflux condenser on a water bath for 30 minutes. Wash the condenser with 10 mL of hot water, and filter through a glass filter (G3). Wash the flask with two 10-mL portions of warm water, and combine the filtrate and the washings. After cooling, add water to make exactly 50 mL, and use this solution as the sample solution. On the other hand, dissolve about 0.2 g of potassium iodide for assay, previously dried at 105°C for 4 hours and accurately weighed, in water to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of acetic acid (100) and water to make exactly 50 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution into 30-mL separatory funnels as directed under Ultraviolet-visible Spectrophotometry (2.24); it exhibits a maximum between 401 nm and 405 nm (phenol).

\[
\text{Amount (mg) of total iodine (I)} = M_S \times A_T/A_S \times 0.764
\]

\( M_S \): Amount (mg) of potassium iodide for assay

(4) Phenol—Measure the specific gravity of Compound Iodine Glycerin according to Method 2. Weigh exactly about 2 mL of it, add 3 mL of 0.1 mol/L sodium thiosulfate VS, and shake. Add 2 mL of dilute hydrochloric acid, and shake with two 10-mL portions of chloroform. Separate the chloroform layer, and shake with two 10-mL portions of 0.5 mol/L sodium hydroxide TS. Separate the water layer, add water to make exactly 500 mL, and use this solution as the sample solution. Dissolve about 0.5 g of phenol for assay, accurately weighed, in ethanol (95) to make exactly 100 mL, shake, and allow to stand for 30 minutes. Add dilute sodium hydroxide solution as the standard solution, proceed in the same manner as the sample solution, and use so obtained solution as the standard solution. Pipet 3 mL each of the sample solution and standard solution, to each add 2 mL of dilute hydrochloric acid, and place in a water bath at 30°C. Allow to stand for 10 minutes, and add exactly 2 mL of a solution of sodium nitrite (1 in 100), shake, and allow to stand at 30°C for 60 minutes. Add dilute potassium hydroxide-ethanol TS to make exactly 25 mL, and determine the absorbances of these solutions, \( A_T \) and \( A_S \), at 403 nm as directed under Ultraviolet-visible Spectrophotometry (2.24), using the solution prepared in the same manner with 3 mL of water instead of the sample solution as the blank.

\[
\text{Amount (mg) of phenol (C}_6\text{H}_5\text{O)} = M_S \times A_T/A_S \times 1/50
\]

\( M_S \): Amount (mg) of phenol for assay

Containers and storage—Tight containers. Storage—Light-resistant.

**Dental Iodine Glycerin**

歯科用ヨード・グリセリン

Dental Iodine Glycerin contains not less than 9.0 w/v% and not more than 11.0 w/v% of iodine (I: 126.90), not less than 7.2 w/v% and not more than 8.8 w/v% of potassium iodide (KI: 166.00), and not less than 0.9 w/v% and not more than 1.1 w/v% of zinc sulfate hydrate (ZnSO₄·7H₂O: 287.55).

**Method of preparation**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g)</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Potassium Iodide</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Zinc Sulfate Hydrate</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Glycerin</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
<td>To make 100 mL</td>
</tr>
</tbody>
</table>

Dissolve and mix the above ingredients.

**Description** Dental Iodine Glycerin is a dark red-brown liquid, having the odor of iodine.
Identification (1) The colored solution obtained in the Assay (1) acquires a red color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits a maximum between 510 nm and 514 nm (iodine).

(2) The colored solution obtained in the Assay (2) acquires a red color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits a maximum between 510 nm and 514 nm (potassium iodide).

(3) Put 1 mL of Dental Iodine Glycerin in a glass-stoppered, test tube, add 10 mL of ethanol (95), and mix. Then add 2 mL of sodium hydroxide TS, add 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color develops (glycerin).

(4) The colored solution obtained in the Assay (3) acquires a red-purple to purple color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits a maximum between 618 nm and 622 nm (zinc sulfate hydrate).

Assay (1) Iodine—Pipet 5 mL of Dental Iodine Glycerin, and add diluted ethanol (3 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the sample solution. On the other hand, weigh accurately about 0.5 g of iodine for assay and add diluted ethanol (3 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, and add 2 mL of sodium hydroxide TS, add 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color develops (glycerin).

(2) Potassium iodide—Separate the water layers of the sample solution and standard solution obtained in (1), pipet 7 mL each of the water layers, and to each add exactly 1 mL of diluted hydrochloric acid (1 in 2), 1 mL of sodium nitrite TS and 10 mL of a mixture of chloroform and hexane (2:1), and shake immediately. Separate the chloroform-hexane layer and filter through a pledget of cotton. Determine the absorbances, A_T and A_S, of the filtrates obtained from the sample solution and standard solution, respectively, at 512 nm as directed under Ultraviolet-visible Spectrophotometry (2.24), using a mixture of chloroform and hexane (2:1) as the blank.

\[
\text{Amount (mg) of iodine (I)} = M_S \times \frac{A_T}{A_S}
\]

M_S: Amount (mg) of iodine for assay

Amount (mg) of potassium iodide (KI) = M_S × A_T/A_S

M_S: Amount (mg) of potassium iodide for assay

(3) Zinc sulfate Hydrate—Pipet 5 mL of Dental Iodine Glycerin, and add diluted ethanol (3 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the sample solution. On the other hand, pipet 10 mL of Standard Zinc Stock Solution, add diluted ethanol (3 in 200) to make exactly 1000 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, to each add 10 mL of a mixture of chloroform and hexane (2:1), shake, and allow to stand. Pipet 3 mL each of the water layers, and to each add 2 mL of boric acid-potassium chloride-sodium hydoxide buffer solution, pH 10.0, 2 mL of zinc TS and water to make exactly 25 mL. Determine the absorbances, A_T and A_S, obtained from the sample solution and standard solution, respectively, at 620 nm as directed under Ultraviolet-visible Spectrophotometry (2.24), using the solution prepared in the same manner with 3 mL of water as the blank.

\[
\text{Amount (mg) of zinc sulfate Hydrate (ZnSO}_4 \cdot 7\text{H}_2\text{O}) = M_S \times \frac{A_T}{A_S} \times 4.398
\]

M_S: Amount (mg) of zinc in 10 mL of Standard Zinc Stock Solution

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Iodine, Salicylic Acid and Phenol Spirit

ヨード・サリチル酸・フェノール精

Iodine, Salicylic Acid and Phenol Spirit contains not less than 1.08 w/v% and not more than 1.32 w/v% of iodine (I: 126.90), not less than 0.72 w/v% and not more than 0.88 w/v% of potassium iodide (KI: 166.00), not less than 4.5 w/v% and not more than 5.5 w/v% of salicylic acid (C7H6O3: 138.12), not less than 1.8 w/v% and not more than 2.2 w/v% of phenol (C6H5O: 94.11), and not less than 7.2 w/v% and not more than 8.8 w/v% of benzoic acid (C7H6O2: 122.12).

Method of preparation

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine Tincture</td>
<td>200 mL</td>
</tr>
<tr>
<td>Salicylic Acid</td>
<td>50 g</td>
</tr>
<tr>
<td>Phenol</td>
<td>20 g</td>
</tr>
<tr>
<td>Benzoid Acid</td>
<td>80 g</td>
</tr>
<tr>
<td>Ethanol for Disinfection</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Spirits, with the above ingredients. It may be prepared with an appropriate quantity of Ethanol and Purified Water or Purified Water in Containers in place of Ethanol for Disinfection.

Description Iodine, Salicylic Acid and Phenol Spirit is a dark red-brown liquid, having the odor of phenol.

Identification (1) To a mixture of 1 mL of starch TS and 9 mL of water add 1 drop of Iodine, Salicylic Acid and Phenol Spirit: a dark blue-purple color develops (iodine).

(2) To 1 mL of Iodine, Salicylic Acid and Phenol Spirit add 5 mL of ethanol (95) and water to make 50 mL. To 1 mL of this solution add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 50 mL, and to 15 mL of this solution add 5 mL of a solution of iron (III) nitrate
enneaehydrate (1 in 200): a red-purple color is produced (salicylic acid).

(3) Shake 1 mL of Iodine, Salicylic Acid and Phenol Spirit with 1 mL of sodium thiosulfate TS, add 20 mL of water and 5 mL of dilute hydrochloric acid, and extract with 25 mL of diethyl ether. Wash the diethyl ether extract with two 25-mL portions of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, and add 3 mL of sodium hydroxide TS: a yellow color is developed (phenol).

(4) Shake 1 mL of Iodine, Salicylic Acid and Phenol Spirit with 1 mL of sodium thiosulfate TS, add 20 mL of water and 5 mL of dilute hydrochloric acid, extract with 10 mL of diethyl ether, and use the diethyl ether extract as the sample solution. Dissolve 25 mg of salicylic acid, 10 mg of phenol and 40 mg of benzoic acid in 5 mL of each of diethyl ether, respectively, and use these solutions as the standard solutions (1), (2) and (3). Perform the test with the sample solution and standard solutions (1), (2) and (3) as directed under Thin-layer Chromatography 2.05. Spot 5 μL of each solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100:45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine spots from the sample solution show the same positions as those from the standard solutions (1), (2) and (3).

**Assay**

(1) Iodine— Pipet 4 mL of Iodine, Salicylic Acid and Phenol Spirit, add ethanol (95) to make exactly 50 mL, and use this solution as the sample solution. On the other hand, weigh accurately about 1.2 g of iodine for assay and about 0.8 g of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 4 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Pipet 3 mL each of the sample solution and standard solution, to each add exactly 25 mL of a mixture of chloroform and hexane (2:1), and shake. Further add exactly 10 mL of water, shake and separate the chloroform-hexane layers [use the water layers in (2)]. Filter through a pledget of absorbent cotton, and determine the absorbances of the filtrates from the sample solution and standard solution, respectively, at 4 and 5 mL at 512 nm as directed under Ultraviolet-visible Spectrophotometry 2.24, using a mixture of chloroform and hexane (2:1) as the blank.

Amount (mg) of iodine (I) = Mₘ × Aₐ / Aₖ × 1/25

**Mₘ:** Amount (mg) of iodine for assay

(2) Potassium iodide—Separate the water layers of the sample solution and standard solution obtained in the Assay (1), pipet 8 mL each of the water layers, and add 1 mL of diluted dilute hydrochloric acid (1 in 2) and 1 mL of sodium nitrite TS. Immediately after shaking, add exactly 10 mL of a mixture of chloroform and hexane (2:1), shake, and proceed in the same manner as for the Assay (1).

Amount (mg) of potassium iodide (KI) = Mₘ × Aₐ / Aₖ × 1/25

**Mₘ:** Amount (mg) of potassium iodide for assay

(3) Salicylic acid, phenol and benzoic acid— Pipet 2 mL of Iodine, Salicylic Acid and Phenol Spirit, add 20 mL of diluted methanol (1 in 2) and 0.1 mol/L sodium thiosulfate VS until the color of iodine disappears, add exactly 20 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 200 mL, and use this solution as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, about 80 mg of phenol for assay, and 0.32 g of benzoic acid, previously dried in a desiccator (silica gel) for 3 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL. Pipet 25 mL of this solution, add exactly 20 mL of the internal standard solution and diluted methanol (1 in 2) to make 200 mL, and use this solution as the standard solution. Perform the test with 3 μL of the sample solution and standard solution as directed under Liquid Chromatography 2.05 according to the following conditions. Calculate the ratios, Qₐ, Qₑ, Q₂₀ and Q₀₂₀, of the peak areas of salicylic acid, phenol and benzoic acid to those of the internal standard of the sample solution, and the ratios, Qₙ₀, Q₀ₙ₀ and Qₙ₀₀, of the peak areas of salicylic acid, phenol and benzoic acid to those of the internal standard of the standard solution.

Amount (mg) of salicylic acid (C₉H₆O₃) = Mₘ × Qₐ / Q₀ × 1/2

Amount (mg) of phenol (C₇H₅O) = Mₘ × Q₁₀ / Q₀ × 1/2

Amount (mg) of benzoic acid (C₇H₆O₂) = Mₘ × Q₂₀ / Q₀ × 1/2

**Mₘ:** Amount (mg) of salicylic acid for assay

**Mₘ:** Amount (mg) of phenol for assay

**Mₘ:** Amount (mg) of benzoic acid

**Internal standard solution—** A solution of theophylline in methanol (1 in 1000).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography, 5 μm in particle diameter.

Column temperature: Room temperature.

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, and methanol (3:1).

Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 6 minutes.

Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 50 mg of theophylline in 100 mL of diluted ethanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with 10 μL of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant.
**Iodoform**

Add 150 mL of water, and titrate shake well, and allow to stand in a dark place over 16 hours. It in 20 mL of ethanol (95). Add exactly 30 mL of 0.1 mol/L previously dried, in a 500-mL glass-stoppered flask, and dissolve Weigh accurately about 0.2 g of Iodoform, previ-

**Description** Iodoform occurs as lustrous, yellow crystals or crystalline powder. It has a characteristic odor. It is freely soluble in diethyl ether, sparingly soluble in ethanol (95), and practically insoluble in water. It is slightly volatile at ordinary temperature. Melting point: about 120°C (with decomposition).

**Identification** Heat 0.1 g of Iodoform: a purple gas is evolved.

**Purity (1)** Water-soluble colored substances and acidity or alkalinity—Shake well 2.0 g of Iodoform, previously powdered, with 5 mL of water for 1 minute, allow to stand, and filter the supernatant liquid: the filtrate is colorless and neutral.

(2) Chloride <1.03>—Shake well 3.0 g of Iodoform, previously powdered, with 75 mL of water for 1 minute, allow to stand, and filter the supernatant liquid. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate <1.14>—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.017%).

**Loss on drying <2.41>** Not more than 0.5% (1 g, silica gel, 24 hours).

**Residue on ignition <2.44>** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Iodoform, previously dried, in a 500-mL glass-stoppered flask, and dissolve it in 20 mL of ethanol (95). Add exactly 30 mL of 0.1 mol/L silver nitrate VS and 10 mL of nitric acid, stopper the flask, shake well, and allow to stand in a dark place over 16 hours. Add 150 mL of water, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 5 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS = 13.12 mg of CHI₃

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Iopamidol**

Iopamidol, when dried, contains not less than 99.0% of C₁₇H₂₂I₃N₃O₈.

**Description** Iopamidol occurs as a white crystalline powder. It is very soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (99.5).

**Identification** (1) To 50 mg of Iopamidol add 5 mL of hydrochloric acid, heat for 10 minutes in a water bath: the test solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Heat 0.1 g of Iopamidol over a flame: a purple gas is evolved.

(3) Determine the infrared absorption spectrum of Iopamidol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation <2.49>** [α]D²₀: -4.6 – -5.2° (after drying, 4 g, water, warm, after cooling, 10 mL, 100 mm).

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Iopamidol in 10 mL of water: the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.60 g of Iopamidol in 8 mL of water, add 1 mL of a solution of sodium nitrite (1 in 50) and 12 mL of 2 mol/L hydrochloric acid TS, shake, and allow to stand for 2 minutes. Add 1 mL of a solution of ammonium amidosulfate (1 in 10), shake well, allow to stand for 1 minute, and add 1 mL of napthyylethylenediamine TS and water to make exactly 50 mL. Determine the absorbance of this solution at 495 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution, prepared in the same manner, as the blank: the absorbance is not more than 0.12 (not more than 0.020%).

(3) Iodine—Dissolve 2.0 g of Iopamidol in 25 mL of water, add 5 mL of 1 mol/L sulfuric acid and 5 mL of toluene, shake well, and allow to stand: the toluene layer is colorless.

(4) Free iodine ion—Weigh accurately about 5.0 g of Iopamidol, dissolve in 70 mL of water, and adjust the pH to about 4.5 with dilute acetic acid. To this solution add 2 mL
of 0.1 mol/L sodium chloride TS, and titrate \( <2.50 \) with 0.001 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.001 mol/L silver nitrate VS
\[ = 0.1269 \text{ mg of I} \]

Content of iodine ion in Iopamidol is not more than 0.001%.

(5) Heavy metals \( <1.07 \) — Moisten 1.0 g of Iopamidol with a small quantity of sulfuric acid, heat gradually to almost incinerate by a possibly lower temperature. After cooling, moisten again with a small quantity of sulfuric acid, heat gradually until white fumes no longer are evolved, and incinerate by ignition between 450 to 550°C. Proceed as directed in Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not rectified in Method 2, and perform the test. Prepare the control solution with exactly 20 mL each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.07 \) according to the following conditions, and determine each peak area of the both solutions by the automatic integration method: each area of the peaks other than the peak of iopamidol from the sample solution is not larger than the peak area of the standard solution, and the total of these areas is not larger than 2.5 times of the peak area of the standard solution.

**Operating conditions**
- Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \text{m} \) in particle diameter).
- Column temperature: A constant temperature of about 35°C.
- Mobile phase: Use water as the mobile phase A, and a mixture of water and methanol (3:1) as the mobile phase B. Change the mixed ratios of the mobile phase A and the mobile phase B stepwise as follows:

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 6</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>6 – 18</td>
<td>92 → 65</td>
<td>8 → 35</td>
</tr>
<tr>
<td>18 – 30</td>
<td>65 → 8</td>
<td>35 → 92</td>
</tr>
<tr>
<td>30 – 34</td>
<td>8</td>
<td>92</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate to 1.5 mL per minute.

Time span of measurement: About 4.3 times as long as the retention time of iopamidol.

**System suitability**
- System performance: Dissolve 1 mL of the sample solution and 10 mg of \( N,N'\text{-bis}[2\text{-hydroxy-1-(hydroxymethyl)ethyl}]\text{-5-hydroxyacetylamo}_2\text{-2,4,6-triiodoisophthalamide} \) in water to make exactly 100 mL. When the procedure is run with 20 \( \mu \text{L} \) of this solution under the above operating conditions, \( N,N'\text{-bis}[2\text{-hydroxy-1-(hydroxymethyl)ethyl}]\text{-5-hydroxyacetylamo}_2\text{-2,4,6-triiodoisophthalamide} \) and iopamidol are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of \( N,N'\text{-bis}[2\text{-hydroxy-1-(hydroxymethyl)ethyl}]\text{-5-hydroxyacetylamo}_2\text{-2,4,6-triiodoisophthalamide} \) is not more than 1.0%.

**Loss on drying \( <2.41 \) Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition \( <2.44 \) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Iopamidol, previously dried, transfer to a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, boil for 30 minutes under a reflux condenser, cool, and filter. Wash the flask and the filter paper with 50 mL of water, and combine the washing with the filtrate. Add 5 mL of acetic acid (100) to this solution, and titrate \( <2.50 \) with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
\[ = 25.90 \text{ mg of C}_17\text{H}_{22}\text{I}_3\text{N}_3\text{O}_8 \]

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

**Iotalamic Acid**

イオタラム酸

\[
\text{C}_{11}\text{H}_{33}\text{I}_3\text{N}_3\text{O}_4: 613.91
\]

3-Acetylamino-2,4,6-triiodo-5-(methylaminocarbonyl)benzoic acid [2276-90-6]

Iotalamic Acid, when dried, contains not less than 99.0% of \( \text{C}_{11}\text{H}_{33}\text{I}_3\text{N}_3\text{O}_4 \).

**Description** Iotalamic Acid occurs as a white powder. It is odorless.

It is sparingly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

It gradually colored by light.

**Identification**
- (1) Heat 0.1 g of Iotalamic Acid over a flame: a purple gas is evolved.
- (2) Determine the infrared spectrum of Iotalamic Acid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
Purity (1) Clarity and color of solution—Dissolve 2.0 g of Iotalamic Acid in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Primary aromatic amines—To 0.50 g of Iotalamic Acid add 15 mL of water, and dissolve it in 1 mL of sodium hydroxide TS while ice-cooling. Add 4 mL of a solution of sodium nitrite (1 in 100) to the solution, immediately add 12 mL of 1 mol/L hydrochloric acid TS, and shake gently. Then allow the mixture to stand for exactly 2 minutes, add 8 mL of ammonium amidosulfate TS, and shake occasionally for 5 minutes. Add 3 drops of a solution of 1-naphthol in ethanol (95) (1 in 10), allow to stand for 1 minute, add 3.5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, mix, and immediately add water to make 50 mL. Determine within 20 minutes the absorbance of this solution at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared in the same manner, as the blank: the absorbance is not more than 0.25.

(3) Soluble halides—Dissolve 0.5 g of Iotalamic Acid in 20 mL of diluted ammonia TS (1 in 40), add 6 mL of dilute nitric acid, shake, allow to stand for 5 minutes, and filter. Transfer the filtrate to a Nessler tube, wash the residue with 20 mL of water, combine the filtrate and the washings, and add water to make 50 mL. Proceed as directed for the Chloride Limit Test <1.07> using this solution as the test solution. Prepare the control solution as follows: to 0.10 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of diluted ammonia TS (1 in 40), 6 mL of dilute nitric acid and water to make 50 mL.

(4) Iodine—Dissolve 0.20 g of Iotalamic Acid in 2.0 mL of sodium hydroxide TS, add 2.5 mL of 0.5 mol/L sulfuric acid TS, and allow to stand for 10 minutes with occasional shaking. Add 5 mL of chloroform, shake well, and allow to stand: the chloroform layer remains colorless.

(5) Heavy metals—Proceed with 1.0 g of Iotalamic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 0.6 g of Iotalamic Acid according to Method 3, and perform the test (not more than 3.3 ppm).

Loss on drying <2.41>—Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44>—Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Iotalamic Acid, previously dried, place it in a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, and heat for 30 minutes under a reflux condenser. Cool, filter, wash the flask and the filter paper with 50 mL of water, and combine the washings and the filtrate. Add 5 mL of acetic acid (100) to this solution, and titrate <2.50> with 0.1 mol/L silver nitrate VS, until the color of the precipitate changes from yellow to green (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS).

Each mL of 0.1 mol/L silver nitrate VS = 20.46 mg of C17H15I3N2O4

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Iotroxic Acid

イオトロクス酸

\[ C_{22}H_{30}I_6N_2O_7 \] 1215.81
3,3′-(3,6,9-Trioxaundecanoyl)diiminobis-(2,4,6-triiodobenzoic acid)

[51022-74-3]

Iotroxic Acid contains not less than 98.5% of \( C_{22}H_{30}I_6N_2O_7 \), calculated on the anhydrous basis.

Description Iotroxic Acid occurs as a white crystalline powder.

It is soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is gradually colored by light.

Identification (1) Heat 0.1 g of Iotroxic Acid over a flame: a purple gas evolves.

(2) Dissolve a suitable amount of Iotroxic Acid in a suitable amount of methanol, evaporate the methanol under reduced pressure, and determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Iotroxic Acid in 10 mL of sodium hydroxide TS (1 in 5): the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.20 g of Iotroxic Acid in 5 mL of water and 1 mL of sodium hydroxide TS, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, mix, and allow to stand for 2 minutes. Add 5 mL of ammonium amidosulfate TS, shake well, allow to stand for 1 minute, then add 0.4 mL of a solution of a-naphthol in ethanol (95) (1 in 10), 15 mL of sodium hydroxide TS and water to make exactly 50 mL. Read the absorbance of this solution at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a blank solution obtained in the same manner as above: the absorbance is not more than 0.22.

(3) Iodine—Dissolve 0.20 g of Iotroxic Acid in 2.0 mL of sodium hydrogen carbonate TS, add 5 mL of toluene, mix well, and allow to stand: the toluene layer is colorless.

(4) Free iodine ion—Weigh accurately about 5.0 g of Iotroxic Acid, dissolve in 12 mL of a solution of meglumine (3 in 20), add water to make 70 mL, and adjust the pH to about 4.5 with acetic acid (100). To this solution add 2 mL of 0.1 mol/L sodium chloride TS, and titrate <2.50> with 0.001 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.001 mol/L silver nitrate VS = 0.1269 mg of I
Ipratropium Bromide Hydrate / Official Monographs

Ipratropium Bromide Hydrate

Ipratropium Bromide Hydrate, when dried, contains not less than 99.0% of ipratropium bromide (C₂₀H₂₅BrNO₃.H₂O; 412.36).

**Description**
Ipratropium Bromide Hydrate occurs as a white, crystalline powder.

It is freely soluble in water, soluble in ethanol (99.5), slightly soluble in acetonitrile and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of Ipratropium Bromide Hydrate (1 in 20) is between 5.0 and 7.5.

Melting point: about 223°C (with decomposition, after drying).

**Identification**
(1) To 5 mg of Ipratropium Bromide Hydrate add 0.5 mL of fuming nitric acid, and evaporate on a water bath to dryness. After cooling, dissolve the residue in 5 mL of acetone, and add 2 drops of potassium hydroxide-ethanol TS: a purple color develops.

(2) Determine the absorption spectrum of a solution of Ipratropium Bromide Hydrate in 0.01 mol/L hydrochloric acid TS (3 in 2000) as directed under Ultraviolet-visible Spectrophotometry \( \leq 2240 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Ipratropium Bromide Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry \( \leq 2250 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) The solution of Ipratropium Bromide Hydrate (1 in 100) responds to the Qualitative Tests \( \leq 1.09 \) for bromide.

**Purity**
(1) Clarity and color of solution—Dissolve 1.0 g of Ipratropium Bromide Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Sulfate \( \leq 1.14 \)—Perform the test with 1.0 g of Ipratropium Bromide Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals \( \leq 1.07 \)—Proceed with 2.0 g of Ipratropium Bromide Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic \( \leq 1.11 \)—Prepare the test solution with 2.0 g of Ipratropium Bromide Hydrate according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 1 ppm).

(5) Isopropylatropine bromide—Dissolve 25 mg of Ipratropium Bromide Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Perform the test with 25 μL of the sample solution as directed under Liquid Chromatography \( \leq 2.01 \) according to the following conditions. Determine the peak area, \( A_p \), of ipratropium and the peak area, \( A_b \), having a relative retention time to ipratropium about 1.3 by the automatic integration method: \( A_p/(A_b + A_0) \) is not more than 0.01, and no peak other than the peak of ipratropium and the peak having a relative retention time to ipratropium about 1.3 appears within about 14 minutes of the retention time after the solvent peak.

**Operating conditions**
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column about 4 mm in inside diameter and 10 to 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: Room temperature.
Mobile phase: A mixture of diluted phosphoric acid (1 in 200), acetonitrile and methanesulfonic acid (1000:120:1).
Flow rate: Adjust the flow rate so that the retention time of ipratropium is about 7 minutes.

Selection of column: Heat a solution of Ipratropium Bromide in 1 mol/L hydrochloric acid TS (1 in 100) at 100°C for 1 hour, and cool. To 2.5 mL of this solution add the mobile phase to make 100 mL. Proceed with 25 μL of this solution under the above operating conditions, and calculate the resolution. Use a column showing a resolution not less than 3 between the peak of ipratropium and the peak having a relative retention time to ipratropium about 0.6.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of ipratropium obtained from 25 μL of the sample solution composes 50 to 80% of the full scale.

(6) Apo-compounds—Dissolve 0.14 g of Ipratropium Bromide Hydrate in 0.01 mol/L hydrochloric acid TS to make 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A<sub>1</sub> and A<sub>2</sub>, at 246 nm and 263 nm, respectively; A<sub>1</sub>/A<sub>2</sub> is not more than 0.91.

Loss on drying <2.41> 3.9 – 4.4% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Ipratropium Bromide Hydrate, previously dried, dissolve in 40 mL of acetic acid (100), add 40 mL of 1,4-dioxane and 2.5 mL of Bromide Hydrate, previously dried, dissolve in 40 mL of Assay. Weigh accurately about 0.3 g of Ipratropium Bromide in 0.01 mol/L hydrochloric acid TS (potentiometric titration). Perform a blank determination, and calculate the absorbances, using the sample solution composes 50 to 80% of the full scale.

System suitability—When the procedure is run with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ipriflavone to that of the internal standard is not more than 2.0%.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Ipriflavone and Ipriflavone RS, previously dried, dissolve separately in

Ipriflavone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ipriflavone RS; both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 116 – 119°C.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Ipriflavone according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ipriflavone according to Method 4, and perform the test. Prepare the test solution with 10 mL of dilute hydrochloric acid instead of using 3 mL of hydrochloric acid. Prepare the standard color with 1.0 mL of Standard Arsenic Solution (not more than 1 ppm).

(3) Related substances—Dissolve 30 mg of Ipriflavone in 50 mL of acetonitrile. To 5 mL of this solution add acetonitrile to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of ipriflavone obtained from the sample solution is not larger than 1/2 times the peak area of ipriflavone from the standard solution, and the total area of the peaks other than the peak of ipriflavone obtained from the sample solution is not larger than the peak area of ipriflavone from the standard solution.

Operating conditions—
Detector, column, column temperature and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 2 times as long as the retention time of ipriflavone, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 2 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of ipriflavone obtained from 20 μL of this solution is equivalent to 7 to 13 times that of ipriflavone from 20 μL of the standard solution.
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ipriflavone are not less than 2000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ipriflavone to that of the internal standard is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Ipriflavone and Ipriflavone RS, previously dried, dissolve separately in

Ipriflavone

C<sub>18</sub>H<sub>16</sub>O<sub>3</sub>: 280.32
7-(1-Methylethyl)oxy-3-phenyl-4H-chromen-4-one [35212-22-7]

Ipriflavone, when dried, contains not less than 98.5% and not more than 101.0% of C<sub>18</sub>H<sub>16</sub>O<sub>3</sub>.

Description Ipriflavone occurs as white to yellowish white crystals or crystalline powder.
It is soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.
It gradually turns yellow on exposure to light.

Identification (1) Determine the absorption spectrum of a solution of Ipriflavone in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ipriflavone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ipriflavone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ipriflavone RS; both spectra exhibit similar intensities of absorption at the same wave numbers.
Ipriflavone Tablets / Official Monographs

976

JP XVI

Acetonitrile (1 in 100).

Standard solution as directed under Liquid Chromatography of the Mass variation test.

Uniformity of dosage units

between 247 nm and 251 nm, and between 297 nm and 301 nm.

Containers—Tight containers.

Storage—Light-resistant.

Ipriflavone Tablets

イプリフラボン錠

Ipriflavone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of ipriflavone (C_{18}H_{16}O_{3}: 280.32).

Method of preparation

Prepare as directed under Tablets, with Ipriflavone.

Identification

To a quantity of powdered Ipriflavone Tablets, equivalent to 11 mg of Ipriflavone according to the labeled amount, add 100 mL of methanol, shake vigorously for 10 minutes, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (<2.0>) according to the following conditions, and calculate the ratio of the peak area of ipriflavone to that of the internal standard.

Internal standard solution—A solution of di-n-butyl phthalate in acetonitrile (1 in 100).

Internal standard solution

- Detectors: A solution of di-(2,5-dichlorophenyl)-1,3,5-triazine-2,4-diamine (C_{18}H_{15}N_{5}: 372.16) and 6-(2,5-Dichlorophenyl)-1,3,5-triazine-2,4-diamine monomaleate [84504-69-8].

- System suitability:
  - System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ipriflavone and the internal standard are eluted within 6 minutes.
  - System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ipriflavone to that of the internal standard is not more than 1.0%.

- Containers and storage—Tight containers.

- Storage—Light-resistant.

Irsogladine Maleate

イルソグラジンマレイン酸塩

Irsogladine Maleate contains not less than 99.0% and not more than 101.0% of C_{9}H_{7}Cl_{2}N_{5}.C_{4}H_{4}O_{4}.

Description

Irsogladine Maleate occurs as white crystals or crystalline powder. It has a slightly bitter taste. It is sparingly soluble in acetic acid (100) and in ethylene glycol, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification

(1) Dissolve 20 mg of Irsogladine Maleate inmethanol to make 20 mL. Take 2 mL of this solution, and add water to make 20 mL. To 2 mL of this solution add water to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (<2.2>) and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Irsogladine Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry (<2.25>) and compare the spectrum with the Reference Spectrum: both spectra
exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 10 mg of Irsogladine Maleate in 1 mL of dilute hydrochloric acid and 4 mL of water, and add 3 drops of potassium permanganate TS: the color of the solution is discharged immediately.

Purity (1) Heavy metals \( <1.0 \% \)—Proceed with 2.0 g of Irsogladine Maleate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Irsogladine Maleate in 10 mL of ethyleneglycol, and use this solution as the sample solution. Pipet 1 mL of this solution, add ethyleneglycol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 \) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peaks of maleic acid and irsogladine obtained from the sample solution is not larger than 1/10 times the peak area of irsogladine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanesulfonic acid solution (1 in 1000) and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of irsogladine is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of irsogladine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add ethyleneglycol to make exactly 10 mL. Confirm that the peak area of irsogladine obtained from 5 \( \mu \)L of the solution is equivalent to 7 to 13\% of that of irsogladine from 5 \( \mu \)L of the standard solution.

System performance: When the procedure is run with 5 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of irsogladine are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of irsogladine is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying \( <2.4 \% \) Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition \( <2.4 \% \) Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Irsogladine Maleate, previously dried, dissolve in 25 mL of acetic acid (100), and titrate \( <2.5 \% \) with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 18.61 mg of \( \text{C}_9\text{H}_7\text{Cl}_2\text{N}_5\cdot\text{C}_4\text{H}_4\text{O}_4\).

Containers and storage Containers—Well-closed containers.

Irsogladine Maleate Fine Granules

イルソグラジンマレイン酸塩細粒

Irsogladine Maleate Fine Granules contain not less than 93.0% and not more than 107.0% of irsogladine maleate (\( \text{C}_9\text{H}_7\text{Cl}_2\text{N}_5\cdot\text{C}_4\text{H}_4\text{O}_4 \); 372.16).

Method of preparation Prepare as directed under Granules, with Irsogladine Maleate.

Identification To a quantity of powdered Irsogladine Maleate Fine Granules, equivalent to 2 mg of Irsogladine Maleate according to the labeled amount, add 5 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of irsogladine maleate in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.03 \). Spot 10 \( \mu \)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of petroleum ether, acetone and acetic acid (100) (12:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution has the same \( R_f \) value as the spot from the standard solution.

Uniformity of dosage units \( <6.0 \% \) Perform the test according to the following method: Irsogladine Maleate Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

Take out the total contents of 1 pack of Irsogladine Maleate Fine Granules, add 2 mL of water, add 2 mL methanol per mg of irsogladine maleate (\( \text{C}_9\text{H}_7\text{Cl}_2\text{N}_5\cdot\text{C}_4\text{H}_4\text{O}_4 \)), treat with ultrasonic waves for 10 minutes with occasional shaking, and add water to make exactly \( V \) mL so that each mL contains about 40 \( \mu \)g of irsogladine maleate (\( \text{C}_9\text{H}_7\text{Cl}_2\text{N}_5\cdot\text{C}_4\text{H}_4\text{O}_4 \)). Centrifuge this solution, pipet 1 mL of the supernatant liquid, and add water to make exactly 20 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 \( \mu \)m, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of irsogladine maleate for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \( <2.2 \) using water as the blank, and determine the absorbances, \( A_T \) and \( A_S \), at 210 nm.
Amount (mg) of irsogladine maleate \((C_9H_7Cl_2N_5.C_4H_4O_4)\)

\[
M_3: \text{Amount (mg) of irsogladine maleate for assay}
\]

**Dissolution**<6.10> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Irsogladine Maleate Fine Granules is not less than 70%.

Start the test with an accurately weighed amount of Irsogladine Maleate Fine Granules, equivalent to about 4 mg of irsogladine maleate \((C_9H_7Cl_2N_5.C_4H_4O_4)\) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 \(\mu\)m. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of irsogladine maleate for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, and add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and determine the absorbance, \(A_1\) and \(A_2\), at 210 nm.

Dissolution rate (%) with respect to the labeled amount of irsogladine maleate \((C_9H_7Cl_2N_5.C_4H_4O_4)\)

\[
\frac{M_3 \times A_1}{A_2 \times 1/C \times 9}
\]

\(M_3\): Amount (mg) of irsogladine maleate for assay

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (750:250:3).

Flow rate: Adjust the flow rate so that the retention time of irsogladine is about 9 minutes.

**System suitability**—

System performance: When the procedure is run with 5 \(\mu\)L of the standard solution under the above operating conditions, irsogladine and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of irsogladine to that of the internal standard is not more than 1.0%.

**Containers and storage**—

Containers—Tight containers.

### Irsogladine Maleate Tablets

**イルソグラジンマレイン酸塩錠**

Irsogladine Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of irsogladine maleate \((C_9H_7Cl_2N_5.C_4H_4O_4): 372.16\).

**Method of preparation**—

Prepare as directed under Tablets, with Irsogladine Maleate.

**Identification**—

To a quantity of powdered Irsogladine Maleate Tablets, equivalent 2 mg of Irsogladine Maleate according to the labeled amount, add 5 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of irsogladine maleate in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of petroleum ether, acetone and acetic acid (100) (12:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution has the same \(R_f\) value as the spot from the standard solution.

**Uniformity of dosage units**<6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Irsogladine Maleate Tablets add 2 mL of water, add 2 mL of methanol per mg of irsogladine maleate \((C_9H_7Cl_2N_5.C_4H_4O_4)\), treat with ultrasonic waves for 10
minutes with occasional shaking, add water to make exactly
V mL so that each mL contains about 40 \( \mu g \) of irsogladine
maleate (\( C_9H_7Cl_2N_5C_4H_4O_4 \)). Centrifuge this solution, pipet
1 mL of the supernatant liquid, and add water to make ex-
actly 20 mL. Filter this solution through a membrane filter
with a pore size not exceeding 0.5 \( \mu m \), discard the first 10
mL of the filtrate, and use the subsequent filtrate as the sam-
ple solution. Separately, weigh accurately about 20 mg of ir-
sogladine maleate for assay, previously dried at 105 °C for 4
hours, and dissolve in methanol to make exactly 20 mL.
Pipet 2 mL of this solution, and add water to make exactly
20 mL. Pipet 2 mL of this solution, add water to make ex-
actly 100 mL, and use this solution as the standard solution.
Perform the test with the sample solution and standard solu-
tion as directed under Ultraviolet-visible Spectrophotometry.

Amount (mg) of irsogladine maleate (\( C_9H_7Cl_2N_5C_4H_4O_4 \))
= \( M_5 \times A_5/A_3 \times V/500 \)

M₅: Amount (mg) of irsogladine maleate for assay

Dissolution 6.10 When the test is performed at 50 revolu-
tions per minute according to the Paddle method using 900
mL of water as the dissolution medium, the dissolution rate
in 30 minutes of Irsogladine Maleate Tablets is not less than
80%.

Start the test with 1 tablet of Irsogladine Maleate Tablets,
withdraw not less than 20 mL of the medium at the specified
minute after starting the test, and filter through a membrane
filter with a pore size not exceeding 0.5 \( \mu m \). Discard the first
10 mL of the filtrate, pipet \( V \) mL of the subsequent filtrate,
add water to make exactly \( V \) mL so that each mL contains
about 2.2 \( \mu g \) of irsogladine maleate (\( C_9H_7Cl_2N_5C_4H_4O_4 \)) ac-
cording to the labeled amount, and use this solution as the sam-
plesolution. Separately, weigh accurately about 20 mg of
irsogladine maleate for assay, previously dried at 105 °C
for 4 hours, and dissolve in methanol to make exactly 20
mL. Pipet 2 mL of this solution, and add water to make ex-
actly 20 mL. Pipet 2 mL of this solution, add water to make
exactly 100 mL, and use this solution as the standard solution.
Perform the test with the sample solution and standard solu-
tion as directed under Ultraviolet-visible Spectrophotometry,

Amount (mg) of irsogladine maleate (\( C_9H_7Cl_2N_5C_4H_4O_4 \))
= \( M_5 \times Q_s/Q_s \times 1/5 \)

M₅: Amount (mg) of irsogladine maleate for assay

Internal standard solution—A solution of ethyl parahy-
droxybenzoate in methanol (1 in 2500).

Operating conditions—
Detector: An ultraviolet absorption photometer (wave-
length: 250 nm).

Column: A stainless steel column 4.6 mm in inside dia-
ter and 15 cm in length, packed with octadecylsilanized silica
gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about
25°C.

Mobile phase: A mixture of water, acetonitrile and acetic
acid (100) (750:250:3).

Flow rate: Adjust the flow rate so that the retention time
of irsogladine is about 9 minutes.

System suitability—
System performance: When the procedure is run with 5 \( \mu L \)
of the standard solution under the above operating condi-
tions, irsogladine and the internal standard are eluted in this
order with the resolution between these peaks being not less
than 10.

System repeatability: When the test is repeated 6 times
with 5 \( \mu L \) of the standard solution under the above operating
conditions, the relative standard deviation of the ratio of the
peak area of irsogladine to that of the internal standard is
not more than 1.0%.

Containers and storage Containers—Tight containers.
Isepamicin Sulfate

Iseパマイシン硫酸塩

\[
\text{C}_{22}\text{H}_{43}\text{N}_5\text{O}_{12} \times \text{H}_2\text{SO}_4
\]

6-Amino-6-deoxy-α-D-glucopyranosyl-(1→4)-
[3-deoxy-4-C-methyl-3-methylamino-β-L-arabinopyranosyl-
(1→6)]-2-deoxy-1-N-[25]-3-amino-2-hydroxypropanoyl]-
D-streptamine sulfate

67814-76-0

Isepamicin Sulfate is the sulfate of a derivative of gentamycin B, an aminoglycoside substance, having antibacterial activity produced by the growth of Micromonospora purpurea.

It contains not less than 680 µg (potency) and not more than 780 µg (potency) per mg, calculated on the anhydrous basis. The potency of Isepamicin Sulfate is expressed as mass (potency) of isepamicin (C\(_{22}\)H\(_{43}\)N\(_5\)O\(_{12}\)) 569.60.

**Description** Isepamicin Sulfate occurs as a white to pale yellowish white powder.

It is very soluble in water, and practically insoluble in methanol and in ethanol (95).

It is hygroscopic.

**Identification** (1) Dissolve 20 mg of Isepamicin Sulfate in 1 mL of water, add 3 mL of anthrone TS, shake, and allow to stand: a blue-purple color develops.

(2) Dissolve 10 mg each of Isepamicin Sulfate and Isepamicin Sulfate RS in 5 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer chromatography (2.03). Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia water (28), ethanol (99.5), 1-buthanol and chloroform (5:5:2:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at about 100°C for about 10 minutes: the principal spots from the sample solution and the standard solution exhibit a red-brown color and show the same Rf value.

(3) Dissolve 10 mg of Isepamicin Sulfate in 1 mL of water, and add 1 drop of barium chloride TS: a white precipitate is produced.

**Optical rotation** (2.49) [α]\(_D\) = +100 – +120° (0.25 g calculated on the anhydrous bases, water, 25 mL, 100 mm).

**pH** (2.54) Dissolve 0.5 g of Isepamicin Sulfate in 5 mL of water: the pH of the solution is between 5.5 and 7.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Isepamicin Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals (1.07)—Proceed with 1.0 g of Isepamicin Sulfate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Use the sample solution obtained in the Assay as the sample solution. Perform the test with 5 µL of the sample solution as directed under Liquid Chromatography (2.07) according to the following conditions. Determine each peak area of the sample solution by the automatic integration method, and calculate the amounts of their peaks by the area percentage method: the amount of HAPA-gentamine-B equivalent to about 0.4 of the relative retention time to isepamicin is not more than 5.0%, and gentamicin B equivalent to about 1.3 of that is not more than 3.0%. Correct the peak area of gentamicin B by multiplying the relative response factor, 1.11.

**Operating conditions**—

Apparatus, detector, column, column temperature, reaction coil, mobile phase, reagent, reaction temperature, flow rate of the mobile phase, and flow rate of the reagent: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of isepamicin.

**System suitability**—

Test for required detection: Pipet 1 mL of the sample solution, add water to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of isepamicin obtained from 5 µL of this solution is equivalent to 7 to 13% of that from 5 µL of the solution for system suitability test.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

**Water** (2.48) Not more than 12.0% (0.2 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

**Residue on ignition** (2.44) Not more than 1.0% (1 g).

**Assay** Weigh accurately an amount of Isepamicin Sulfate and Isepamicin Sulfate RS, equivalent to about 20 mg (potency), dissolve each in water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.07) according to the following conditions, and determine the peak areas, A1 and A2, of isepamicin of the solutions.

Amount [µg (potency)] of isepamicin (C\(_{22}\)H\(_{43}\)N\(_5\)O\(_{12}\)) = \(M_5 \times \frac{A_1}{A_2} \times 1000\)

\(M_5\): Amount [mg (potency)] of Isepamicin Sulfate RS

**Operating conditions**—

Apparatus: Consist of two pumps for the mobile phase and the reagent transport, inject port, column, reaction coil, detector and recorder. Use a reaction coil with thermostat.

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Reaction coil: A column 0.25 μm in inside diameter and 5 m in length.

Mobile phase: Dissolve 28.41 g of anhydrous sodium sulfate and 5.23 g of sodium 1-pentane sulfonate in 900 mL of water, add 1 mL of acetic acid (100), and add water to make exactly 1000 mL.

Reagent: To 500 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, add 5 mL of a solution of o-phthalaldehyde in ethanol (95) (2 in 25), 1 mL of 2-mercaptoethanol and 2 mL of a solution of lauramacrogol (1 in 4).

Reaction temperature: A constant temperature of about 45°C.

Flow rate of the mobile phase: About 0.6 mL per minute.

Flow rate of the reagent: About 0.5 mL per minute.

System suitability—

System performance: Dissolve 2 mg of Gentamicin B in 10 mL of the standard solution. When the procedure is run with 5 μL of this solution under the above operating conditions, isepamicin and gentamicin B are eluted in this order with the resolution between these peaks being not less than 1.0.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of isepamicin is not more than 3.0%.

Containers and storage — Tight containers.

Isepamicin Sulfate Injection

イセパマイシン硫酸塩注射液

Isepamicin Sulfate Injection is an aqueous injection. It contains not less than 90.0% and not more than 110.0% of the labeled potency of isepamicin (C_{22}H_{43}N_{5}O_{12}: 569.60).

Method of preparation — Prepare as directed under Injections, with Isepamicin Sulfate.

Description — Isepamicin Sulfate Injection is a clear, colorless liquid.

Identification — To a volume of Isepamicin Sulfate Injection, equivalent to 20 mg (potency) of Isepamicin Sulfate according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve an amount of Isepamicin Sulfate RS, equivalent to 20 mg (potency) in 10 mL of water, and use this solution as the standard solution. Proceed with these solutions as directed in the Identification (2) under Isepamicin Sulfate.

Osmotic pressure ratio — Being specified separately.

pH <2.54> 5.5 – 7.5.

Purity — Related substances—Use the sample solution obtained in the Assay as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amounts of these peaks by the area percentage method: the amount of isoserine, having the relative retention time of about 0.3 with respect to isepamicin, is not more than 2.0%, and the amount of gentamicin B, having the relative retention time of about 1.3 with respect to isepamicin, is not more than 4.0%. For this calculation, use the peak area of gentamicin B after multiplying by the relative response factor, 1.11.

Operating conditions—

Apparatus, detector, column, column temperature, reaction coil, mobile phase, reaction reagent, reaction temperature, flow rate of the mobile phase, and flow rate of the reaction reagent: Proceed as directed in the operating conditions in the Assay under Isepamicin Sulfate.

Time span of measurement: About 2 times as long as the retention time of isepamicin.

System suitability—

Test for required detectability: To 1 mL of the sample solution add water to make 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of isepamicin obtained from 5 μL of this solution is equivalent to 7 to 13% of that of isepamicin from the solution for system suitability test.

System performance and system repeatability: Proceed as directed in the Assay under Isepamicin Sulfate.

Bacterial endotoxins <4.01> Less than 0.50 EU/mg (potency).

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay — Pipet a volume of Isepamicin Sulfate Injection, equivalent to about 0.2 g (potency) of Isepamicin Sulfate, add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Isepamicin Sulfate RS, equivalent to about 20 mg (potency), dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Isepamicin Sulfate.

Amount [mg (potency)] of isepamicin (C_{22}H_{43}N_{5}O_{12})

\[ M_\text{s} = M_\text{S} = \text{Amount [mg (potency)] of Isepamicin Sulfate RS} \]

Containers and storage — Hermetic containers.

Expiration date — 24 months after preparation.
Isoflurane

Isoflurane contains not less than 99.0% and not more than 101.0% of C₃H₂ClF₅O, calculated on the anhydrous basis.

**Description** Isoflurane occurs as a clear, colorless fluid liquid.
- It is miscible with ethanol (99.5), with methanol and with o-xylene.
- It is slightly soluble in water.
- It is volatile, and has no inflammability.
- It shows no optical rotation.
- Refractive index nD: about 1.30
- Boiling point: about 47 – 50°C

**Identification**
1. The test solution obtained by the Oxygen Flask Combustion Method <1.06> with 50 µL of Isoflurane, using 40 mL of water as the absorbing liquid, responds to the Qualitative Tests <2.24>, using 40 mL of water, shake thoroughly, and separate the water layer. To the standard solution add 5 mL of sodium fluoride in water to make exactly 1000 mL, and use this solution as the standard solution.

2. Determine the infrared absorption spectrum of Isoflurane as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Isoflurane RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Specific gravity** <2.56> dD₂₀: 1.500 – 1.520

**Purity** (1) Acidity or alkalinity—To 10 mL of Isoflurane add 5 mL of freshly boiled and cooled water, and shake for 1 minute: the water layer is neutral.

(2) Soluble chloride—To 60 g of Isoflurane add 40 mL of water, shake thoroughly, and separate the water layer. To 20 mL of the layer add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as directed under Chloride Limit Test <1.07>. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 3 ppm).

(3) Soluble fluoride—To 6 g of Isoflurane add 12 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), and shake for 10 minutes. Transfer 4.0 mL of the water layer into a Nessler tube, add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, allow to stand for 60 minutes, and use this solution as the sample solution. Separately, to 0.4 mL of the fluoride standard solution and 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in a Nessler tube add 30 mL of the mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1), then proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, obtained by proceeding in the same manner as above with 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), as the blank: the absorbance of the sample solution is not more than that of the standard solution (not more than 2 ppm).

**Purity** (2) Related substances—Use Isoflurane as the sample solution. To exactly 1 mL of the sample solution add o-xylene to make exactly 100 mL. Pipet 1 mL of this solution, add o-xylene to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than isofoxurane is not larger than the peak area of isofoxurane from the standard solution, and the total area of the peaks other than isofoxurane is not larger than 3 times the peak area of isofoxurane from the standard solution.

**Purity** (3) Peroxide—To 10 mL of Isoflurane add 1 mL of a freshly prepared solution of potassium iodide (1 in 10), shake vigorously, and allow to stand in a dark place for 1 hour: the water layer is not yellow.

**Operating conditions**—
Detector, column, column temperature, carrier gas, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of isofoxurane after injection of the sample solution.

**System suitability**—
Test for required detectability: To exactly 1 mL of the standard solution add o-xylene to make exactly 2 mL. Confirm that the peak area of isofoxurane obtained with 5 µL of this solution is equivalent to 35 to 65% of that with 5 µL of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

**Purity** (6) Residue on evaporation—Pipet 65 mL of Isoflurane, evaporate on a water bath, and dry the residue at 105°C for 1 hour: not more than 1.0 mg.

**Water** <2.48> Not more than 0.1% (2 g, Coulometric titration).

**Assay**
To exactly 5 mL each of Isoflurane and Isoflurane RS (separately determined water content <2.48> in the same manner as Isoflurane), add exactly 3 mL of ethyl acetate as the internal standard, then add o-xylene to make exactly 50 mL each. To 5 mL each of these solutions add o-xylene to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 2 µL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q₁ and Q₃, of the peak area of isofoxurane to that of the internal standard.
Amount (mg) of isoflurane (C₃H₇ClF₃O) in 5 mL of Isoflurane
\[ V_n = \frac{Q_n}{Q_S} \times 1000 \times 1.506 \]

\( V_n \): Amount (mL) of Isoflurane RS, calculated on the anhydrous basis.

1.506: Specific gravity \( (d_3^{40}) \) of isoflurane

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A stainless steel column 3 mm in inside diameter and 3.5 m in length, packed with silica gel for gas chromatography (125 – 149 μm in particle diameter), coated in 10% with nonylphenoxypoly(ethyleneoxide)ethanol for gas chromatography and in 15% with polyalkylene glycol for gas chromatography.

Column temperature: A constant temperature of about 80°C.
Carrier gas: Nitrogen.
Flow rate: Adjust the flow rate so that the retention time of isoflurane is about 7 minutes.

System suitability—
System performance: When the procedure is run with 2 \( \mu \)L of the standard solution under the above operating conditions, isoflurane and the internal standard are eluted in this order with the resolution between these peaks being not less than 3
System repeatability: When the test is repeated 6 times with 2 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoflurane is not more than 1.0%.

Containers and storage — Containers—Tight containers.
Storage—At a temperature not exceeding 30°C.

L-Isoleucine

L-イソロイシン

\( C_6H_{13}NO_2 \): 131.17
(2S,3S)-2-Amino-3-methylpentanoic acid
[73-32-5]

L-Isoleucine, when dried, contains not less than 98.5% of \( C_6H_{13}NO_2 \).

Description  L-Isoleucine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Isoleucine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> \([\alpha]_D^{20^o}: +39.5 – +41.5^o\) (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of L-Isoleucine in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Isoleucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of L-Isoleucine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Isoleucine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Isoleucine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.002%).

(5) Heavy metals <1.07>—Dissolve 1.0 g of L-Isoleucine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Isoleucine according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Isoleucine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50), and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.13 g of L-Isoleucine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 13.12 mg of \( C_6H_{13}NO_2 \)

Containers and storage — Containers—Tight containers.
L-Isoleucine, L-Leucine and L-Valine Granules

イソロイシン・ロイシン・バリン顆粒

L-Isoleucine, L-Leucine and L-Valine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of L-Isoleucine (C₆H₁₃NO₂: 131.17), L-Leucine (C₆H₁₃NO₂: 131.17) and L-Valine (C₅H₁₁NO₂: 117.15).

Method of preparation
Prepare as directed under Granules, with L-Isoleucine, L-Leucine and L-Valine.

Identification
Dissolve an amount of pulverized L-Isoleucine, L-Leucine and L-Valine Granules, equivalent to about 0.95 g of L-isoleucine (C₆H₁₃NO₂), add exactly 0.46 g of L-leucine for assay and about 0.24 g of L-valine for assay, previously these are dried at 105°C for 3 hours, add exactly 2 mL of the internal standard solution, dissolve in 0.1 mol/L hydrochloric acid TS to make 100 mL, and use this solution as the sample solution. Perform the test with exactly 20 µL each of the sample solution and the standard solution as directed under Liquid Chromatography according to the following conditions: the retention times of the peak obtained from the sample solution and the standard solution are the same.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 31.2 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust to pH 2.8 with phosphoric acid. To 970 mL of this solution add 30 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of L-valine is about 2.5 minutes.

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, valine, isoleucine and leucine are eluted in this order, and the resolution between the peaks of isoleucine and leucine is not less than 1.5.
System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operation conditions, the relative standard deviations of the retention time of isoleucine, leucine and valine are not more than 1.0%, respectively.

Uniformity of dosage units
Perform the test according to the following method: the Granules in single-unit container meets the requirement of the Content uniformity test.
To the total content of 1 container of L-Isoleucine, L-Leucine and L-Valine Granules add exactly V/25 mL of the internal standard solution, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 3.8 mg of L-isoleucine (C₆H₁₃NO₂). To 2 mL of this solution add 0.02 mol/L hydrochloric acid TS to make 200 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of L-isoleucine (C₆H₁₃NO₂)
= \( M_{SA} \times Q_{V} / Q_{S} \times V / 50 \)
Amount (mg) of L-leucine (C₆H₁₃NO₂)
= \( M_{SA} \times Q_{V} / Q_{S} \times V / 50 \)
Amount (mg) of L-valine (C₅H₁₁NO₂)
= \( M_{SB} \times Q_{V} / Q_{S} \times V / 50 \)

\( M_{SA}: \) Amount (mg) of L-isoleucine for assay
\( M_{SB}: \) Amount (mg) of L-leucine for assay
\( M_{SC}: \) Amount (mg) of L-valine for assay

Internal standard solution—A solution of glycine in 0.1 mol/L hydrochloric acid TS (1 in 20).

Disintegration
It meets the requirement. Carry out the test for 15 minutes.

Assay
Pulverize the total amount of the content of not less than ten containers of L-Isoleucine, L-Leucine and L-Valine Granules. Weigh accurately a portion of the powder, equivalent to about 0.95 g of L-isoleucine (C₆H₁₃NO₂), add exactly 10 mL of the internal standard solution, dissolve in 0.1 mol/L hydrochloric acid TS to make 250 mL. To 2 mL of this solution add 0.02 mol/L hydrochloric acid TS to make 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.2 g of L-isoleucine for assay, about 0.4 g of L-leucine for assay and about 0.24 g of L-valine for assay, previously these are dried at 105°C for 3 hours, add exactly 2 mL of the internal standard solution, dissolve in 0.1 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.02 mol/L hydrochloric acid TS to make 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions: the ratios, Qₚ, Qₜ and Qₑ of the peak area of L-isoleucine, L-leucine and L-valine to that of the internal standard obtained from the sample solution, and the ratios, Qₛ₁, Qₛ₂ and Qₛ₃ of the peak area of L-isoleucine, L-leucine and L-valine to that of the internal standard from the standard solution.

Amount (mg) of L-isoleucine (C₆H₁₃NO₂)
= \( M_{SA} \times Q_{V} / Q_{S} \times 5 \)
Amount (mg) of L-leucine (C₆H₁₃NO₂)
= \( M_{SA} \times Q_{V} / Q_{S} \times 5 \)
Amount (mg) of L-valine (C₅H₁₁NO₂)
= \( M_{SB} \times Q_{V} / Q_{S} \times 5 \)

\( M_{SA}: \) Amount (mg) of L-isoleucine for assay
\( M_{SB}: \) Amount (mg) of L-leucine for assay
\( M_{SC}: \) Amount (mg) of L-valine for assay

Internal standard solution—A solution of glycine in 0.1 mol/L hydrochloric acid TS (1 in 20).

Operation conditions—
Detector: A visible absorption photometer (wavelength: 570 nm).
Column temperature: A constant temperature of about 57°C.
Reaction vessel temperature: A constant temperature of about 130°C.
Reaction time: About 1 minute.
Mobile phase: After prepare the mobile phases A, B, C, D, and E according to the following table, add 0.1 mL caprylic acid to each mobile phase.

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
<th>Mobile phase C</th>
<th>Mobile phase D</th>
<th>Mobile phase E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid monohydrate</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>12.80 g</td>
<td>6.10 g</td>
<td>—</td>
</tr>
<tr>
<td>Trisodium citrate dihydrate</td>
<td>6.19 g</td>
<td>7.74 g</td>
<td>13.31 g</td>
<td>26.67 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>3.74 g</td>
<td>54.35 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>8.00 g</td>
</tr>
<tr>
<td>Ethanol (99.5)</td>
<td>130 mL</td>
<td>20 mL</td>
<td>4 mL</td>
<td>—</td>
<td>100 mL</td>
</tr>
<tr>
<td>Thiodiglycol</td>
<td>5 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5 mL</td>
<td>—</td>
</tr>
<tr>
<td>Lauromacrogol solution (1 in 4)</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
</tr>
<tr>
<td>Water</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>amount</td>
</tr>
</tbody>
</table>

Total amount 1000 mL 1000 mL 1000 mL 1000 mL 1000 mL

Switching of mobile phase: Switch the mobile phases A, B, C, D and E sequentially so that when proceed with 20 mL of the standard solution under the conditions above, the internal standard, valine, isoleucine and leucine are eluted in this order with the resolution between the peaks of isoleucine and leucine being not less than 1.2.

Reaction reagent: Dissolve 407 g of lithium acetate dihydrate in an appropriate amount of water, add 245 mL of acetic acid (100), 801 mL of 1-methoxy-2-propanol and water to make 2000 mL, pass nitrogen for 10 minutes, and use this solution as Solution (I). Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin, pass nitrogen for 5 minutes, add 0.161 g of sodium borohydride, and pass nitrogen for 30 minutes. To this solution add an equal amount of Isoniazid according to Method 3, and perform the test. In this case, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and ignite the ethanol to burn (not more than 5 ppm).

Isoniazid, when dried, contains not less than 98.5% of C₉H₇N₃O₆.

**Identification** (1) Dissolve about 20 mg of Isoniazid in water to make 200 mL. To 5 mL of the solution add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry 2.245, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Isoniazid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.251, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**pH** 2.5–6.0

**Melting point** 170 – 173°C.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Isoniazid in 20 mL of water: the solution is clear and colorless.

**Heavy metals** 0.07—Proceed with 1.0 g of Isoniazid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic 0.11—Prepare the test solution with 0.40 g of Isoniazid according to Method 3, and perform the test. In this case, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and ignite the ethanol to burn (not more than 5 ppm).

(4) Hydrazine—Dissolve 0.10 g of isoniazid in 5 mL of water, add 0.1 mL of a solution of salicylaldehyde in ethanol (95) (1 in 20), shake immediately, and allow to stand for 5 minutes: no turbidity is produced.

**Loss on drying** 0.4%—Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** 2.44—Not more than 0.1% (1 g).
**Isoniazid Injection**

イソニアジド注射液

Isoniazid Injection is an aqueous solution for injection. It contains not less than 95.0% and not more than 105.0% of the labeled amount of isoniazid (C₆H₇N₃O: 137.14).

**Method of preparation** Prepare as directed under Injections, with Isoniazid.

**Description** Isoniazid Injection occurs as a clear, colorless liquid. pH: 6.5 – 7.5.

**Identification** To a volume of Isoniazid Injection, equivalent to 20 mg of Isoniazid according to the labeled amount, and add water to make 200 mL. To 5 mL of the solution add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 264 nm and 268 nm.

**Bacterial endotoxins**<4.07> Less than 0.50 EU/mg.

**Extractable volume**<6.07> It meets the requirement.

**Foreign insoluble matter**<6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter**<6.07> It meets the requirement.

**Sterility**<4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** To an exactly measured volume of Isoniazid Injection, equivalent to about 50 mg of isoniazid (C₆H₇N₃O), add water to make exactly 100 mL. Pipet 5 mL of the solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of isoniazid for assay, previously dried at 105°C. Titrate 0.1 mol/L perchloric acid VS until the color of the solution changes from yellow to green (indicator: 0.5 mL of p-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 13.71 mg of C₆H₇N₃O

**Containers and storage** Containers—Tight containers.

**Storage**—Light-resistant.

**Isoniazid Tablets**

イソニアジド錠

Isoniazid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of isoniazid (C₆H₇N₃O: 137.14).

**Method of preparation** Prepare as directed under Tablets, with Isoniazid.

**Identification** Take a quantity of powdered Isoniazid Tablets, equivalent to 20 mg of Isoniazid according to the labeled amount, add 200 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 264 nm and 268 nm.

**Uniformity of dosage units**<6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Isoniazid Tablets add exactly V mL of water so that each mL contains about 0.5 mg of isoniazid (C₆H₇N₃O), and shake well to disintegrate. Filter this solu-
tion, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of isoniazid (C}_6\text{H}_7\text{N}_3\text{O)} = M_s \times A_T/A_S \times V/100
\]

\(M_s\): Amount (mg) of isoniazid for assay

**Assay**

Weigh accurately and powder not less than 20 Isoniazid Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.1 g of isoniazid (C\(_6\)H\(_7\)N\(_3\)O), add 150 mL of water, shake for 30 minutes, then add water to make exactly 200 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, and add mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of isoniazid for assay, previously dried at 105°C for 2 hours, dissolve in water to make exactly 100 mL, then pipet 5 mL of this solution, add water to make exactly 50 mL, and then pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), of the sample solution and standard solution at 267 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of isoniazid (C\(_6\)H\(_7\)N\(_3\)O)

\[
M_s \times A_T/A_S \times V \times C \times 90
\]

\(M_s\): Amount (mg) of isoniazid for assay

**C**: Labeled amount (mg) of isoniazid (C\(_6\)H\(_7\)N\(_3\)O) in 1 tablet

**System suitability**

System suitability— Dissolve 5 mg of Isoniazid and 5 mg of isonicotinic acid in 100 mL of the mobile phase. When the procedure is run with 10 \(\mu\)L of this solution under the above operating conditions, isonicotinic acid and isoniazid are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoniazid is not more than 1.0%.

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant.

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**-Isoprenaline Hydrochloride**

-イソプレナリン塩酸塩

C\(_{11}\)H\(_{17}\)NO\(_3\).HCl: 247.72

4-[(1R)-1-Hydroxy-2-[(1-methylethyl)amino]ethyl]benzene-1,2-diol monohydrochloride

[5984-95-2]

-Isoprenaline Hydrochloride, when dried, contains not less than 98.0% of C\(_{11}\)H\(_{17}\)NO\(_3\).HCl.

**Description**

-Isoprenaline Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95), and practically insoluble in acetic acid (100), in acetic anhydride, in diethyl ether and in chloroform.

It gradually changes in color by air and by light.

**Identification**

(1) Dissolve 10 mg of -Isoprenaline Hydrochloride in 5 mL of water, and add 1 drop of iron (III) chloride TS: a deep green color develops, and changes through yellow-green to brown on standing.

(2) Dissolve 1 mg each of -Isoprenaline Hydrochloride in 1 mL of water in the test tubes A and B. Add 10 mL of potassium hydrogen phthalate buffer solution, pH 3.5 to A, and add 10 mL of phosphate buffer solution, pH 6.5 to B. To each of the test tubes add 1 mL of iodine TS, allow to stand for 5 minutes, and add 2 mL each of sodium thiosulfate TS: a red color develops in the test tube A, and a deep red color develops in the test tube B.

(3) Dissolve 10 mg of -Isoprenaline Hydrochloride in 1
mL of water, and add 1 mL of phosphotungstic acid TS: a light brown precipitate is produced.

(4) Determine the absorption spectrum of a solution of \( l \)-Isoprenaline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry \(< 2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) A solution of \( l \)-Isoprenaline Hydrochloride (1 in 10) responds to the Qualitative Tests \(< 1.09>\) (2) for chloride.

**Optical rotation** \(< 2.49>\) \([\alpha]_{20}^{D}: -36 \text{ to } -41^\circ\) (after drying, 0.25 g, water, 25 mL, 100 mm).

**pH** \(< 2.54>\) Dissolve 0.10 g of \( l \)-Isoprenaline Hydrochloride in 10 mL of water: the pH of the solution is between 4.5 and 5.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of \( l \)-Isoprenaline Hydrochloride in 20 mL of 0.1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Sulfate \(< 1.14>\) Perform the test with 0.10 g of \( l \)-Isoprenaline Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.192%).

(3) Heavy metals \(< 1.07>\) Proceed with 1.0 g of \( l \)-Isoprenaline Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Isoproterenone—Dissolve 50 mg of \( l \)-Isoprenaline Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and determine the absorbance of the solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry \(< 2.24>\) not more than 0.040.

**Loss on drying** \(< 2.41>\) Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** \(< 2.44>\) Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.5 g of \( l \)-Isoprenaline Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic acid (100) and acetic anhydride (3:2) by warming, cool, and titrate \(< 2.50>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 24.77 mg of C\(_{11}\)H\(_{17}\)NO\(_3\).HCl

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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### Isopropanol

**Isopropyl Alcohol**

\[ \text{C}_3\text{H}_8\text{O} : 60.10 \]

Propan-2-ol

[67-63-0]

**Description** Isopropanol is a clear, colorless liquid. It has a characteristic odor.

It is miscible with water, with methanol, with ethanol (95), and with diethyl ether.

It is flammable and volatile.

**Identification** (1) To 1 mL of Isopropanol add 2 mL of iodine TS and 2 mL of sodium hydroxide TS, and shake: a light yellow precipitate is formed.

(2) To 5 mL of Isopropanol add 20 mL of potassium dichromate and 5 mL of sulfuric acid with caution, and warm gently on a water bath: the produced gas has the odor of acetone, and the gas turns the filter paper, previously wetted with a solution of salicylaldehyde in ethanol (95) (1 in 10) and with a solution of sodium hydroxide (3 in 10), to reddish brown.

**Specific gravity** \(< 2.56>\) \(d_{20}^{20} : 0.785 \text{ to } 0.788\)

**Purity** (1) Clarity of solution—To 2.0 mL of Isopropanol add 8 mL of water, and shake: the solution is clear.

(2) Acidity—To 15.0 mL of Isopropanol add 50 mL of freshly boiled and cooled water and 2 drops of phenolphthalein TS, and add 0.40 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Residue on evaporation—Evaporate 20.0 mL of Isopropanol on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

**Water** \(< 2.48>\) Not more than 0.75 w/v% (2 mL, volumetric titration, direct titration).

**Distilling range** \(< 2.57>\) 81 – 83°C, not less than 94 vol%.

**Containers and storage** Containers—Tight containers. Storage—Remote from fire.
Isopropylantipyrine

Propyphenazone

イソプロピルアンチピリン

C₆H₁₈N₂O: 230.31
1,5-Dimethyl-4-(1-methylphenyl)-1,2-dihydro-3H-pyrazol-3-one

Isopropylantipyrine, when dried, contains not less than 98.0% of C₁₄H₁₈N₂O₂.

Description Isopropylantipyrine occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in acetic acid (100), freely soluble in ethanol (95) and in acetone, soluble in diethyl ether, and slightly soluble in water.

Identification (1) To 2 mL of a solution of Isopropylantipyrine (1 in 500) add 1 drop of iron (III) chloride TS: a light red color develops. Further add 3 drops of sulfuric acid to this solution: the color changes to pale yellow.

(2) Add 5 mL of a solution of Isopropylantipyrine (1 in 500) to a mixture of 5 mL of potassium hexacyanoferrate (III) TS and 1 to 2 drops of iron (III) chloride TS: a dark green color gradually develops.

(3) To 2 mL of a solution of Isopropylantipyrine (1 in 500) add 2 to 3 drops of tannic acid TS: a white precipitate is produced.

Melting point <2.60> 103 – 105°C.

Purity (1) Chloride <1.07>—Dissolve 1.0 g of Isopropylantipyrine in 30 mL of dilute sulfuric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute sulfuric acid, 30 mL of dilute ethanol and water to make 50 mL (not more than 0.014%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Isopropylantipyrine in 30 mL of dilute ethanol, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and 30 mL of dilute ethanol, and dilute with water to make 50 mL (not more than 0.019%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Isopropylantipyrine in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, 25 mL of acetone, and dilute with water to make 50 mL (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Isopropylantipyrine according to Method 3, and perform the test (not more than 2 ppm).

(5) Antipyrine—Dissolve 1.0 g of Isopropylantipyrine in 10 mL of dilute ethanol, and add 1 mL of sodium nitrite TS and 1 mL of dilute sulfuric acid: no green color develops.

Loss on drying <2.47> Not more than 0.5% (1 g, in vacuum, silica gel, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Isopropylantipyrine, previously dried, dissolve in 60 mL of a mixture of acetic acid (100) and acetic anhydride (2:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 23.03 mg of C₁₄H₁₈N₂O₂

Containers and storage Containers—Tight containers.

Isosorbide

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C₆H₁₀O₄: 146.14
1,4:3,6-Dianhydro-D-glucitol

Isosorbide contains not less than 98.5% of C₆H₁₀O₄, calculated on the anhydrous basis.

Description Isosorbide occurs as white crystals or masses. It is odorless, or has a faint, characteristic odor, and has a bitter taste.

It is very soluble in water and in methanol, freely soluble in ethanol (95), and slightly soluble in diethyl ether. It is hygroscopic.

Identification (1) To 0.1 g of Isosorbide add 6 mL of dilute sulfuric acid (1 in 2), and dissolve by heating in a water bath. After cooling, shake well with 1 mL of a solution of potassium permanganate (1 in 30), and heat in a water bath until the color of potassium permanganate disappears. To this solution add 10 mL of 2,4-dinitrophenyl-hydrazine TS, and heat in a water bath: an orange precipitate is formed.

(2) To 2 g of Isosorbide add 30 mL of pyridine and 4 mL of benzoyl chloride, boil under a reflux condenser for 50 minutes, cool, and pour gradually the solution into 100 mL of cold water. Filter the formed precipitate by suction through a glass filter (G3), wash with water, recrystallize twice from ethanol (95), and dry in a desiccator (in vacuum, silica gel) for 4 hours: it melts <2.60> between 102°C and 103°C.

(3) Determine the infrared absorption spectrum of Isosorbide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit...
Isosorbide Dinitrate

硝酸イソソルビド

\[
\text{C}_6\text{H}_8\text{N}_2\text{O}_8: 236.14
\]

1,4;3,6-Dianhydro-D-glucitol dinitrate

\[
[\text{87-33-2}]
\]

Isosorbide Dinitrate contains not less than 95.0% of \( \text{C}_6\text{H}_8\text{N}_2\text{O}_8 \), calculated on the anhydrous basis.

**Description** Isosorbide Dinitrate occurs as white crystals or crystalline powder. It is odorless or has a faint odor like that of nitric acid.

It is very soluble in \( \text{N},\text{N}\)-dimethylformamide and in acetone, freely soluble in chloroform and in toluene, soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

It explodes if heated quickly or subjected to percussion.

**Identification** (1) Dissolve 10 mg of Isosorbide Dinitrate in 1 mL of water, and dissolve by adding 2 mL of sulfuric acid cautiously. After cooling, superimpose 3 mL of iron (II) sulfate TS, and allow to stand for 5 to 10 minutes; a brown ring is produced at the zone of contact.

(2) Dissolve 0.1 g of Isosorbide Dinitrate in 6 mL of diluted sulfuric acid (1 in 2) by heating in a water bath. After cooling, add 1 mL of a solution of potassium permanganate (1 in 30), stir well, and heat in a water bath until the color of potassium permanganate disappears. Add 10 mL of 2,4-dinitro-phenylhydrazine TS, and heat in a water bath: an orange precipitate is produced.

**Optical rotation** \( <2.49^0; [\alpha]_D^{20} + 134 - + 139^0 \) (1 g, calculated on the anhydrous basis, ethanol (95), 100 mL, 100 mm).

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Isosorbide Dinitrate in 10 mL of acetone: the solution is clear and colorless.

(2) Sulfate \( <1.1^0 \)—Dissolve 1.5 g of Isosorbide Dinitrate in 15 mL of \( \text{N},\text{N}\)-dimethylformamide, add 60 mL of water, cool, and filter. Wash the filter paper with three 20-mL portions of water, combine the washings with the filtrate, and add water to make 150 mL. To 40 mL of this solution add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Nitrate—Dissolve 50 mg of Isosorbide Dinitrate in 30 mL of toluene, and extract with three 20-mL portions of water. Combine the aqueous layers, and wash with two 20-mL portions of toluene. To the aqueous layer add water to make 100 mL, and use this solution as the sample solution. Pipet 5.0 mL of Standard Nitric Acid Solution and 25 mL of the sample solution in each Nessler tube, and add water to make 50 mL, respectively. To each of them add 2.5 mL of Standard Lead Solution (not more than 5 ppm).

**Containers and storage** Containers—Tight containers.

**Residue on ignition** \( <2.44^0 \) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 10 g of Isosorbide, calculated on the anhydrous basis, and dissolve in water to make exactly 100 mL. Determine the optical rotation \( <2.49^0; [\alpha]_D^{20} \), of this solution at 20 ± 1°C in a 100-mm cell.

\[
\text{Amount (g) of } \text{C}_6\text{H}_8\text{N}_2\text{O}_8 = [\alpha]_D \times 2.1978
\]

**Purity (1)** Clarity and color of solution—Take 25 g of Isosorbide in a Nessler tube, and dissolve in 50 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To a mixture of 1.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add water to make 10.0 mL. To 3.0 mL of this solution add water to make 50 mL.

(2) Sulfate \( <1.1^0 \)—Perform the test with 2.0 g of Isosorbide. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals \( <1.0^0 \) —Proceed with 5.0 g of Isosorbide according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(4) Arsenic \( <1.1^0 \) —Prepare the test solution with 1.0 g of Isosorbide according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Isosorbide in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.0^0 \). Spot 10 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95) and cyclohexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of ethanol (95) and sulfuric acid (9:1) on the plate, and heat at 150°C for 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Water** \( <2.48^0 \) Not more than 1.5% (2 g, volumetric titration, direct titration).

**Optical rotation** \( <2.49^0; [\alpha]_D^{20} + 45.0 - + 46.0^0 \) (5 g, calculated on the anhydrous basis, water, 50 mL, 100 mm).

**Purity** (1) Clarity and color of solution—Take 25 g of Isosorbide in a Nessler tube, and dissolve in 50 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To a mixture of 1.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add water to make 10.0 mL. To 3.0 mL of this solution add water to make 50 mL.

(2) Sulfate \( <1.1^0 \)—Perform the test with 2.0 g of Isosorbide. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals \( <1.0^0 \) —Proceed with 5.0 g of Isosorbide according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(4) Arsenic \( <1.1^0 \) —Prepare the test solution with 1.0 g of Isosorbide according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Isosorbide in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.0^0 \). Spot 10 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95) and cyclohexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of ethanol (95) and sulfuric acid (9:1) on the plate, and heat at 150°C for 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Residue on ignition** \( <2.44^0 \) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 10 g of Isosorbide, calculated on the anhydrous basis, and dissolve in water to make exactly 100 mL. Determine the optical rotation \( <2.49^0; [\alpha]_D^{20} \), of this solution at 20 ± 1°C in a 100-mm cell.

\[
\text{Amount (g) of } \text{C}_6\text{H}_8\text{N}_2\text{O}_8 = [\alpha]_D \times 2.1978
\]

**Containers and storage** Containers—Tight containers.

similar intensities of absorption at the same wave numbers.
60 mg of Griss-Romijin’s nitric acid reagent, stir well, allow to stand for 30 minutes, and observe from the side of the Nessler tube: the sample solution has no more color than the standard solution.

(4) Heavy metals <1.07>—Dissolve 1.0 g of Isosorbide Dinitrate in 30 mL of acetone, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

Water <2.48> Not more than 1.5% (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Isosorbide Dinitrate in a Kjeldahl flask as described under the Nitrogen Determination <1.08>, dissolve in 10 mL of methanol, add 3 g of Devarda’s alloy and 50 mL of water, and connect the flask with the distillation apparatus as described under the Nitrogen Determination <1.08>. Measure exactly 25 mL of 0.05 mol/L sulfuric acid VS in an absorption flask, add 5 drops of bromocresol green-methyl red TS, and immerse the lower end of the condenser tube in it. Add 15 mL of a solution of sodium hydroxide (1 in 2) through the funnel, cautiously rinse the funnel with 20 mL of water, immediately close the clamp attached to the rubber tubing, then begin the distillation with steam gradually, and continue the distillation until the distillate measures 100 mL. Remove the absorption flask, rinse the end of the condenser tube with a small quantity of water, and titrate <2.50> the distillate and the rinsings with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from red through light red-purple to light blue-green. Perform a blank determination.

Each mL of 0.05 mol/L sulfuric acid VS

\[= 11.81 \text{ mg of } \text{C}_6\text{H}_8\text{N}_2\text{O}_8\]

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Isosorbide Dinitrate Tablets
硝酸イソソルビド錠

Isosorbide Dinitrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of isosorbide dinitrate (C₆H₈N₂O₈; 236.14).

Method of preparation Prepare as directed under Tablets, with Isosorbide Dinitrate.

Identification Weigh a quantity of powdered Isosorbide Dinitrate Tablets, equivalent to 0.1 g of Isosorbide Dinitrate according to the labeled amount, add 50 mL of diethyl ether, shake well, and filter. Measure 5 mL of the filtrate, evaporate to dryness cautiously, add 1 mL of water to the residue, and dissolve by adding 2 mL of sulfuric acid cautiously. After cooling, superimpose 3 mL of iron (II) sulfate TS, and allow to stand for 5 to 10 minutes: a brown ring is produced at the zone of contact.

Purity Free nitrate ion—Weigh accurately a quantity of powdered Isosorbide Dinitrate Tablets, equivalent to 50 mg of Isosorbide Dinitrate according to the labeled amount, transfer to a separator, add 30 mL of toluene, shake thoroughly, extract with three 20-mL portions of water, and proceed as directed in Purity (3) under Isosorbide Dinitrate.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Isosorbide Dinitrate Tablets add 1 mL of water, and shake to disintegrate. To this solution add a mixture of water and methanol (1:1) to make exactly V mL so that each mL contains about 0.1 mg of isosorbide dinitrate (C₆H₈N₂O₈), and shake for 10 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of isosorbide dinitrate (C₆H₈N₂O₈)
\[= M_5 \times A_T/A_S \times V \times 1/500\]

\[M_5: \text{Amount (mg) of isosorbide dinitrate for assay, calculated on the anhydrous basis}\]

Disintegration <6.09> It meets the requirement.

For Sublingual Tablets, the time limit of the test is 2 minutes, and omit the use of the disk.

Assay Weigh accurately the mass of not less than 20 tablets of Isosorbide Dinitrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of isosorbide dinitrate (C₆H₈N₂O₈), add a mixture of water and methanol (1:1) to make exactly 50 mL, and shake for 10 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of isosorbide dinitrate for assay (separately, determine the water <2.48> in the same manner as Isosorbide Dinitrate), dissolve in a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, A₇ and A₅, of isosorbide dinitrate in each solution.

Amount (mg) of isosorbide dinitrate (C₆H₈N₂O₈)
\[= M_5 \times A_T/A_S \times 1/10\]

\[M_5: \text{Amount (mg) of isosorbide dinitrate for assay, calculated on the anhydrous basis}\]

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of water and methanol (1:9).
Flow rate: Adjust the flow rate so that the retention time of isosorbide dinitrate is about 6 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosorbide dinitrate are not less than 3000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isosorbide dinitrate is not more than 1.0%.

Containers and storage  Containers—Tight containers.

**Isoxsuprine Hydrochloride**

イソクスプリン塩酸塩

C₁₈H₂₃NO₃.HCl: 337.84

(1R5,2SR)-1-(4-Hydroxyphenyl)-2-[(2SR)-1-phenoxypropan-2-yl]amino]propan-1-ol monohydrochloride [579-56-6]

Isoxsuprine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C₁₈H₂₃NO₃.HCl.

**Description**  Isoxsuprine Hydrochloride occurs as a white, powder or crystalline powder. It is soluble in formic acid and in methanol, and slightly soluble in water and in ethanol (99.5).

Melting point: about 204°C (with decomposition).

A solution of Isoxsuprine Hydrochloride in methanol (1 in 50) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Isoxsuprine Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry 2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Isoxsuprine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry 2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.5 g of Isoxsuprine Hydrochloride in 50 mL of water by warming, and cool: the solution responds to the Qualitative Tests 1.09> (2) for chloride.

**pH 2.54>** Dissolve 0.5 g of Isoxsuprine Hydrochloride in 50 mL of water by warming, and cool: the pH of the solution is between 4.5 and 6.0.

**Purity (1)** Clarity and color of solution—Dissolve 0.1 g of Isoxsuprine Hydrochloride in 10 mL of water, warm if necessary, and cool: the solution is clear and colorless.

(2) Heavy metals 1.07>—Proceed with 1.0 g of Isoxsuprine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Isoxsuprine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01> according to the following conditions. Determine each peak area by the automatic integration method: each peak area other than isoxsuprine obtained from the sample solution is not larger than the peak area of isoxsuprine from the standard solution, and the total area of the peaks other than the peak of isoxsuprine is not larger than 2 times the peak area of isoxsuprine from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 269 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.3 g of diammonium hydrogen phosphate and 3.2 g of sodium 1-pentane sulfonate in water to make 1000 mL, and adjust to pH 2.5 with phosphoric acid. To 770 mL of this solution add 230 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of isoxsuprine is about 18 minutes.

Time span of measurement: About 3 times as long as the retention time of isoxsuprine, beginning after the solvent peak.

**System suitability—**

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of isoxsuprine obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: To 1 mL of the sample solution add 2.5 mL of a solution of methyl parahydroxybenzoate (1 in 25,000) and the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoate and isoxsuprine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoxsuprine is not more than 2.5%.

**Loss on drying 2.41>** Not more than 0.5% (1 g, 105°C, 1 hour).

**Residue on ignition 2.44>** Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.3 g of Isoxsuprine Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 50 mL of a mixture of acetic anhydride and acetic acid (100:7:3), and titrate 2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 33.78 mg of C₁₈H₂₃NO₃.HCl

**Containers and storage** Containers—Well-closed containers.
Isosuprine Hydrochloride Tablets

イソクスプリン塩酸塩錠

Isosuprine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of isosuprine hydrochloride (C₁₈H₂₃NO₃.HCl: 337.84).

**Method of preparation** Prepare as directed under Tablets, with Isosuprine Hydrochloride.

**Identification** To a quantity of powdered Isosuprine Hydrochloride Tablets, equivalent to 10 mg of Isosuprine Hydrochloride according to the labeled amount, add 150 mL of water, shake, and then add water to make 200 mL. Centrifuge this solution, filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of filtrate, and determine the absorption of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry (C₂₂₄nm and C₂₇₆nm). It exhibits maxima between 267 nm and 271 nm, and between 272 nm and 276 nm.

**Uniformity of dosage units** (6.02) Perform the test according to the following method: it meets the requirements of the Content uniformity test.

Add methanol to 1 tablet of Isosuprine Hydrochloride Tablets, and shake to disintegrate. Add methanol to make exactly V mL so that each mL contains about 0.4 mg of isosuprine hydrochloride (C₁₈H₂₃NO₃.HCl). Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of isosuprine hydrochloride (C₁₈H₂₃NO₃.HCl)

\[ M_S = \frac{S \times V}{100} \]

\( A_T / A_S \times V'/V \times \frac{1}{C} \times 36 \)

\( M_S \): Amount (mg) of isosuprine hydrochloride for assay

\( M_c \): Amount (mg) of isosuprine hydrochloride for assay

**Dissolution** (6.10) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Isosuprine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Isosuprine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 11 μg of isosuprine hydrochloride (C₁₈H₂₃NO₃.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of isosuprine hydrochloride for assay, previously dried at 105°C for 1 hour, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the isosuprine peak areas, \( A_T \) and \( A_S \), of both solutions.

Dissolution rate (%) with respect to the labeled amount of isosuprine hydrochloride (C₁₈H₂₃NO₃.HCl)

\[ \frac{M_c \times A_T / A_S \times V'/V \times \frac{1}{C} \times 36}{M} \]

\( M_c \): Amount (mg) of isosuprine hydrochloride for assay

\( C \): Labeled amount (mg) of isosuprine hydrochloride (C₁₈H₂₃NO₃.HCl) in 1 tablet

**Operating conditions**—

Proceed as directed in the operating conditions in the Assay.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosuprine are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isosuprine is not more than 2.0%.

**Assay** Weigh accurately not less than 20 Isosuprine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder equivalent to about 40 mg of isosuprine hydrochloride (C₁₈H₂₃NO₃.HCl), add 60 mL of methanol, shake for 20 minutes, and then add methanol to make exactly 100 mL. Centrifuge a portion of this solution, filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of isosuprine hydrochloride for assay, previously dried at 105°C for 1 hour, and dissolve in methanol to make exactly 100 mL. Filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of filtrate, and use the subsequent filtrate as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of isosuprine in each solution.

Amount (mg) of isosuprine hydrochloride (C₁₈H₂₃NO₃.HCl)

\[ M_S = \frac{A_T / A_S \times V}{100} \]

\( M_S \): Amount (mg) of isosuprine hydrochloride for assay

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 269 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.3 g of diammonium hydrogen phosphate and 3.2 g of sodium 1-pentane sulfonate in water to make 1000 mL, and adjust to pH 2.5 with phosphoric acid. To 600 mL of this solution add 400 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of isosuprine is about 9 minutes.

**System suitability**—

System performance: To exactly 1 mL of the standard solution add the mobile phase to make exactly 50 mL. When the procedure is run with 10 μL of this solution under the
above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isoxsuprine are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoxsuprine is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Itraconazole

イトラコンゾール

\[
\text{C}_{35}\text{H}_{38}\text{Cl}_{2}\text{N}_{8}\text{O}_{4}: 705.63 \\
4-(4-[4-(4-[(2RS,4SR)-2-(2,4-Dichlorophenyl)]-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-y][methoxy]phenyl)piperazin-1-ylphenyl)-2-[(1RS)-1-methylpropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one \\
4-(4-[4-[4-[(2SR,4RS)-2-(2,4-Dichlorophenyl)]-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-y][methoxy]phenyl)piperazin-1-ylphenyl)-2-[(1RS)-1-methylpropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one [84625-61-6]
\]

Itraconazole contains not less than 98.5% and not more than 101.0% of \( \text{C}_{35}\text{H}_{38}\text{Cl}_{2}\text{N}_{8}\text{O}_{4} \), calculated on the dried basis.

Description Itraconazole occurs as a white powder.

It is soluble in \( \text{N},\text{N}\)-dimethylformamide, very slightly soluble in ethanol (99.5), and practically insoluble in water and in 2-propanol.

A solution of Itraconazole in \( \text{N},\text{N}\)-dimethylformamide (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Itraconazole in 2-propanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Itraconazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Itraconazole as directed under Flame Coloration Test \(\text{<1.07>}(2): \) a green color appears.

Melting point \(<2.60\rangle\ 166 – 170^\circ\text{C}\.

Purity (1) Heavy metals \(\text{<1.07>}: \) Proceed with 1.0 g of Itraconazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Itraconazole in 10 mL of a mixture of methanol and tetrahydrofuran (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of methanol and tetrahydrofuran (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add the mixture of methanol and tetrahydrofuran (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07\rangle\) according to the following conditions. Determine each peak area of each solution by the automatic integration method: the area of each peak other than itraconazole obtained from the sample solution is not larger than the peak area of itraconazole from the standard solution. Furthermore, the total area of the peaks other than itraconazole is not larger than 2.5 times the peak area of itraconazole from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: A solution of Tetrabutylammonium hydrogensulfate (17 in 625).

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 20</td>
<td>0 – 80</td>
<td>80 – 50</td>
</tr>
<tr>
<td>20 – 25</td>
<td>20 – 50</td>
<td>100 – 50</td>
</tr>
</tbody>
</table>

Flow rate: 1.5 mL per minute.

Time span of measurement: About 2 times as long as the retention time of itraconazole, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mixture of methanol and tetrahydrofuran (1:1) to make exactly 10 mL. Confirm that the peak area of itraconazole obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 1 mg of Itraconazole and 1 mg of miconazole nitrate in 20 mL of the mixture of methanol and tetrahydrofuran (1:1). When the procedure is run with 10 μL of this solution under the above operating condi-
lations, miconazole and itraconazole are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of itraconazole is not more than 2.0%.

Loss on drying <2.4% Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.4% Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Itraconazole, dissolve in 70 mL of a mixture of 2-butanone and acetic acid (100) (7:1), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 35.28 mg of C₃₅H₃₈Cl₂N₈O₄

Containers and storage Containers—Tight containers.

Japanese Encephalitis Vaccine

日本脳炎ワクチン

Japanese Encephalitis Vaccine is a liquid for injection containing inactivated Japanese encephalitis virus.

It conforms to the requirements of Japanese Encephalitis Vaccine in the Minimum Requirements for Biological Products.

Description Japanese Encephalitis Vaccine is a clear or a slightly whitish turbid and colorless liquid.

Freeze-dried Japanese Encephalitis Vaccine

乾燥日本脳炎ワクチン

Freeze-dried Japanese Encephalitis Vaccine is a preparation for injection which is dissolved before use.

It contains inactivated Japanese encephalitis virus.

It conforms to the requirements of Freeze-dried Japanese Encephalitis Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Japanese Encephalitis Vaccine is a clear or a slightly whitish turbid and colorless liquid on addition of solvent.

Josamycin

ジョサマイシン

Josamycin is a macrolide substance having antibacterial activity produced by the growth of Streptomyces narboensis var. josamyceticus.

It contains not less than 900 µg (potency) and not more than 1100 µg (potency) per mg, calculated on the dried basis. The potency of Josamycin is expressed as mass (potency) of josamycin (C₄₂H₆₉NO₁₅).

Description Josamycin occurs as a white to yellowish white powder.

It is very soluble in methanol and in ethanol (99.5), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Josamycin in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Josamycin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Josamycin and Josamycin RS in 1 mL of methanol, add diluted methanol (1 in 2) to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions: the retention time of the main peak obtained from the sample solution is the same as that the peak of josamycin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2).

Purity (1) Heavy metals <1.07%—Proceed with 1.0 g of Josamycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 50 mg of Josamycin in
Josamycin Tablets / Official Monographs

5 mL of methanol, add diluted methanol (1 in 2) to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography &gt;2.01&lt; according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of josamycin and the related substances by the area percentage method: the amounts of the peaks other than josamycin are not more than 6%, and the total of these peaks is not more than 20%.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 231 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadeylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 119 g of sodium perchlorate monohydrate in water to make 1000 mL, and adjust the pH to 2.5 with 1 mol/L hydrochloric acid TS. To 600 mL of this solution add 400 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of josamycin is about 10 minutes.

Time span of measurement: About 4 times as long as the retention time of josamycin beginning after the solvent peak.

**System suitability—**

Test for required detectability: Measure exactly 3 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the solution for system suitability test. Measure exactly 2 mL of the solution for system suitability test, and add diluted methanol (1 in 2) to make exactly 20 mL. Confirm that the peak area of josamycin obtained from 10 μL of this solution is equivalent to 8 to 12% of that from 10 μL of the solution for system suitability test.

System performance: Dissolve about 0.05 g of Josamycin in 50 mL of 0.1 mol/L potassium dihydrogen phosphate TS, pH 2.0, and allow to stand at 40°C for 3 hours. Adjust the pH of this solution to 6.8 to 7.2 with 2 mol/L sodium hydroxide TS, and add 50 mL of methanol. This solution contains both josamycin and josamycin S1. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of josamycin S1, which relative retention time to josamycin is about 0.9, and josamycin is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of josamycin is not more than 1.5%.

**Loss on drying &lt;2.41&gt;** Not more than 1.0% (0.5 g, in vacuum, phosphorus(V) oxide, 60°C, 3 hours).

**Residue on ignition &lt;2.44&gt;** Not more than 0.1% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics &lt;4.02&gt; according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Josamycin RS, equivalent to about 30 mg (potency), dissolve in 5 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add water to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Josamycin, equivalent to about 30 mg (potency), dissolve in 5 mL of methanol, and add water to make exactly 100 mL. Take exactly a suitable amount of this solution, add water to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Josamycin Tablets**

Josamycin Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of josamycin (C₄₂H₆₉NO₁₅: 827.99).

**Method of preparation** Prepare as directed under Tablets, with Josamycin.

**Identification** To a quantity of powdered Josamycin Tablets, equivalent to 10 mg (potency) of Josamycin according to the labeled amount, add 100 mL of methanol, shake vigorously, and centrifuge. To 5 mL of the supernatant liquid, add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry &lt;2.24&gt;: it exhibits a maximum between 229 nm and 233 nm.

**Loss on drying &lt;2.41&gt;** Not more than 5.0% (0.5 g, in vacuum, 60°C, 3 hours).

**Uniformity of dosage units &lt;6.02&gt;**—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Josamycin Tablets, add 5 mL of water, and shake vigorously to disintegrate the tablet. Add methanol and then use ultrasonic waves to disperse the particles, add methanol to make exactly 5 mL so that each mL contains about 2 mg (potency) of Josamycin, and centrifuge. Pipet 3 mL of the supernatant liquid, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, accurately weigh about 50 mg (potency) of Josamycin RS, dissolve in 5 mL of water and methanol to make exactly 25 mL. Pipet 3 mL of this solution, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and Aₜ, of the sample solution and standard solution at 231 nm as directed under Ultraviolet-visible Spectrophotometry &lt;2.24&gt;. However, X in the formula for
calculation of acceptance value is the result of the assay.

\[
\text{Amount (mg (potency)) of josamycin (C}_{42}\text{H}_{69}\text{NO}_{15}) = M_S \times \frac{A_I}{A_S} \times \frac{V}{25}
\]

M_S: Amount (mg (potency)) of Josamycin RS

**Disintegration** <0.07> Perform the test using the disk: it meets the requirement.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.07> according to the following conditions.

(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Josamycin.

(ii) Sample solutions—Weigh accurately the mass of not less than 20 Josamycin Tablets and pulverize into a powder. Weigh accurately a portion of the powder, equivalent to about 0.3 g (potency) of Josamycin, add 50 mL of methanol, shake vigorously, and add water to make exactly 1000 mL. Take exactly an appropriate amount of this solution, add water to prepare solutions containing 30 \(\mu\)g (potency) and 7.5 \(\mu\)g (potency) per mL, and use these solutions as the high and the low concentration sample solutions, respectively.

**Containers and storage** Containers—Tight containers.

**Josamycin Propionate**

ジョサマイシンプロピオン酸エステル

\[
\text{C}_{42}\text{H}_{69}\text{NO}_{15}: \quad 884.06
\]

(3R,4R,5S,6R,8R,9R,10E,12E,15R)-3-Acetoxy-5-
[2,6-dideoxy-4-O-(3-methylbutanoyl)-3-C-methyl-
\(\alpha\)-l-ribo-hexopyranosyl-(1→4)-3,6-dideoxy-3-
dimethylamino-\(\beta\)-D-glucopyranosyloxy]-6-formylmethyl-4-
methoxy-8-methyl-9-propanoyloxyhexadeca-10,12-
dien-15-olide

[16846-24-5, Josamycin]

Josamycin Propionate is a derivative of josamycin.

It contains not less than 843 \(\mu\)g (potency) and not more than 1000 \(\mu\)g (potency) per mg, calculated on the dried basis. The potency of Josamycin Propionate is expressed as mass (potency) of josamycin (C\(_{42}\)H\(_{69}\)NO\(_{15}\): 827.99).

**Description** Josamycin Propionate occurs as a white to light yellowish white crystalline powder.

It is very soluble in acetonitrile, freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Josamycin Propionate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Josamycin Propionate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Josamycin Propionate and Josamycin Propionate RS in 50 mL of diluted acetonitrile (1 in 2), and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the retention time of the peak of josamycin propionate obtained from the sample solution is the same with that of the peak of josamycin propionate from the standard solution.

**Operating conditions**

- Detector: column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2).

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Josamycin Propionate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 50 mg of Josamycin Propionate in the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 10 \(\mu\)L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of each peak other than josamycin propionate by the area percentage method: the amount of any peak other than josamycin is not more than 6%, and the total of these peaks is not more than 22%.

**Operating conditions**

- Detector: An ultraviolet absorption photometer (wavelength: 234 nm).
- Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: To 10 mL of triethylamine add water to make 1000 mL, and adjust the pH to 4.3 with acetic acid (100). To 500 mL of this solution add 500 mL of acetonitrile.
- Flow rate: Adjust the flow rate so that the retention time of josamycin propionate is about 24 minutes.
- Time span of measurement: About 3.5 times as long as the retention time of josamycin propionate beginning after the solvent peak.

**System suitability**

- Test for required detectability: Measure exactly 3 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the solution for system suitability test. Measure exactly 2 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of josamycin propionate obtained from 10 \(\mu\)L of this solution is equivalent to 8 to 12% of that from 10 \(\mu\)L of the solution for system suitability test.

**System performance:** Dissolve 5 mg of josamycin pro-
Kainic Acid Hydrate

Kainic Acid Hydrate, when dried, contains not less than 99.0% of kainic acid (C_{10}H_{15}NO_{4}: 213.23).

**Description**
Kainic Acid Hydrate occurs as white crystals or crystalline powder. It is odorless, and has an acid taste. It is sparingly soluble in water and in warm water, very slightly soluble in acetic acid (100) and in ethanol (95), and practically insoluble in diethyl ether. It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

The pH of its solution (1 in 100) is between 2.8 and 3.5. Melting point: about 252°C (with decomposition).

**Identification**
(1) To 5 mL of a solution of Kainic Acid Hydrate (1 in 5000) add 1 mL of ninhydrin TS, and warm in a water bath at a temperature between 60°C and 70°C for 5 minutes: a yellow color is produced.

(2) Dissolve 50 mg of Kainic Acid Hydrate in 5 mL of acetic acid (100), and add 0.5 mL of bromine TS: the color of bromine disappears immediately.

**Optical rotation**
\[ \alpha^D_{\text{D}}: -13\text{ to }-17^\circ \] (0.5 g, water, 50 mL, 200 mm).

**Purity**
(1) Clarity and color of solution—Dissolve 0.10 g of Kainic Acid Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Chloride
Take 0.5 g of Kainic Acid Hydrate in a platinum crucible, dissolve in 5 mL of sodium carbonate TS, and evaporate on a water bath to dryness. Heat the crucible slowly at first, and then ignite until the sample is almost incinerated. After cooling, add 12 mL of dilute nitric acid to the residue, dissolve by warming, and filter. Wash the residue with 15 mL of water, combine the washings and the filtrate, and add water to make 50 mL. Perform the test using this solution as the test solution.

Control solution: Add 5 mL of sodium carbonate TS to 0.30 mL of 0.01 mol/L hydrochloric acid VS, and proceed as directed above (not more than 0.021%).

(3) Sulfate
Dissolve 0.5 g of Kainic Acid Hydrate in 40 mL of water by warming. Cool, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium
Take 0.25 g of Kainic Acid Hydrate, and perform the test. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).
(5) Heavy metals <1.07>—Proceed with 1.0 g of Kainic Acid Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.17>—Dissolve 1.0 g of Kainic Acid Hydrate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Amino acid and other imino acid—Dissolve 0.1 g of Kainic Acid Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03> with these solutions. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the supernatant liquid of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 6.5–8.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.4 g of Kainic Acid Hydrate, previously dried, and dissolve in 50 mL of warm water, cool and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS).

Each mL of 0.1 mol/L sodium hydroxide VS = 21.32 mg of C_{10}H_{15}NO_{4}

Containers and storage Containers—Tight containers.

Kainic Acid and Santonin Powder

カイニン酸・サントニン散

Kainic Acid and Santonin Powder contains not less than 9.0% and not more than 11.0% of santonin (C_{12}H_{14}O_{5}: 246.30), and not less than 1.80% and not more than 2.20% of kainic acid hydrate (C_{10}H_{15}NO_{4}.H_{2}O: 231.25).

Method of preparation

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santonin</td>
<td>100 g</td>
</tr>
<tr>
<td>Kainic Acid Hydrate</td>
<td>20 g</td>
</tr>
<tr>
<td>Starch, Lactose Hydrate or their mixture</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Kainic Acid and Santonin Powder occurs as a white powder.

Identification (1) Shake 1 g of Kainic Acid and Santonin Powder with 10 mL of chloroform, and filter [use the residue for the test (2)]. Distil off the chloroform of the filtrate, and dissolve the residue in 2 mL of potassium hydroxide-ethanol TS: a red color is produced (kainic acid).

Assay (1) Santonin—Weigh accurately about 0.25 g of Kainic Acid and Santonin Powder and about 25 mg of santonin for assay, add 20 mL each of ethanol (95), shake thoroughly for 5 minutes, and filter. Wash the residue with three 10-mL portions of ethanol (95), and filter. Combine the filtrate and the washings, and add ethanol (95) to make exactly 50 mL. Pipet 2 mL each of these solutions, add ethanol (95) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_s and A_S, of the sample solution and the standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Amount (mg) of santonin (C_{12}H_{14}O_{5})} = M_s \times \frac{A_s}{A_S}
\]

M_s: Amount (mg) of santonin for assay

(2) Kainic acid—Weigh accurately about 1.25 g of Kainic Acid and Santonin Powder, add 20 mL of diluted pyridine (1 in 10), shake thoroughly for 5 minutes, and filter. Wash the residue with three 10-mL portions of diluted pyridine (1 in 10), and filter. Combine the filtrate and the washings, and add diluted pyridine (1 in 10) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted pyridine (1 in 10) to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve about 25 mg of kainic acid for assay, previously dried at 105°C for 4 hours and accurately weighed, in diluted pyridine (1 in 10) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted pyridine (1 in 10) to make exactly 25 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and standard solution, add 2 mL of ninhydrin-\(\text{L}\)-ascorbic acid TS, and heat on a water bath for 30 minutes. After cooling immediately, shake vigorously for 2 minutes, add water to make exactly 20 mL, and allow to stand for 15 minutes. Determine the absorbances, A_T and A_S, of these solutions at 425 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the solution prepared in the same manner with 2 mL of diluted pyridine (1 in 10) instead of the sample solution as the blank.

\[
\text{Amount (mg) of kainic acid hydrate (C_{10}H_{15}NO_{4}.H_{2}O)} = M_s \times \frac{A_T}{A_S} \times 1.085
\]

M_s: Amount (mg) of kainic acid for assay

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.
Kallidinogenase

カリジノゲナーゼ

[K001-01-8]

Kallidinogenase is an enzyme obtained from healthy porcine pancreas, and has kinin-releasing activity based on cleavage of kininogen.

It contains not less than 25 Kallidinogenase Units per mg. Usually, it is diluted with Lactose Hydrate or the like.

Kallidinogenase contains not less than 90% and not more than 110% of the labeled Units.

**Description** Kallidinogenase occurs as a white to light brown powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Kallidinogenase (1 in 300) is between 5.5 and 7.5.

**Identification** (1) Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 10 Kallidinogenase Units per mL. Pipet 5 mL of this solution, and add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Pipet 4 mL each of this solution into two separate test tubes, add exactly 1 mL each of aprotinin TS and 0.05 mol/L phosphate buffer solution, pH 7.0 separately to each test tube, allow them to stand at room temperature for 20 minutes, and use these solutions as the sample solutions 1 and 2. Separately, pipet 1 mL of trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Pipet 4 mL each of this solution into two separate test tubes, add exactly 1 mL each of aprotinin TS and 0.05 mol/L phosphate buffer solution, pH 7.0 separately to each test tube, allow them to stand at room temperature for 20 minutes, and use these solutions as the sample solutions 3 and 4. Then, pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at 30.0 ± 0.5°C for 5 minutes, place in a 10-mm cell, add exactly 0.1 mL of the sample solutions 1 and 2, respectively, mix. Pipet 0.1 mL of this solution, add exactly 0.9 mL of trichloroacetic-acid-gelatin-phosphate buffer solution, and mix. Pipet 0.05 mL of this solution, add exactly 0.5 mL of trichloroacetic acid-gelatin-tris buffer solution, and mix. Pipet 0.2 mL of this solution, add exactly 0.6 mL of trichloroacetic acid-gelatin-tris buffer solution, and mix. Perform the test at 30.0 ± 0.5°C as directed under Ultraviolet-visible Spectrophotometry (2,24), and determine the change of the absorbance at 253 nm for 4 to 6 minutes. Separately, pipet 1 mL of trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Add exactly 0.1 mL of this solution to exactly 2.9 mL of substrate TS for kallidinogenase assay (2), previously warmed at 30.0 ± 0.5°C for 5 minutes, and use this solution as the blank. If the rate of change in the absorbance remains constant, determine the change of absorbance per 1 minute, A, and calculate R by using the following equation: the value of R is between 0.12 and 0.16.

\[
R = A/0.0383 \times 1/(a \times b)
\]

a: Amount (mg) of Kallidinogenase in 1 mL of the sample solution
b: Amount (Unit) of kallidinogenase in 1 mg of Kallidinogenase obtained in the Assay

**Specific activity** Perform the test with Kallidinogenase as directed under Nitrogen Determination (1,08) to determine the nitrogen content, convert 1 mg of nitrogen (N: 14.01) into 6.25 mg of protein, and calculate the specific activity using the amount (Units) of Kallidinogenase obtained in the Assay: it is not less than 100 Kallidinogenase Units per 1 mg of protein.

**Purity** (I) Fat—To 1.0 g of Kallidinogenase add 20 mL of diethyl ether, extract with occasional shaking for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, combine the washing with the filtrate, evaporate the diethyl ether, and dry the residue at 105°C for 2 hours: the mass of the residue is not more than 1 mg.

(2) Kininase—
(i) Bradykinin solution: Weigh an appropriate amount of bradykinin, and dissolve in gelatin-phosphate buffer solution, pH 7.4 to prepare a solution containing 0.200 μg of bradykinin per mL.
(ii) Kallidinogenase solution: Weigh accurately a suitable amount of Kallidinogenase according to the labeled unit, dissolve in gelatin-phosphate buffer solution, pH 7.4 to make a solution containing 1 unit of kallidinogenase per mL.
(iii) Sample solution: Pipet 0.5 mL of bradykinin solution, warm at 30 ± 0.5°C for 5 minutes, then add exactly 0.5 mL of kallidinogenase solution previously warmed at 30 ± 0.5°C for 5 minutes, and mix immediately. After allow this solution to stand at 30 ± 0.5°C for exactly 150 seconds, add exactly 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Boil for 3 minutes, then cool in ice immediately, centrifuge, and allow to stand at a room temperature for 15 minutes. Pipet 0.5 mL of the supernatant liquid, add exactly 0.5 mL of gelatin-tris buffer solution, pH 8.0, and mix. Pipet 0.1 mL of this solution, add exactly 0.9 mL of trichloroacetic acid-gelatin-tris buffer solution, and mix. Pipet 0.2 mL of this solution, add exactly 0.6 mL of trichloroacetic acid-gelatin-tris buffer solution, shake, and use this solution as the sample solution.

(iv) Control solution: Proceed with 0.5 mL of gelatin-phosphate buffer solution, pH 7.4 as described in (iii), and use the solution so obtained as the control solution.

(2) Pipet 2.9 mL of substrate TS for kallidinogenase assay (2), previously warmed at 30.0 ± 0.5°C for 5 minutes, place in a 10-mm cell, add exactly 0.1 mL of the sample solution obtained in the Assay, and start simultaneously a chronograph. Perform the test at 30.0 ± 0.5°C as directed under Ultraviolet-visible Spectrophotometry (2,24), and determine the change of the absorbance at 253 nm for 4 to 6 minutes. Separately, pipet 1 mL of trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Add exactly 0.1 mL of this solution to exactly 2.9 mL of substrate TS for kallidinogenase assay (2), previously warmed at 30.0 ± 0.5°C for 5 minutes, and use this solution as the blank. If the rate of change in the absorbance remains constant, determine the change of absorbance per 1 minute, A, and calculate R by using the following equation: the value of R is between 0.12 and 0.16.

\[
R = A/0.0383 \times 1/(a \times b)
\]

a: Amount (mg) of Kallidinogenase in 1 mL of the sample solution
b: Amount (Unit) of kallidinogenase in 1 mg of Kallidinogenase obtained in the Assay

[9001-01-8]
microplate washing to the wells, then remove. Repeat this procedure 3 times, take off the washings thoroughly, then add 100 μL each of the sample solution and control solution, and 50 μL of gelatin-phosphate buffer solution, pH 7.0, shake, and allow to stand at a constant temperature of about 25°C for 1 hour. Then add 50 μL of peroxidase-labeled bradykinin TS, shake, and allow to stand in a cold place for a night.

Take off the solution, add 0.3 mL of phosphate buffer solution for microplate washing, and remove. Repeat this procedure 4 times, take off the washings thoroughly, add 100 μL of substrate solution for peroxidase determination, and allow to stand at a constant temperature of about 25°C for exactly 30 minutes while protecting from light. Then add 100 μL of diluted sulfuric acid (23 in 500), shake, and determine the absorbance at 490 – 492 nm.

Separately, dissolve a suitable amount of bradykinin in gelatin-phosphate buffer solution, pH 7.0 to make solutions containing exactly 100 ng, 25 ng, 6.25 ng, 1.56 ng, 0.39 ng and 0.098 ng of bradykinin per mL, and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3), the standard solution (4), the standard solution (5) and the standard solution (6), respectively. Use 1 mL of gelatin-phosphate buffer solution, pH 7.0 as the standard solution (7). To each of the well add 50 μL each of the standard solutions and 100 μL of trichloroacetic acid-gelatin-tris buffer solution, and proceed in the same manner as for the sample solution and for the control solution.

Prepare the standard curve from the amounts of bradykinin in the standard solutions and their absorbances, and determine the amount of bradykinin, B1 (pg) and B2 (pg), of the sample solution and the control solution.

The absorbance is usually determined by using a spectrophotometer for microplate. Since the wells are used as the cell for absorbance determination, take care for dirt and scratch of the well. Light pass length of the well is changeable according to the labeled Units, take care for dirt and scratch of the well.

The absorbance at 490 – 492 nm is not less than 0.8.

\[ R = B_1/B_2 \]

(3) Trypsin-like substances—Pipet 4 mL of the sample stock solution prepared for the Assay, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the sample solution. Pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed to 35 ± 0.5°C for 5 minutes, place in a 10-mm cell, add exactly 0.5 mL of the sample solution, warm at 30 ± 0.5°C for 5 minutes, and start simultaneously a chronograph. Perform the test at 30 ± 0.5°C as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank, and determine the absorbances at 405 nm, A1 and A0, of this solution after having allowed it to stand for exactly 2 and 6 minutes. Separately, pipet 4 mL of the sample stock solution prepared for the Assay, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the control solution. Perform the same test with the control solution, and determine the absorbances, A1 and A0. Calculate T by using the following equation: the value of T does not exceed 0.05.

\[ T = (A_0 - A_1) / (A_0 - A_1) \]

(4) Protease—Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 1 Kallidinogenase Unit per mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, place in a test tube, and allow to stand at 35 ± 0.5°C for 5 minutes. Then, pipet 5 mL of substrate TS for kallidinogenase assay (3), previously warmed to 35 ± 0.5°C, add quickly to the sample solution in the test tube, and allow to stand at 35 ± 0.5°C for exactly 20 minutes. Then add exactly 5 mL of trichloroacetic acid TS, shake well, allow to stand at room temperature for 1 hour, and filter through a membrane filter (5 μm in pore size). Discard the first 3 mL of the filtrate, and determine the absorbance, A, of the subsequent filtrate at 280 nm within 2 hours as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS, shake well, and add exactly 5 mL of the substrate TS for kallidinogenase assay (3). Proceed in the same manner as described for the sample solution, and determine the absorbance, A0, of this solution. Calculate the value of \((A - A_0)\): it is not more than 0.2.

Loss on drying <2.41> Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 3% (0.5 g, 650 – 750°C).

Kinin-releasing activity

(i) Kallidinogenase solution: Weigh accurately a suitable amount of Kallidinogenase, according to the labeled unit, dissolve in 0.02 mol/L phosphate buffer solution, pH 8.0 to make a solution containing 0.1 unit of kallidinogenase per mL. Perform this procedure by using glassware.

(ii) Sample solution: Pipet 0.5 mL of kininogen TS, warm at 30 ± 0.5°C for 5 minutes, then add exactly 0.5 mL of kallidinogenase solution previously warmed at 30 ± 0.5°C for 5 minutes, and mix immediately. After allow this solution to stand at 30 ± 0.5°C for exactly 2 minutes, add exactly 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Boil for 3 minutes, then cool in ice immediately, centrifuge, and allow to stand at a room temperature for 15 minutes. Pipet 0.5 mL of the supernatant liquid, add exactly 0.5 mL of gelatin-tris buffer solution, pH 8.0, and shake. Pipet 0.1 mL of this solution, add exactly 1.9 mL of trichloroacetic acid-gelatin-tris buffer solution, shake, and use this solution as the sample solution.

(iii) Procedure: Perform the test with the sample solution as directed in the Purity (2), and determine the amount, B (pg), of kinin per well. The kinin-releasing activity per 1 unit of Kallidinogenase calculated by the following equation is not less than 500 ng bradykinin equivalent/min/unit.

Kinin-releasing activity (ng bradykinin equivalent/min/ unit) per 1 unit of Kallidinogenase = \( B \times 4.8 \)

Assay Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing about 10 Kallidinogenase Units per mL, and use this solution as the sample stock solution. Pipet 4 mL of
the sample stock solution, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the sample solution. Pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at 30 ± 0.5°C for 5 minutes, place in a 1-cm cell, add exactly 0.5 mL of the sample solution, warmed at 30 ± 0.5°C for 5 minutes, and start simultaneously a chronograph. Perform the test at 30 ± 0.5°C as directed under the Ultraviolet-visible Spectrophotometry <2.20> using water as the blank, and determine the absorbances at 405 nm, $A_{T2}$ and $A_{T6}$, of this solution after allowing to stand for exactly 2 and 6 minutes. Separately, dissolve Kallidinogenase RS in 0.05 mol/L phosphate buffer solution, pH 7.0 to make a solution so that each mL contains exactly 10 Units, and use this solution as the standard stock solution. Pipet 4 mL of the stock solution, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the standard solution. Take exactly 0.5 mL of the standard solution, perform the test in the same manner as described for the sample solution, and determine the absorbances, $A_{S2}$ and $A_{S6}$, of the solution after allowing to stand for exactly 2 and 6 minutes. Separately, take exactly 1 mL of the trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution to make exactly 10 mL. Pipet 0.5 mL of this solution, perform the test in the same manner as described for the sample solution, and determine the absorbances, $A_{O2}$ and $A_{O6}$, of the solution after allowing to stand for exactly 2 and 6 minutes.

$$\text{Units per 1 mg of Kallidinogenase} = \frac{(A_{T6} - A_{T2}) - (A_{O6} - A_{O2})}{(A_{S6} - A_{S2}) - (A_{O6} - A_{O2})} \times \frac{M_S}{a} \times \frac{1}{b}$$

$M_S$: Amount (Units) of Kallidinogenase RS  
$a$: Volume (mL) of the standard stock solution  
$b$: Amount (mg) of Kallidinogenase in 1 mL of the sample stock solution

**Containers and storage**  Containers—Tight containers.

---

**Kanamycin Monosulfate**

<table>
<thead>
<tr>
<th>C$<em>{18}$H$</em>{36}$N$<em>{4}$O$</em>{11}$.H$_2$SO$_4$: 582.58</th>
<th>3-Amino-3-deoxy-$\alpha$-D-glucopyranosyl-(1→6)-[6-amino-6-deoxy-$\alpha$-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine monosulfate</th>
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<tbody>
<tr>
<td>[25389-94-0]</td>
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</table>

Kanamycin Monosulfate is the sulfamate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces kanamyceticus*.  
It contains not less than 750 µg (potency) and not more than 832 µg (potency) per mg, calculated on the dried basis. The potency of Kanamycin Monosulfate is expressed as mass (potency) of kanamycin (C$_{18}$H$_{36}$N$_{4}$O$_{11}$: 484.50).

**Description**  Kanamycin Monosulfate occurs as a white crystalline powder.  
It is freely soluble in water, and practically insoluble in ethanol (99.5).

**Identification (1)**  Dissolve 50 mg of Kanamycin Monosulfate in 3 mL of water, and add 6 mL of anthrone TS: a blue-purple color develops.  
(2) Dissolve 20 mg each of Kanamycin Monosulfate and Kanamycin Monosulfate RS in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.  
(3) To a solution of Kanamycin Monosulfate dissolved in 100 mL of water, add 1 drop of barium chloride TS: a blue precipitate is formed.

**Optical rotation**  <2.49>  $\left[a\right]_{D}^{20} = +112^\circ - +123^\circ$ (0.2 g calculated on the dried basis, water, 20 mL, 100 mm).

**Sulfuric acid**  Weigh accurately about 0.25 g of Kanamycin Monosulfate, dissolve in 100 mL of water, adjust the pH to 11.0 with ammonia solution (28), add exactly 10 mL of 0.1
mol/L barium chloride VS, and titrate <2.50> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution, blue-purple, disappears (indicator: 0.5 mg of phthalain purple). At a near of the end-point add 50 mL of ethanol (99.5). Perform a blank determination in the same manner. The amount of sulfuric acid (SO$_4$) is not less than 15.0% and not more than 17.0%, calculated on the dried basis.

Each mL of 0.1 mol/L barium chloride VS $= 9.606$ mg of SO$_4$

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 g of Kanamycin Monosulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Kanamycin Monosulfate according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.30 g of Kanamycin Monosulfate in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 45 mg of Kanamycin Monosulfate RS in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.02>. Spot 1 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in 1-butanol (1 in 100) on the plate, and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <2.44> Not more than 4.0% (5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.5% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Kanamycin Monosulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5 and 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 µg (potency) and 5 µg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kanamycin Monosulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 µg (potency) and 5 µg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Well-closed containers.

### Kanamycin Sulfate

**Description** Kanamycin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

**Identification** (1) Dissolve 20 mg each of Kanamycin Sulfate and Kanamycin Monosulfate RS in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol (1 in 100) on the plate, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same Rf value. The potency of Kanamycin Sulfate is expressed as mass (potency) of kanamycin (C$_{18}$H$_{36}$N$_{4}$O$_{11}$·xH$_{2}$SO$_{4}$: 484.50).

**Optical rotation** <2.49> [a]$_D^{[2]}$: $+103^\circ$ + $115^\circ$ (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

**pH** <2.50> The pH of a solution obtained by dissolving 1.0 g of Kanamycin Sulfate in 20 mL of water is between 6.0
and 7.5.

Purity (1) Clarity and color of solution—Dissolve 1.5 g of Kanamycin Sulfate in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Kanamycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic—Prepare the test solution with 2.0 g of Kanamycin Sulfate according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.30 g of Kanamycin Sulfate in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 9.0 mg of Kanamycin Monosulfate RS in water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in 1-butanol (1 in 100) on the plate, and heat at 110°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying—Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay—Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer having pH 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Kanamycin Monosulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kanamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage—Containers—Tight containers.

Kaolin

Kaolin is a native, hydrous aluminum silicate.

Description—Kaolin occurs as white or nearly white, fragmentary masses or powder. It has a slightly clay-like odor. It is practically insoluble in water, in ethanol (99.5) and in diethyl ether.

It is insoluble in dilute hydrochloric acid and in sodium hydroxide TS.

When moistened with water, it darkens and becomes plastic.

Identification (1) Heat 1 g of Kaolin with 10 mL of water and 5 mL of sulfuric acid in a porcelain dish, and evaporate the mixture nearly to dryness. Cool, add 20 mL of water, boil for 2 to 3 minutes, and filter: the color of the residue is gray.

(2) The filtrate obtained in (1) responds to the Qualitative Tests for Kaolin, atic, and colorless to pale yellow.

Purity (1) Acid or alkali—Add 25 mL of water to 1.0 g of Kaolin, agitate thoroughly, and filter: the pH <2.5 of the filtrate is between 4.0 and 7.5.

(2) Acid-soluble substances—Add 20 mL of dilute hydrochloric acid to 1.0 g of Kaolin, agitate for 15 minutes, and filter. Evaporate 10 mL of the filtrate to dryness, and heat strongly between 450°C and 550°C to constant mass: the mass of the ignited residue is not more than 10 mg.

(3) Carbonate—Stir 1.0 g of Kaolin with 5 mL of water, then add 10 mL of diluted sulfuric acid (1 in 2): no effervescence occurs.

(4) Heavy metals—Proceed with 10 mL of water and 5 mL of hydrochloric acid for 20 minutes with frequent agitation, cool, centrifuge, and separate the supernatant liquid. Wash the precipitate twice with 10 mL of water, centrifuge each time, and combine the supernatant liquid and the washings. Add dropwise ammonia solution to this solution until a slight precipitate occurs, then add dilute hydrochloric acid dropwise while agitating strongly to complete solution. Add 0.45 g of hydroxylammonium chloride, and heat. Cool, add 0.45 g of sodium acetate trihydrate and 6 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Combine the filtrate and the washings, and add water to make 150 mL. Perform the test using 50 mL of this solution as the test solution. To 2.5 mL of Standard Lead Solution add 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, 2 mL of acetic acid (31) and water to make 50 mL, and use this solution as the control solution (not more than 50 ppm).

(5) Iron—Add 10 mL of dilute hydrochloric acid to 40 mg of Kaolin, and heat for 10 minutes with shaking in a water bath. After cooling, add 0.5 g of L-tartaric acid, dissolve with shaking, prepare the test solution with this solution according to Method 2, and perform the test according to Method B. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(6) Arsenic—Add 5 mL of water and 1 mL of sulfuric acid to 1.0 g of Kaolin, and heat on a sand bath until white fumes begin to evolve. Cool, and add water to make 5 mL. Perform the test with this solution as the test solution.
(not more than 2 ppm).

(7) Foreign matter—Place 5 g of Kaolin in a beaker, add 100 mL of water, stir, and decant to leave sand. Repeat this procedure several times with 100-mL portions of water: no sandy residue remains.

Loss on ignition <2.45> Not more than 15.0% (1 g, 600°C, 5 hours).

Plasticity Add 7.5 mL of water to 5.0 g of Kaolin, and agitate thoroughly: the resultant mass has no remarkable fluidity.

Containers and storage Containers—Well-closed containers.

Ketamine Hydrochloride

ケタミン塩酸塩

C13H16ClNO.HCl: 274.19
(2RS)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone monohydrochloride [1867-66-9]

Ketamine Hydrochloride, when dried, contains not less than 99.0% of C13H16ClNO.HCl.

Description Ketamine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in formic acid, freely soluble in water and in methanol, sparingly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

A solution of Ketamine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 258°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ketamine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 3000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ketamine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ketamine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> (2) for chloride.

Absorbance <2.24> $E_{1\text{cm}}^{1\text{dm}}$ (269 nm): 22.0 – 24.5 (after drying, 30 mg, 0.1 mol/L hydrochloric acid TS, 100 mL).

pH <2.54> Dissolve 1.0 g of Ketamine Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 3.5 and 4.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ketamine Hydrochloride in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ketamine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ketamine Hydrochloride, according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.5 g of Ketamine Hydrochloride in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and isopropylamine (49:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate, dry the plate, and then spray evenly hydrogen peroxide TS: the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Ketamine Hydrochloride, previously dried, dissolve in 1 mL of formic acid, add 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate $<2.50>$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 27.42 mg of C13H16ClNO.HCl

Containers and storage Containers—Tight containers.

Ketoconazole

ケトコナゾール

C26H28Cl2N4O4: 531.43
1-{Acetyl-4-[(2RS,4SR)-2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazine [65277-42-1]

Ketoconazole, when dried, contains not less than 99.0% and not more than 101.0% of C26H28Cl2N4O4.

Description Ketoconazole occurs as a white to light yellowish white powder.

It is soluble in methanol, sparingly soluble in ethanol
Ketoconazole Cream / Official Monographs

(99.5), and practically insoluble in water.

A solution of Ketoconazole in methanol (1 in 20) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Ketoconazole in methanol (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ketoconazole as directed in the potassium bromide disk under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Ketoconazole as directed under Flame Coloration Test <1.04> (2): a green color appears.

**Melting point** <2.60> 148 – 152°C.

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Ketoconazole according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related Substances—Dissolve 0.10 g of Ketoconazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 5 mL of this solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of ketoconazole obtained from the sample solution is not larger than 2/5 times the peak area of ketoconazole from the standard solution, and the total area of the peaks other than the peak of ketoconazole obtained from the sample solution is not larger than the peak area of ketoconazole from the standard solution.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Acetonitrile for liquid chromatography.
Mobile phase B: A solution of tetrabutylammonium hydrogensulfate (17 in 5000).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>5 → 50</td>
<td>95 → 50</td>
</tr>
<tr>
<td>10 – 15</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Flow rate: 2.0 mL per minute.
Time span of measurement: Beginning after the solvent peak to 15 minutes after injection.

**System suitability—**
Test for required detectability: Pipet 2 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of ketoconazole obtained from 10 µL of this solution is equivalent to 7 to 13% of that of ketoconazole from 10 µL of the standard solution.

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ketoconazole are not less than 40,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ketoconazole is not more than 2.5%.

(3) Residual solvent—Being specified separately.

**Loss on drying** <2.4> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.4> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Ketoconazole, previously dried, dissolve in 70 mL of a mixture of 2-butanol and acetic acid (100:7:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS is equivalent to 26.57 mg of C_{26}H_{28}Cl_{2}N_{4}O_{4}.

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

**Ketoconazole Cream**

ケトコナゾールクリーム

Ketoconazole Cream contains not less than 95.0% and not more than 105.0% of the labeled amount of ketoconazole (C_{26}H_{28}Cl_{2}N_{4}O_{4}: 531.43).

**Method of preparation** Prepare as directed under Creams, with Ketoconazole.

**Identification** To a quantity of Ketoconazole Cream, equivalent to 0.1 g of Ketoconazole according to the labeled amount, add 20 mL of 2-propanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of ketoconazole in 5 mL of 2-propanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL of each sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol, water and ammonia solution (28:40:40:25:2:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same Rf value as the spot from the standard solution.

**Assay** Weigh accurately an amount of Ketoconazole
Ketoconazole Lotion

カートコンゾールローション

Ketoconazole Lotion is an emulsion lotion.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of ketoconazole (C26H28Cl2N4O4; 531.43).

Method of preparation Prepare as directed under Lotions, with Ketoconazole.

Description Ketoconazole Lotion occurs as a white emulsion.
Containers and storage  Containers—Tight containers.

Ketoconazole Solution

Ketoconazole Solution is a liquid for external use. Ketoconazole Solution contains not less than 95.0% and not more than 105.0% of the labeled amount of ketoconazole (C_{26}H_{28}Cl_{2}N_{4}O_{4}: 531.43).

Method of preparation  Prepare as directed under Liquids and Solutions for Cutaneous Application, with Ketoconazole.

Description  Ketoconazole Solution is a clear liquid.

Identification  To a volume of Ketoconazole Solution, equivalent to 10 mg of Ketoconazole according to the labeled amount, add methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of ketoconazole in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 \mu L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol, water and ammonia solution (28:40:40:30:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution shows no optical rotation. From the sample solution and standard solution, show the same Rf values as the spot obtained from the standard solution.

pH  Being specified separately.

Assay  To an exact amount of Ketoconazole Solution, equivalent to about 10 mg of Ketoconazole as directed under the labeled amount, add methanol to make 10 mL, and use this solution as the sample solution. Separately, add methanol to make 25 mL, and use this solution as the sample solution. Perform the test with 20 \mu L each of the sample solution and standard solution as directed under Liquid Chromatography <2.03> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ketoconazole to that of the internal standard.

\[
\text{Amount (mg) of ketoconazole (C_{26}H_{28}Cl_{2}N_{4}O_{4})} = M_S \times Q_T/Q_S \times 1/5
\]

\(M_S\): Amount (mg) of ketoconazole for assay

Internal standard solution—A solution of bifonazole in methanol (3 in 2000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \mu m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of diisopropylamine in methanol (1 in 500), ammonium acetate solution (1 in 200) and acetic acid (100) (1800:600:1).

Flow rate: Adjust the flow rate so that the retention time of ketoconazole is about 11 minutes.

System suitability—
System performance: When the procedure is run with 20 \mu L of the standard solution under the above operating conditions, ketoconazole and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 \mu L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ketoconazole to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Tight containers.

Ketoprofen

Ketoprofen occurs as a white, crystalline powder. It is very soluble in methanol, freely soluble in ethanol (95) and in acetone, and practically insoluble in water.

A solution of Ketoprofen in ethanol (99.5) (1 in 100) shows no optical rotation.

It is colored to pale yellow by light.

Identification (1)  Determine the absorption spectrum of a solution of Ketoprofen in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)  Determine the infrared absorption spectrum of Ketoprofen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point  <2.60>  94 – 97°C.
Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ketoprofen in 10 mL of aceton: the solution is clear, and has no more color than the following control solution.

Control solution: To a mixture of 0.6 mL of Cobalt (II) Chloride CS and 2.4 mL of Iron (III) Chloride CS add diluted hydrochloric acid (1 in 10) to make 10 mL. To 5.0 mL of this solution add diluted hydrochloric acid (1 in 10) to make 100 mL.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Ketoprofen according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure with a minimum of exposure to light, using light-resistant vessels. Dissolve 20 mg of Ketoprofen in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2,017> according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks, having the relative retention time of about 1.5 and about 0.3 with respect to ketoprofen, are not larger than 4.5 times and not larger than 2 times the peak area of ketoprofen from the standard solution, respectively, the area of the peak other than ketoprofen and the peaks mentioned above is not larger than the peak area of ketoprofen from the standard solution, and the total area of these peaks is not larger than 2 times the peak area of ketoprofen from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 233 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 68.0 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with phosphoric acid. To 20 mL of this solution add 430 mL of acetonitrile and 550 mL of water.
Flow rate: Adjust the flow rate so that the retention time of ketoprofen is about 7 minutes.
Time span of measurement: About 7 times as long as the retention time of ketoprofen.

System suitability—
Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ketoprofen obtained with 20 µL of this solution is equivalent to 9 to 11% of that with 20 µL of the standard solution.

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ketoprofen are not less than 8000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ketoprofen is not more than 2.0%.

Loss on drying <2,417> Not more than 0.5% (0.5 g, in vacuum, 60°C, 24 hours).
Residue on ignition <2,447> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Ketoprofen, previously dried, dissolve in 25 mL of ethanol (95), add 25 mL of water, and titrate <2,507> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 25.43 mg of C₁₉H₁₉NO₃

Containers and storage Containers—Light-resistant. Storage—Light-resistant.

Ketotifen Fumarate

ケトチフェンフマル酸塩

C₁₉H₁₉NOS.C₄H₄O₄: 425.50
4-(1-Methylpiperidin-4-ylidene)-4
thiophen-10(9H)-one mono fumarate [34580-14-8]

Ketotifen Fumarate, when dried, contains not less than 99.0% and not more than 101.0% of ketotifen fumarate (C₁₉H₁₉NOS.C₄H₄O₄).

Description Ketotifen Fumarate occurs as a white to light yellowish white crystalline powder. It is sparingly soluble in methanol and in acetic acid (100), and slightly soluble in water, in ethanol (99.5) and in acetic anhydride.

Melting point: about 190°C (with decomposition).

Identification (1) Prepare the test solution with 30 mg of Ketotifen Fumarate as directed under Oxygen Flask Combustion Method <1.06> using 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <1.09> for sulfate.

(2) Determine the absorption spectrum of a solution of Ketotifen Fumarate in methanol (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry <2,247>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Ketotifen Fumarate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,257>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
Purity (1) Chloride <[1.03]—Dissolve 0.6 g of Ketotifen Fumarate in 2.5 mL of sodium carbonate TS in a crucible, heat on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter if necessary, neutralize with diluted nitric acid (3 in 10), and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of sodium carbonate TS, the used amount of diluted nitric acid (3 in 10) for the neutralization, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.015%).

(2) Heavy metals <[1.07]—Proceed with 1.0 g of Ketotifen Fumarate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Ketotifen Fumarate in 10 mL of a mixture of methanol and ammonia TS (99:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of methanol and ammonia TS (99:1) to make exactly 25 mL. Pipet 1 mL of this solution, add a mixture of methanol and ammonia TS (99:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <[2.03]>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, water and ammonia solution (28) (90:10:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying and then hydrogen peroxide TS on the plate: the number of the spot other than the principal spot obtained from the sample solution is not more than four, and they are not more intense than the spot from the standard solution.

Loss on drying <[2.41]—Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <[2.44]—Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Ketotifen Fumarate, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <[2.50] with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 42.55 mg of C₁₉H₁₉NOS.C₄H₄O₄

Containers and storage Containers—Tight containers.
It contains not less than 1450 μg (potency) and not more than 1700 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin is expressed as mass (potency) of kitasamycin corresponding to the mass of leucomycin A₅ (C₄₉H₆₅NO₁₄: 771.93). One mg (potency) of kitasamycin is equivalent to 0.530 mg of leucomycin A₅ (C₄₉H₆₅NO₁₄).

**Description** Kitasamycin occurs as a white to light yellow-white powder. It is very soluble in acetonitrile, in methanol and in ethanol (95), and practically insoluble in water.

**Identification** Determine the absorption spectrum of a solution of Kitasamycin in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Content ratio of the active principle** Dissolve 20 mg of Kitasamycin in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area by the automatic integration method. Calculate the amounts of leucomycin A₅, leucomycin A₄ and leucomycin A₁ by the area percentage method: the amounts of leucomycin A₅, leucomycin A₄ and leucomycin A₁ are 40 to 70%, 5 to 25% and 3 to 12%, respectively. Relative retention times of leucomycin A₄ and leucomycin A₁ with respect to leucomycin A₅ are 1.2 and 1.5, respectively.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 232 nm).
Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To a volume of a solution of ammonium acetate (77 in 5000) add diluted phosphoric acid (1 in 150) to adjust to pH 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of leucomycin A₅ is about 8 minutes.
Time span of measurement: About 3 times as long as the retention time of leucomycin A₅.

**System suitability**—
System performance: Dissolve about 20 mg each of Leucomycin A₅ RS and Josamycin RS in 20 mL of diluted acetonitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A₅ and josamycin are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leucomycin A₅ is not more than 1.0%.

**Water** <2.48> Not more than 3.0% (0.1 g, volumetric titration, direct titration).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.
(i) Test organism—*Bacillus subtilis* ATCC 6633
(ii) Culture medium—Use the medium 1 in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.
(iii) Standard solutions—Weigh accurately an amount of Leucomycin A₅ RS equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.
(iv) Sample solutions—Weigh accurately an amount of Kitasamycin equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Take exactly a suitable amount of the solution, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.
Kitasamycin Acetate

**Leucomycin Acetate**

キタサマイシン酢酸エステル

\[
(3R,4R,5S,6R,8R,9R,10E,12E,15R)-3,9-
\text{Diacetoxy}-5-[4-O-acyl-2,6-dideoxy-3-C-methyl-\alpha-
\text{l-ribo-hexopyranosyl-(1→4)}-2-O-acetyl-3,6-dideoxy-
3-dimethylamino-\beta-D-glucopyranosyloxy]-6-formylmethyl-
4-methoxy-8-methylhexadeca-10,12-dien-15-olide
\]

Leucomycin A₁ and A₃ Acetates: acyl = 3-methylbutanoyl
Leucomycin A₄ and A₅ Acetates: acyl = butanoyl
Leucomycin A₆ and A₇ Acetates: acyl = propanoyl

[178234-32-7, Kitasamycin Acetate]

Kitasamycin Acetate is a derivative of kitasamycin.

It contains not less than 680 μg (potency) and not more than 790 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin Acetate is expressed as mass (potency) of kitasamycin corresponding to the mass of leucomycin A₅ \((C_{39}H_{65}NO_{14} \cdot 771.93)\). One mg (potency) of kitasamycin is equivalent to 0.530 mg of leucomycin A₅ \((C_{39}H_{65}NO_{14})\).

**Description** Kitasamycin Acetate occurs as a white to light yellow-white powder.

It is very soluble in methanol and in ethanol (95), and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Kitasamycin Acetate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Water** <2.48> Not more than 5.0 % (0.1 g, volumetric titration, direct titration).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633
(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.
(iii) Standard solution—Weigh accurately an amount of Leucomycin A₅ RS equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.
(iv) Sample solution—Weigh accurately an amount of Kitasamycin Acetate equivalent to about 30 mg (potency), dissolve in 25 mL of methanol, add water to make exactly 50 mL, shake well, and allow to stand at 37 ± 2°C for 24 hours. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.
Kitasamycin Tartrate
Leucomycin Tartrate

キタサマイシン酒石酸塩

Kitasamycin Tartrate is the tartrate of kitasamycin. It contains not less than 1300 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin Tartrate is expressed as mass (potency) of kitasamycin based on the amount of leucomycin A₅ (C₉₀H₁₅₄NO₂₄: 771.93). One mg (potency) of Kitasamycin Tartrate is equivalent to 0.530 mg of leucomycin A₅ (C₉₀H₁₅₄NO₂₄).

**Description** Kitasamycin Tartrate occurs as a white to light yellowish white powder.
It is very soluble in water, in methanol and in ethanol (99.5).

**Identification** (1) Determine the absorption spectrum of a solution of Kitasamycin Tartrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Kitasamycin Tartrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 1 g of Kitasamycin Tartrate in 20 mL of water, add 3 mL of sodium hydroxide TS, add 20 mL of n-butyl acetate, shake well, and discard the n-butyl acetate layer. To the aqueous layer add 20 mL of n-butyl acetate, and shake well. The aqueous layer so obtained responds to the Qualitative Tests <1.09> (1) for tartrate.

**pH** <2.5> Dissolve 3.0 g of Kitasamycin Tartrate in 100 mL of water: the pH of the solution is between 3.0 and 5.0.

**Content ratio of the active principle** Dissolve 20 mg of Kitasamycin Tartrate in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amounts of leucomycin A₅, leucomycin A₂, and leucomycin A₆ by the area percentage method: the amount of leucomycin A₅ is 40 – 70%, leucomycin A₂ is 5 – 25%, and leucomycin A₆ is 3 – 12%. The relative retention times of leucomycin A₂ and leucomycin A₅ with respect to leucomycin A₁ are 1.2 and 1.5, respectively.

**Operating conditions**
Detector: An ultraviolet absorption photometer (wavelength: 232 nm).
Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To a suitable amount of a solution of ammonium acetate (77 in 5000) add diluted phosphoric acid (1 in 150) to adjust the pH to 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of leucomycin A₁ is about 8 minutes.
Time span of measurement: About 3 times as long as the retention time of leucomycin A₁.

**System suitability**
System performance: Dissolve about 20 mg each of Leucomycin A₁ RS and Josamycin RS in 20 mL of diluted aceto-
nitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A₅ and josamycin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leucomycin A₅ is not more than 1.0%.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Kitasamycin Tartrate in 10 mL of water: the solution is clear and colorless or light yellow.

(2) Heavy metals <1.07—Proceed with 1.0 g of Kitasamycin Tartrate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

Water <2.48 Not more than 3.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Leucomycin A₅ RS, equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kitasamycin Tartrate, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

### Labetalol Hydrochloride

![Labetalol Hydrochloride](image)

**Labetalol Hydrochloride**

ラベタロール塩酸塩

C₁₉H₂₄N₂O₃.HCl: 364.87

2-Hydroxy-5-\{(1RS)-1-hydroxy-2-[(1RS)-1-methyl-3-phenylpropylamino]ethyl\}benzamide monohydrochloride

2-Hydroxy-5-\{(1RS)-1-hydroxy-2-[(1SR)-1-methyl-3-phenylpropylamino]ethyl\}benzamide monohydrochloride (32780-64-6)

Labetalol Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of C₁₉H₂₄N₂O₃.HCl.

**Description** Labetalol Hydrochloride occurs as a white crystalline powder.

It is freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5).

It dissolves in 0.05 mol/L sulfuric acid TS.

Melting point: about 181°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Labetalol Hydrochloride in 0.05 mol/L sulfuric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Labetalol Hydrochloride as directed in the potassium chloride disc method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Labetalol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

**pH <2.34** The pH of a solution prepared by dissolving 0.5 g of Labetalol Hydrochloride in 50 mL of water is between 4.0 and 5.0.

**Purity (1) Heavy metals <1.07>**—Proceed with 1.0 g of Labetalol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.8 g of Labetalol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add
methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water, and ammonia solution (28) (25:15:8:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot from the sample solution do not exceed 2 in number and are not more intense than the spot obtained from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Isomer ratio Dissolve 5 mg of Labetalol Hydrochloride in 0.7 mL of a solution of n-butylboronic acid in anhydrous pyridine (3 in 250), allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 µL of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the areas of two adjacent peaks, A<sub>a</sub> and A<sub>b</sub>, where A<sub>a</sub> is the peak area of the shorter retention time and A<sub>b</sub> is the peak area of the longer retention time, using the automatic integration method: the ratio A<sub>a</sub>/ (A<sub>a</sub> + A<sub>b</sub>) is between 0.45 and 0.55.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.53 mm in inside diameter and 25 m in length, coated inside with methyl silicone polymer for gas chromatography in 5 µm thickness.

Column temperature: A constant temperature of about 290°C.

Injection port temperature: A constant temperature of about 350°C.

Detector temperature: A constant temperature of about 350°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of the peak showing earlier elution of the two peaks of labetalol is about 9 minutes.

System suitability—

System performance: Proceed with 2 µL of the sample solution under the above conditions: the resolution between the two labetalol peaks is not less than 1.5.

System repeatability: Repeat the test 6 times under the above conditions with 2 µL of the sample solution: the relative standard deviation of the ratio of the peak area of labetalol with the shorter retention time to that of the longer retention time is not more than 2.0%.

Assay Weigh accurately about 0.3 g of Labetalol Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 36.49 mg of C<sub>19</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>HCl

Containers and storage Containers—Tight containers.

**Labetalol Hydrochloride Tablets**

Labetalol Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of labetalol hydrochloride (C<sub>19</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>HCl: 364.87).

**Method of preparation** Prepare as directed under Tablets, with Labetalol Hydrochloride.

**Identification (1)** To a quantity of powdered Labetalol Hydrochloride Tablets equivalent to 5 mg of Labetalol Hydrochloride according to the labeled amount, add 100 mL of 0.05 mol/L sulfuric acid TS, shake, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 300 nm and 304 nm.

(2) To a quantity of powdered Labetalol Hydrochloride Tablets equivalent to 0.25 g of Labetalol Hydrochloride according to the labeled amount, add 25 mL of methanol, shake vigorously for 30 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of labetalol hydrochloride in 1 mL of methanol, and use this solution as the standard solution. Perform the test using these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water, and ammonia solution (28) (25:15:8:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same R<sub>f</sub> value.

**Uniformity of dosage units**<6.10> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Labetalol Hydrochloride Tablets add 5 mL of 0.5 mol/L sulfuric acid TS and 30 mL of water, shake vigorously for 30 minutes, add water to make exactly 50 mL, and filter. Discard the first 5 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL contains about 40 µg of labetalol hydrochloride (C<sub>19</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>HCl), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A<sub>1</sub> and A<sub>2</sub>, of the sample solution and standard solution at 302 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of labetalol hydrochloride (C<sub>19</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>HCl) = M<sub>S</sub> = A<sub>1</sub>/A<sub>2</sub> × V/40

M<sub>S</sub>: Amount (mg) of labetalol hydrochloride for assay

**Dissolution**<6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900
mL of water as the dissolution medium, the dissolution rate in 30 minutes of Labetalol Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Labetalol Hydrochloride Tablets, withdraw not less than 20 mL of the medium at specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V mL so that each mL contains about 50 μg of labetalol hydrochloride (C\textsubscript{19}H\textsubscript{24}N\textsubscript{2}O\textsubscript{3}.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, \(A_T\) and \(A_S\), at 302 nm.

\[
\text{Dissolution rate} \% = \frac{M_S}{M \times A_T/A_S \times V/V \times 1/C \times 90}
\]

\(M_S\): Amount (mg) of labetalol hydrochloride for assay
\(C\): Labeled amount (mg) of labetalol hydrochloride (C\textsubscript{19}H\textsubscript{24}N\textsubscript{2}O\textsubscript{3}.HCl) in 1 tablet

**Assay**
Weigh accurately not less than 20 Labetalol Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of labetalol hydrochloride (C\textsubscript{19}H\textsubscript{24}N\textsubscript{2}O\textsubscript{3}.HCl), add 100 mL of 0.5 mol/L sulfuric acid TS and 600 mL of water, shake vigorously for 30 minutes, add water to make exactly 1000 mL, and filter. Discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, and add 0.05 mol/L sulfuric acid TS to make exactly 25 mL. Pipet 5 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.01 mol/L hydrochloric acid VS for lactate.

\[
\text{Amount (mg) of labetalol hydrochloride (C\textsubscript{19}H\textsubscript{24}N\textsubscript{2}O\textsubscript{3}.HCl)} = M_S \times A_T/A_S \times 25
\]

\(M_S\): Amount (mg) of labetalol hydrochloride for assay

**Containers and storage**
Containers—Tight containers.

## Lactic Acid

乳酸

\[
\text{C}_3\text{H}_6\text{O}_3: 90.08
\]

\(2\RS\)-2-Hydroxypropanoic acid

[50-21-5]

Lactic Acid is a mixture of lactic acid and lactic anhydride.

It contains not less than 85.0% and not more than 92.0% of C\textsubscript{3}H\textsubscript{6}O\textsubscript{3}.

**Description**
Lactic Acid occurs as a clear, colorless or light yellow, viscous liquid. It is odorless or has a faint, unpleasant odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

It is hygroscopic.

Specific gravity \(d_2^{16}: \) about 1.20

**Identification**
A solution of Lactic Acid (1 in 50) changes blue litmus paper to red and responds to the Qualitative Tests <1.09> for lactate.

**Purity**

1. Chloride <1.03>—Perform the test with 1.0 g of Lactic Acid. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

2. Sulfate <1.14>—Perform the test with 2.0 g of Lactic Acid. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

3. Heavy metals <1.07>—To 2.0 g of Lactic Acid add 10 mL of water and 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red color appears. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).

4. Iron <1.10>—Prepare the test solution with 4.0 g of Lactic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 5 ppm).

5. Sugars—To 1.0 g of Lactic Acid add 10 mL of water, and neutralize with sodium hydroxide TS. Boil the mixture with 10 mL of Fehling’s TS for 5 minutes: no red precipitate is produced.

6. Citric, oxalic, phosphoric and L-tartaric acid—To 1.0 g of Lactic Acid add 1.0 mL of water, followed by 40 mL of calcium hydroxide TS. Boil the mixture for 2 minutes: no change occurs.

7. Glycerin or mannitol—Shake 10 mL of Lactic Acid with 12 mL of diethyl ether: no turbidity is produced.

8. Volatile fatty acids—Warm Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.

9. Cyanide—Transfer 1.0 g of Lactic Acid to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, add dropwise a solution of sodium hydroxide (1 in 10) by shaking until a pale red color develops, add 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. Cool, add...
L-Lactic Acid

**L-乳酸**

\[ C_3H_6O_3 \]

- **Specific gravity**: 1.14
- **Volatile fatty acids**: Warm L-Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.
- **Cyanide**: Transfer 1.0 g of L-Lactic Acid to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and heat in a water bath for 15 minutes. Cool, and adjust to pH 7.0 with 1 mol/L hydrochloric acid VS. Dissolve 5.0 g of hexammonium heptamolybdate tetrahydrate in this solution, add water to make exactly 50 mL, and determine the optical rotation using a 100-mm cell.

### Purity

<table>
<thead>
<tr>
<th>Test</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>Chloride: Perform the test with 1.0 g of L-Lactic Acid. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).</td>
</tr>
<tr>
<td>(2)</td>
<td>Sulfate: Perform the test with 2.0 g of L-Lactic Acid. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).</td>
</tr>
<tr>
<td>(3)</td>
<td>Heavy metal: To 2.0 g of L-Lactic Acid add 10 mL of water and 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red color appears. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).</td>
</tr>
<tr>
<td>(4)</td>
<td>Iron: Prepare the test solution with 4.0 g of L-Lactic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 5 ppm).</td>
</tr>
<tr>
<td>(5)</td>
<td>Sugars: To 1.0 g of L-Lactic Acid add 10 mL of water, and neutralize with sodium hydroxide TS. Boil the mixture with 10 mL of Fehling’s TS for 5 minutes: no red precipitate is produced.</td>
</tr>
<tr>
<td>(6)</td>
<td>Citric, oxalic, phosphoric and l-tartaric acid: To 1.0 g of L-Lactic Acid add 1.0 mL of water, followed by 40 mL of calcium hydroxide TS. Boil the mixture for 2 minutes: no change occurs.</td>
</tr>
<tr>
<td>(7)</td>
<td>Glycerin or mannitol: Shake 10 mL of L-Lactic Acid with 12 mL of diethyl ether: no turbidity is produced.</td>
</tr>
<tr>
<td>(8)</td>
<td>Volatile fatty acids: Warm L-Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.</td>
</tr>
</tbody>
</table>

### Identification

- **Color**: Clear, colorless or light yellow, viscous liquid. It is odorless or has a faint, no unpleasant odor.
- **Specific gravity**: 1.20

### Optical rotation

\[ [\alpha]_D^{20} = -46^\circ \text{ to } -52^\circ \]

### Assay

Weigh accurately about 3 g of L-Lactic Acid, transfer in a conical flask, add accurately measured 40 mL of 1 mol/L sodium hydroxide VS, cover with a watch glass, and heat on a water bath for 15 minutes. Cool, and adjust to pH 7.0 with 1 mol/L hydrochloric acid VS. Dissolve 5.0 g of hexammonium heptamolybdate tetrahydrate in this solution, add water to make exactly 50 mL, and determine the optical rotation using a 100-mm cell.

### Containers and storage

Containers—Tight containers.
Anhydrous Lactose / Official Monographs

mol/L sodium hydroxide VS, invert a watch glass over the flask, and heat on a water bath for 10 minutes. Titrate \(\text{C}_2\text{H}_2\text{O}_3\) the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS immediately (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS = 90.08 mg of \(\text{C}_3\text{H}_6\text{O}_3\)

Containers and storage  Containers—Tight containers.

Anhydrous Lactose

無水乳糖

\(\text{C}_12\text{H}_{22}\text{O}_{11} \cdot 342.30\)

\(\beta\)-D-Galactopyranosyl-(1\(\rightarrow\)4)-\(\beta\)-D-glucopyranose

(\(\beta\)-lactose)

\(\beta\)-D-Galactopyranosyl-(1\(\rightarrow\)4)-\(\alpha\)-D-glucopyranose

(\(\alpha\)-lactose)

[63-42-3, Anhydrous Lactose]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (\(\bullet\)).

Anhydrous Lactose is \(\beta\)-lactose or a mixture of \(\beta\)-lactose and \(\alpha\)-lactose.

*The relative quantities of \(\alpha\)-lactose and \(\beta\)-lactose in Anhydrous Lactose is labeled as the isomer ratio.\(\bullet\)

*Description  Anhydrous Lactose occurs as white crystals or powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).\(\bullet\)

*Identification  Determine the infrared absorption spectrum of Anhydrous Lactose, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(\leq 2.25\), and compare the spectrum with the Reference Spectrum or the spectrum of Anhydrous Lactose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.\(\bullet\)

Optical rotation  \(\leq 2.49\)  \(\alpha\): +54.4° +55.9°. Weigh accurately about 10 g of Anhydruous Lactose, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

Purity  (1) Clarity and color of solution—Dissolve 1.0 g of Anhydrous Lactose in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry \(\leq 2.4\), using water as the control solution: not more than 0.04.

(2) Acidity or alkalinity—Dissolve 6 g of Anhydrous Lactose by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pale red color or red color.

* (3) Heavy metals  \(\leq 1.07\)—Proceed with 4.0 g of Anhydrous Lactose according to Method 2, and perform the test. Prepare the control solution with 2 mL of Standard Lead Solution (not more than 5 ppm).\(\bullet\)

(4) Proteins and light absorbing substances—Dissolve 1.0 g of Anhydrous Lactose in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry \(\leq 2.24\), using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

Loss on drying  \(\leq 2.4\)  Not more than 0.5% (1 g, 80°C, 2 hours).

Water  \(\leq 2.4\)  Not more than 1.0% (1 g, volumetric titration, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

Residue on ignition  \(\leq 2.4\)  Not more than 0.1% (1 g).

*Microbial limit  \(\leq 0.5\)  The acceptance criteria of TAMC and TYMC are \(10^2\) CFU/g and \(5 \times 10^2\) CFU/g, respectively. Salmonella and Escherichia coli are not observed.\(\bullet\)

Isomer ratio  Place 1 mg of Anhydrous Lactose in a 5-mL screw capped reaction vial for gas chromatography, add 0.45 mL of dimethylsulfoxide, stopper, and shake well. Add 1.8 mL of a mixture of pyridine and trimethylsilylimidazole (18:7), seal the vial tightly with a screw cap, and mix gently. Allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 \(\mu\)L of the sample solution as directed under Gas Chromatography \(\leq 2.02\) according to the following conditions. Determine the peak areas of \(\alpha\)-lactose and \(\beta\)-lactose, \(A_\alpha\) and \(A_\beta\), and calculate the contents (%) of \(\alpha\)-lactose and \(\beta\)-lactose in Anhydrous Lactose by the following equations.

\[\text{Content (\% of } \alpha\text{-lactose) } = \frac{A_\alpha}{(A_\alpha + A_\beta)} \times 100\]

\[\text{Content (\% of } \beta\text{-lactose) } = \frac{A_\beta}{(A_\alpha + A_\beta)} \times 100\]

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Injection port temperature: A constant temperature of about 275°C.

Detector temperature: A constant temperature of about 275°C.

Column: A glass column 4 mm in inside diameter and 90 cm in length, packed with siliceous earth for gas chromatography coated at the ratio of 3% with 25% phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography.

Column temperature: A constant temperature of about 215°C.
Carrier gas: Helium.
Flow rate: A constant flow rate of about 40 mL per minute.

System suitability—
System performance: Prepare a solution with 1 mg of a mixture of α-lactose and β-lactose (1:1) in the same manner as for preparing the sample solution, and proceed with 2 μL of this solution under the above operating conditions, and determine the retention times of the peaks of α-lactose and β-lactose: the relative retention time of α-lactose with respect to that of β-lactose is about 0.7 with the resolution between these peaks being not less than 3.0.

*Containers and storage* Containers—Well-closed containers.

**Lactose Hydrate**

**Lactose**

乳糖水和物

C\(_12\)H\(_{22}\)O\(_{11}\), H\(_2\)O: 360.31
β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate

[64044-51-5, Mixture of α- and β-lactose monohydrate]

This monograph is harmonized with the European Pharmacopeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (* •).

Lactose Hydrate is the monohydrate of β-D-galactopyranosyl-(1→4)-α-D-glucopyranose.

*It is a disaccharide obtained from milk, consist of one unit of glucose and one unit of galactose.*

*The label states the effect where it is the granulated powder.*

*Description* Lactose Hydrate occurs as white, crystals, powder or granulated powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

**Identification** Determine the infrared absorption spectrum of Lactose Hydrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with *the Reference Spectrum or the spectrum of Lactose Hydrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [\(\alpha\)]\(_D\)\(_2\) = 54.4° + 55.9°. Weigh accurately about 10 g of Lactose Hydrate, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Lactose Hydrate in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.04.

(2) Acidity or alkalinity—Dissolve 6 g of Lactose Hydrate by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pale red color or red color.

* (3) Heavy metals <4.05>—Dissolve 4.0 g of Lactose Hydrate in 20 mL of warm water, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Proceed with this solution according to Method 1, and perform the test. Prepare the control solution with 1 mL of 0.1 mol/L hydrochloric acid TS and 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(4) Proteins and light absorbing substances—Dissolve 1.0 g of Lactose Hydrate in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

*Loss on drying* <2.41> Not more than 0.5%. For the granulated powder, not more than 1.0% (1 g, 80°C, 2 hours).

**Water** <2.40> 4.5 – 5.5%. *For the granulated powder, 4.0 – 5.5% (1 g, volumetric titration, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).*

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

*Microbial limit* <4.05> The acceptance criteria of TAMC and TYMC are 10² CFU/g and 5 × 10² CFU/g, respectively. *Salmonella and Escherichia coli* are not observed.

*Containers and storage* Containers—Well-closed containers.
Lactulose

ラクツロース

C_{12}H_{22}O_{11}: 342.30
β-D-Galactopyranosyl-(1→4)-D-fructose
[4618-18-2]

Lactulose is a solution of lactulose prepared by isomerizing lactose under the existing of alkaline and purified by ion-exchange resin. It contains not less than 50.0% and not more than 56.0% of C_{12}H_{22}O_{11}.

Description Lactulose occurs as a clear, colorless or light yellow, viscous liquid. It is odorless, and has a sweet taste. It is miscible with water and with formamide.

Identification (1) To 0.7 g of Lactulose add 10 mL of water, 10 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 25) and 0.2 mL of acetic acid (100), and heat in a water bath for 5 to 10 minutes: a blue color develops.

(2) Mix 0.3 g of Lactulose and 30 mL of water, add 16 mL of 0.5 mol/L iodine TS, then immediately add 2.5 mL of 8 mol/L sodium hydroxide TS, allow to stand for 7 minutes, and add 2.5 mL of diluted sulfuric acid (3 in 20). To this solution add a saturated solution of sodium sulfite heptahydrate until the solution turns light yellow, then add 3 drops of methyl orange TS, neutralize with a solution of sodium hydroxide (4 in 25), and add water to make 100 mL. To 10 mL of this solution add 5 mL of Fehling’s TS, and boil for 5 minutes: a red precipitate is produced.

pH <2.54 To 2.0 g of Lactulose add water to make 15 mL: the pH of the solution is between 3.5 and 5.5.

Specific gravity <2.56  \( d^{20}_{20}: 1.320 - 1.360 \)

Purity (1) Heavy metals <1.07—Proceed with 5.0 g of Lactulose according to Method 4, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(2) Arsenic <1.17—Prepare the test solution with 1.0 g of Lactulose according to Method 1, and perform the test (not more than 2 ppm).

(3) Galactose and lactose—Determine the heights of the peaks corresponding to galactose and lactose respectively, on the chromatogram obtained in Assay from the sample solution and the standard solution, and calculate the ratios of the peak heights of galactose and lactose to that of the internal standard from the sample solution, \( Q_{Ta} \) and \( Q_{Tb} \), and then from the standard solution, \( Q_{Sa} \) and \( Q_{Sb} \): it contains galactose of not more than 11%, and lactose of not more than 6%.

Assay Weigh accurately about 1 g of Lactulose, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of Lactulose RS, accurately about 80 mg of D-galactose and accurately about 40 mg of lactose monohydrate, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_{T} \) and \( Q_{S} \), of the peak height of lactulose to that of the internal standard, respectively.

\[
\text{Amount (mg) of galactose (C}_6\text{H}_12\text{O}_6) = M_S \times \frac{Q_{Ta}}{Q_{Sa}}
\]

\[
\text{Amount (mg) of lactose (C}_{12}\text{H}_{22}\text{O}_{11}\cdot\text{H}_2\text{O}) = M_S \times \frac{Q_{Tb}}{Q_{Sb}}
\]

\[
\text{Amount (mg) of lactose (C}_{12}\text{H}_{22}\text{O}_{11}) = M_S \times \frac{Q_T}{Q_S}
\]

Loss on drying <2.41> Not more than 35% (0.5 g, in vacuum, 80°C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers.
Lanatoside C

ラナトシド C

Lanatoside C, when dried, contains not less than 90.0% and not more than 102.0% of \( C_{49}H_{76}O_{20} \).

**Description** Lanatoside C occurs as colorless or white crystals or a white, crystalline powder. It is odorless.

It is soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is hygroscopic.

**Identification** Place 1 mg of Lanatoside C to a small test tube having an internal diameter of about 10 mm, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetone (1 in 10), shake well, and add methanol to make 25 mL. Allow these solutions to stand between 18°C and 22°C for 25 minutes, and determine the absorbances, \( A_T \) and \( A_S \), of the solutions at 485 nm as directed under Ultraviolet-visible Spectrophotometry (2.24), using a solution prepared with 5 mL of methanol in the same manner as the blank solution.

Amount (mg) of \( C_{49}H_{76}O_{20} \) = \( M_S \times A_T/A_S \)

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Lanatoside C Tablets**

ラナトシド C錠

Lanatoside C Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of lanatoside C (\( C_{49}H_{76}O_{20} \): 985.12).

**Method of preparation** Prepare as directed under Tablets, with Lanatoside C.

**Identification** (1) Shake a quantity of powdered Lanatoside C Tablets, equivalent to 1 mg of Lanatoside C according to the labeled amount, with 3 mL of diethyl ether, and filter. Wash the residue with two 3-mL portions of diethyl ether, and air-dry. To the remaining residue add 10 mL of a mixture of chloroform and methanol (9:1), shake, and filter. Wash the residue with two 5-mL portions of a mixture of chloroform and methanol (9:1), combine the filtrate and washings, and evaporate on a water bath to a smaller volume. Transfer the solution to a small test tube having an internal diameter of about 10 mm, further evaporate on a water bath to dryness, and proceed as directed in the Identification under Lanatoside C.

(2) Perform the test with the sample solution and the standard solution obtained in the Assay as directed under Thin-layer Chromatography (2.03). Spot 25 \( \mu L \) each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly with 2,4,6-trinitrophenol TS and 0.5 mL of a solution of sodium hydroxide (1 in 10), shake well, and add methanol to make 25 mL. Allow these solutions to stand between 18°C and 22°C for 25 minutes, and determine the absorbances, \( A_T \) and \( A_S \), of the solutions at 485 nm as directed under Ultraviolet-visible Spectrophotometry (2.24), using a solution prepared with 5 mL of methanol in the same manner as the blank solution.

Amount (mg) of \( C_{49}H_{76}O_{20} \) = \( M_S \times A_T/A_S \)

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

Related substances—Dissolve 10 mg of Lanatoside C in exactly 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1.0 mg of Lanatoside C RS in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography (2.03) with these solutions. Spot 20 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: any spots other than the principal spot from the sample solution are neither larger nor darker than the spot from the standard solution.

**Optical rotation** \( \angle \psi \): +32° to +35° (after drying, 0.5 g, methanol, 25 mL, 100 mm).

**Loss on drying** Not more than 7.5% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

**Residue on ignition** Not more than 0.5% (0.1 g).

**Assay** Weigh accurately about 50 mg each of Lanatoside C and Lanatoside C RS, previously dried, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of the sample solution and standard solution into 25-mL light-resistant, volumetric flasks, and add 5 mL of 2,4,6-trinitrophenol TS and 0.5 mL of a solution of sodium hydroxide (1 in 10), shake well, and add methanol to make 25 mL. Allow these solutions to stand between 18°C and 22°C for 25 minutes, and determine the absorbances, \( A_T \) and \( A_S \), of the solutions at 485 nm as directed under Ultraviolet-visible Spectrophotometry (2.24), using a solution prepared with 5 mL of methanol in the same manner as the blank solution.

Amount (mg) of \( C_{49}H_{76}O_{20} \) = \( M_S \times A_T/A_S \)

**Containers and storage** Containers—Light-resistant.

**Purity** Related substances—Dissolve 10 mg of Lanatoside C in exactly 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1.0 mg of Lanatoside C RS in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography (2.03) with these solutions. Spot 20 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: any spots other than the principal spot from the sample solution are neither larger nor darker than the spot from the standard solution.
Hydrous Lanolin / Official Monographs

Uniformity of dosage unit <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Warm 1 tablet of Lanatoside C Tablets with 5 mL of water until the tablet is disintegrated, add 30 mL of ethanol (95), disperse finely the particles with the aid of ultrasonic waves, add ethanol (95) to make exactly 5 mL of a solution containing about 5 µg of lanatoside C (C_{49}H_{76}O_{20}) in each mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Lanatoside C RS, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL of each sample solution, the standard solution and diluted ethanol (95) (17 in 20) into three brown glass-stoppered test tubes T, S and B, previously containing exactly 10 mL of 0.012 w/v% l-ascorbic acid-hydrochloric acid TS, and add exactly 1 mL each of dilute hydrogen peroxide TS immediately, shake vigorously, and allow to stand at a constant temperature between 25°C and 30°C for 40 minutes. Determine the fluorescence intensities, F_T, F_S and F_B, of the subsequent solutions from the sample solution and the standard solution and the diluted ethanol (95) (17 in 20) at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry <2.22>, respectively.

\[
\text{Amount (mg) of lanatoside C (C}_{49}\text{H}_{76}\text{O}_{20}) = M_S \times \frac{(F_T - F_B)/(F_S - F_B)}{V} \times \frac{100}{5000}
\]

\(M_S\): Amount (mg) of Lanatoside C RS

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 500 mL of diluted hydrochloric acid (3 in 500) as the dissolution medium, the dissolution rate in 60 minutes of Lanatoside C Tablets is not less than 65%. No retest requirement is applied to Lanatoside C Tablets.

Start the test with 1 tablet of Lanatoside C Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 µm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 0.5 µg of lanatoside C (C_{49}H_{76}O_{20}) according to the labeled amount, and use this solution as the sample solution. Separately, dry Lanatoside C RS in vacuum over phosphorus (V) oxide at 60°C for 4 hours, weigh accurately a portion of it, equivalent to 100 times an amount of the labeled amount of lanatoside C (C_{49}H_{76}O_{20}), dissolve in ethanol (95) to make exactly 100 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 500 mL, warm at 37 ± 0.5°C for 60 minutes, and use this solution as the standard solution. Pipet 3 mL each of the sample solution, the standard solution and the dissolution medium, and transfer to glass-stoppered brown test tubes T, S and B, respectively. To these solutions add exactly 10 mL each of 0.012 w/v% l-ascorbic acid-hydrochloric acid TS, and shake. Immediately add exactly 0.2 mL each of diluted hydrogen peroxide TS (1 in 100), shake well, and allow to stand at a constant temperature between 30°C and 37°C for 45 minutes. Determine immediately the fluorescence intensities, F_T, F_S and F_B, of the sample solution and the standard solution at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry <2.22>.

\[
\text{Dissolution rate} = \frac{M_S \times (F_T - F_B)/(F_S - F_B)}{V} \times \frac{V}{100} / \text{Time} \times 100
\]

\(M_S\): Amount (mg) of Lanatoside C RS

C: Labeled amount (mg) of lanatoside C (C_{49}H_{76}O_{20}) in 1 tablet

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Hydrous Lanolin

Hydrous Lanolin is Purified Lanolin to which water is added. It contains not less than 70% and not more than 75% of Purified Lanolin (as determined by the test for Residue on evaporation).

Description Hydrous Lanolin is a yellowish white, ointment-like substance, and has a slight, characteristic odor, which is not rancid.

It is soluble in diethyl ether and in cyclohexane, with the separation of water.

When melted by heating on a water bath, it separates into a clear oily layer and a clear water layer.

Melting point: about 39°C.

Identification Dissolve 1 g of Hydrous Lanolin in 50 mL of cyclohexane, and remove the separated water. Superimpose carefully 1 mL of the cyclohexane solution on 2 mL of sulfuric acid: a red-brown color develops at the zone of contact.
and sulfuric acid layer shows a green fluorescence.

**Acid value** \(<1.13>\) Not more than 1.0.

**Iodine value** 18 – 36 Heat a suitable amount of Hydrous Lanolin on a water bath to remove its almost moisture, then weigh accurately about 0.8 g of the treated Hydrous Lanolin in a glass-stoppered 500-mL flask, and add 10 mL of cyclohexane to dissolve, and add exactly 25 mL of Hanus’s TS, and mix well. If a clear solution is not obtained, add more cyclohexane to make clear, and allow the mixture to stand for 1 hour between 20°C and 30°C in a light-resistant, well-closed container while occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate \(<2.5D\) the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

\[
\text{Iodine value} = (a - b) \times 1.269/M
\]

*M:* amount (g) of sample

\(a\): Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination

\(b\): Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the titration

**Purity** (1) Acidity or alkalinity—To 5 g of Hydrous Lanolin add 25 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and separate the aqueous layer: the aqueous layer is neutral.

(2) Chloride \(<1.07>\)—To 2.0 g of Hydrous Lanolin add 40 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Ammonia—To 10 mL of the aqueous layer obtained in (1) add 1 mL of sodium hydroxide TS, and boil: the gas evolved does not turn moistened red litmus paper to blue.

(4) Water-soluble organic substances—To 5 mL of the aqueous layer obtained in (1) add 0.25 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color of the solution does not disappear.

(5) Petrolatum—Dissolve 1.0 g of the dried residue obtained in the Residue on evaporation in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution. Add dissolve 20 mg of baseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03>\). Spot 25 \(\mu\)L of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with isooctane to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, heat the plate at 80°C for 5 minutes, cool, and examine under ultraviolet light (main wavelength: 365 nm): no fluorescent spot is observed in the same level with the spot of standard solution. For this test use a thin-layer plate previously developed with isooctane to the upper end, dried in air, and heated at 110°C for 60 minutes.

**Residue on evaporation** Weigh accurately about 12.5 g of Hydrous Lanolin, dissolve in 50 mL of diethyl ether, place it in a separator, transfer the separated aqueous layer to another separator, add 10 mL of diethyl ether, shake, and combine the diethyl ether layer and diethyl ether in the first separator. Shake the diethyl ether layer with 3 g of anhydrous sodium sulfate, and filter through dry filter paper. Wash the separator and the filter paper with two 20-mL portions of diethyl ether, combine the washings with the filtrate, evaporate on a water bath until the odor of diethyl ether is no longer perceptible, and dry in a desiccator (in vacuum, silica gel) for 24 hours: the content is not less than 70% and not more than 75%.

**Containers and storage** Containers—Well-closed containers, with occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate \(<2.5D\) the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

\[
\text{Iodine value} = (a - b) \times 1.269/M
\]

*M:* amount (g) of sample

\(a\): Volume (mL) of 0.1 mol/L sodium thiosulfate VS used in the blank determination

\(b\): Volume (mL) of 0.1 mol/L sodium thiosulfate VS used in the titration of the sample.

**Purity** (1) Acid or alkali—To 5 g of Purified Lanolin add 25 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and separate the aqueous layer:
Lard / Official Monographs

Purity (1) Moisture and coloration—Melt 5 g of Lard by heating on a water bath: it forms a clear liquid, from which no water separates. Observe the liquid in a layer 10 mm thick: the liquid is colorless to slightly yellow.

(2) Alkalinity—To 2.0 g of Lard add 10 mL of water, melt by warming on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: the layer is colorless.

(3) Chloride $\langle 0.05 \rangle$—To 1.5 g of Lard add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the opalescence of the mixture does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, and add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50).

(4) Beef tallow—Dissolve 5 g of Lard in 20 mL of diethyl ether, stopper lightly with absorbent cotton, and allow to stand at 20°C for 18 hours. Collect the separated crystals, moisten them with ethanol (95), and examine under a microscope of 200 magnifications: the crystals are in the form of rhomboidal plates grouped irregularly, and do not contain prisms or needles grouped in fan-shaped clusters.

Contains and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Lard

Adeps Suillus

豚脂

Lard is the fat obtained from Sus scrofa Linné var. domesticus Gray (Suidae).

Description Lard occurs as a white, soft, unctuous mass, and has a faint, characteristic odor and a bland taste. It is freely soluble in diethyl ether and in petroleum ether, very slightly soluble in ethanol (95), and practically insoluble in water.

Melting point: 36 – 42°C

Congealing point of the fatty acids: 36 – 42°C

Acid value $\langle 1.13 \rangle$ Not more than 2.0.

Saponification value $\langle 1.13 \rangle$ 195 – 203

Iodine value $\langle 1.13 \rangle$ 46 – 70

the aqueous layer is neutral.

(2) Chloride $\langle 1.05 \rangle$—To 2.0 g of Purified Lanolin add 40 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Ammonia—To 10 mL of the aqueous layer obtained in (1) add 1 mL of sodium hydroxide TS, and boil: the gas evolved does not turn moistened red litmus paper to blue.

(4) Water-soluble organic substances—To 5 mL of the aqueous layer obtained in (1) add 0.25 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color of the solution does not disappear.

(5) Petrolatum—Dissolve 1.0 g of Purified Lanolin in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution. And dissolve 20 mg of vaseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the standard solution. Perform the test with the sample solution as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with isooctane (1:1), and use this solution as the standard solution. And dissolve 20 mg of vaseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution. And dissolve 20 mg of vaseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 2 hours).

Total ash $\langle 5.01 \rangle$ Not more than 0.1%.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Latamoxef Sodium

ラタモキセフナトリウム

$\text{C}_{20}\text{H}_{20}\text{N}_{6}\text{O}_{9}\text{S}$: 564.44

Disodium (6R,7R)-7-[2-carboxylato-2-(4-hydroxyphenyl)acetylamino]-7-methoxy-3-(1-methyl-1H-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [64953-12-4]

Latamoxef Sodium contains not less than 830 μg (potency) and not more than 940 μg (potency) per mg, calculated on the anhydrous basis. The potency of Latamoxef Sodium is expressed as mass (potency) of latamoxef (C$_{20}$H$_{20}$N$_{6}$O$_{9}$S: 520.47).

Description Latamoxef Sodium occurs as white to light yellowish white, powder or masses. It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Latamoxef Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Latamoxef Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the $^1$H spectrum of a solution of Latamoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around $\delta$ 3.5 ppm and at around $\delta$ 4.0 ppm. The ratio of the integrated intensity of these signals, A:B, is about 1:1.

(4) Latamoxef Sodium responds to the Qualitative Tests <1.099> (1) for sodium salt.

Optical rotation <2.490> $[\alpha]^{20}_D = -32 \pm 40^\circ$ (0.5 g calculated on the anhydrous basis, phosphate buffer solution, pH 7.0, 50 mL, 100 mm).

pH <2.540> The pH of a solution obtained by dissolving 1.0 g of Latamoxef Sodium in 10 mL of water is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Latamoxef Sodium in 10 mL of water: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 3.0 mL of Cobalt (II) Chloride CS and 36 mL of Iron (III) Chloride CS add 11 mL of diluted dilute hydrochloric acid (1 in 10). To 2.5 mL of this solution add 7.5 mL of diluted dilute hydrochloric acid (1:10).

(2) Heavy metals <1.077>—Carbonize 1.0 g of Latamoxef Sodium by heating gently, previously powdered if it is masses. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (1 in 10), and burn the ethanol. After cooling, add 1 mL of sulfuric acid. Proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.117>—Prepare the test solution by dissolving 1.0 g of Latamoxef Sodium in 20 mL of water, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve an amount of Latamoxef Sodium, equivalent to about 25 mg (potency), in water to make exactly 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 1-methyl-1H-tetrazole-5-thiol, having the relative retention time of about 0.5 with respect to the first eluted peak of the two peaks of latamoxef, obtained from the sample solution is not larger than the peak area of latamoxef from the standard solution, and the peak area of decarboxylatamoxef, having the relative retention time of about 1.7 with respect to the first peak of the two peaks of latamoxef, is not larger than 2 times that of latamoxef from the standard solution. For this calculation, use the peak area for 1-methyl-1H-tetrazole-5-thiol after multiplying by its relative response factor, 0.52.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of latamoxef is not more than 2.0%.

Water <2.48> Not more than 5.0% (0.5 g, volumetric titration, back titration).

Isomer ratio Dissolve 25 mg of Latamoxef Sodium in water to make 50 mL, and use this solution as the sample solution. Perform the test with 5 $\mu$L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas, $A_a$ and $A_b$, of the two peaks in order of elution, which appear close to each other at the retention time of about 10 minutes: $A_a/A_b$ is between 0.8 and 1.4.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.7 g of ammonium acetate in water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of the first eluted peak of latamoxef is about 8 minutes.

System suitability—

System performance: When the procedure is run with 5 $\mu$L of the sample solution under the above operating conditions, the resolution between the two peaks of latamoxef is not less than 3.

System repeatability: When the test is repeated 3 times with 5 $\mu$L of the sample solution under the above operating conditions, the relative standard deviation of the area of the first eluted peak of latamoxef is not more than 2.0%.

Assay Weigh accurately an amount of Latamoxef Sodium and Latamoxef Ammonium RS, equivalent to about 25 mg (potency) each, dissolve in exactly 5 mL of the internal standard solution, add water to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_a$ and $Q_b$, of the peak area of latamoxef to that of the internal standard.

Amount [mg (potency)] of latamoxef ($C_{20}H_{20}N_6O_9S$) = $M_5 \times Q_a/Q_b \times 1000$

$M_5$: Amount [mg (potency)] of Latamoxef Ammonium RS

Internal standard solution—A solution of m-cresol (3 in 200).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.60 g of tetra n-butylammonium bromide in water to make exactly 1000 mL. To 750 mL of this solution add 250 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of latamoxef is about 7 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, latamoxef and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of latamoxef to that of the internal standard is not more than 1.0%.
Containers and storage Containers—Tight containers.
Storage—Not exceeding 5°C.

Lauromacrogol
Polyoxyethylene Lauryl Alcohol Ether
ラウロマクロゴール
Lauromacrogol is a polyoxyethylene ether prepared by the polymerization of ethylene oxide with lauryl alcohol.

Description Lauromacrogol is a colorless or light yellow, clear liquid or a white, petrodatum-like or waxy solid. It has a characteristic odor, and a somewhat bitter and slightly irritating taste. It is very soluble in ethanol (95), in diethyl ether and in carbon tetrachloride. It is freely soluble or dispersed as fine oily drops in water.

Identification (1) Shake well 0.5 g of Lauromacrogol with 10 mL of water and 5 mL of ammonium thiocyanate-cobalt nitrate TS, then shake with 5 mL of chloroform, and allow to stand: the chloroform layer becomes blue in color.
(2) Dissolve 0.35 g of Lauromacrogol in 10 mL of carbon tetrachloride, and perform the test as directed in the Solution method under Infrared Spectrophotometry <2.25> using a 0.1-mm fixed cell: it exhibits absorption at the wave numbers of about 1347 cm⁻¹, 1246 cm⁻¹ and 1110 cm⁻¹.

Purity (1) Acidity—Transfer 10.0 g of Lauromacrogol into a flask, and add 50 mL of neutralized ethanol. Heat on a water bath nearly to boil, shaking once or twice while heating. Cool, and add 5.3 mL of 0.1 mol/L sodium hydroxide VS and 5 drops of phenolphthalein TS: a red color develops.

(2) Unsaturated compound—Shake 0.5 g of Lauromacrogol with 10 mL of water, and add 5 drops of bromine TS: the color of the solution does not disappear.

Residue on ignition <2.44> Not more than 0.2% (1 g).

Containers and storage Containers—Tight containers.

Lenampicillin Hydrochloride
レナンピシリン塩酸塩

C₁₂H₂₂N₂O₅·HCl: 497.95
5-Methyl-2-oxo[1,3]dioxol-4-ylmethyl (2S,5R,6R)-6-[(2R)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [80734-02-7]

Lenampicillin Hydrochloride is the hydrochloride of ampicillin methylxoxidoxalenylmethyl ester.
It contains not less than 653 μg (potency) and not more than 709 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of the residual solvents. The potency of Lenampicillin Hydrochloride is expressed as mass (potency) of ampicillin (C₁₆H₁₉N₃O₄S: 349.40).

Description Lenampicillin Hydrochloride occurs as a white to light yellowish white powder. It is very soluble in water, in methanol and in ethanol (95), and freely soluble in N,N-dimethylformamide.

Identification (1) Determine the infrared absorption spectrum of Lenampicillin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Lenampicillin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.
(2) To 1 mL of a solution of Lenampicillin Hydrochloride (1 in 100) add 0.5 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation <2.49> [α]D²⁰: +174° – +194° (0.2 g calculated on the anhydrous basis and corrected on the amount of residual solvent, ethanol (95), 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Lenampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Lenampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).
(3) Free ampicillin—Weigh accurately about 0.1 g of Lenampicillin Hydrochloride, dissolve in exactly 10 mL of the internal standard solution, and use this solution as the
sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 25 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. The sample solution should be used to the following test immediately after the solution is prepared. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and calculate the ratios, \(Q_s\) and \(Q_a\), of the peak height of ampicillin to that of the internal standard:

the amount of ampicillin is not more than 1.0%.

\[
\text{Amount (mg) of Ampicillin RS} = M_s / M_y \times Q_s / Q_a \times 2
\]

\(M_s\): Amount (mg [potency]) of Ampicillin RS

\(M_y\): Amount (mg) of the sample

Internal standard solution—A solution of anhydrous caffeine in the mobile phase (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (10 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.22 g of potassium dihydrogen phosphate in water to make 900 mL, and add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, and ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of ampicillin to that of the internal standard is not more than 5%.

(4) Penicilloic acid—Weigh accurately about 0.1 g of Lenampicillin Hydrochloride, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add 10 mL of potassium hydrogen phthalate buffer solution, pH 4.6 and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 15 minutes while protecting from exposure to light, and titrate \(<2.50\) with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction: the amount of penicilloic acid (C_{16}H_{21}N_{3}O_{5}S: 367.42) is not more than 3.0%.

Each mL of 0.01 mol/L sodium thiosulfate VS = 0.45 mg of C_{16}H_{21}N_{3}O_{5}S

(5) Residual solvent \(<2.46\)—Weigh accurately about 0.25 g of Lenampicillin Hydrochloride, dissolve in exactly 1 mL of the internal standard solution, add N,N-dimethylformamide to make 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of 2-propanol and about 0.12 g of ethyl acetate, and add N,N-dimethylformamide to make exactly 100 mL. Pipet 1 mL and 3 mL of this solution, add exactly 1 mL each of the internal standard solution, add N,N-dimethylformamide to make 5 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with 4 µL each of the sample solution, standard solution (1) and (2) as directed under Gas Chromatography \(<2.02\) according to the following conditions, and calculate the ratios, \(Q_s\) and \(Q_a\), of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the sample solution, the ratios, \(Q_{s2}\) and \(Q_{a2}\), of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (1) and the ratios, \(Q_{s3} \) and \(Q_{a3}\), of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (2). Calculate the amounts of 2-propanol and ethyl acetate by the following equations: not more than 0.7% and not more than 1.7%, respectively.

Amount (mg) of 2-propanol = \(M_s / M_y \times (Q_s - Q_{s1}) / (Q_{s2} - Q_{s1})\)

Amount (mg) of ethyl acetate = \(M_s / M_y \times (Q_a - Q_{a1}) / (Q_{a2} - Q_{a1})\)

\(M_s\): Amount (g) of the sample

\(M_y\): Amount (g) of 2-propanol

\(M_{y}\): Amount (g) of ethyl acetate

\(Q_{s1}\): Amount of 2-propanol

\(Q_{a1}\): Amount of ethyl acetate

Internal standard solution—A solution of cyclohexane in N,N-dimethylformamide (1 in 1000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography (180 – 250 µm in particle diameter) coated with tetra-kishydroxypropylethylenediamine for gas chromatography at the ratio of 10 to 15%.

Column temperature: A constant temperature of about 80°C.

Injection port temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 1 minute.

System suitability—

System performance: When the procedure is run with 4 µL of the standard solution (2) under the above operating conditions, the internal standard, ethyl acetate and 2-propanol are eluted in this order, and the resolution between the peaks of the internal standard and ethyl acetate is not less than 2.0.

System repeatability: When the test is repeated 3 times with 4 µL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak height of ethyl acetate to that of the internal standard is not more than 5.0%.

Water \(<2.48\) Not more than 1.5% (1 g, volumetric titration, direct titration).

Residue on ignition \(<2.44\) Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Lenampicillin Hydrochloride and Lenampicillin Hydrochloride RS, equivalent to about 0.1 g (potency), dissolve each in the internal standard...
ard solution to make exactly 10 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of lenampicillin to that of the internal standard.

\[
\text{Amount (mg potency) of ampicillin (C16H19N3O4S)} = M_S \times Q_1 / Q_2 \times 1000
\]

\[
M_S: \text{Amount (mg potency) of Lenampicillin Hydrochloride RS}
\]

**Internal standard solution**—A solution of ethyl aminobenzoate in the mobile phase (1 in 4000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 9.53 g of potassium dihydrogen phosphate in water to make exactly 700 mL, and add acetonitrile to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of lenampicillin is about 6 minutes.

**System suitability**—

System performance: When the procedure is run with 5 \( \mu L \) of the standard solution under the above operating conditions, lenampicillin and the internal standard are eluted in the mobile phase (1 in 4000).

System repeatability: When the test is repeated 6 times with 5 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of lenampicillin to that of the internal standard is not more than 1.0%.

**Containers and storage** — Containers—Tight containers.

**L-Leucine**

L-レオイシン

C/H\textsubscript{2}H\textsubscript{13}NO\textsubscript{2}: 131.17

(2S)-2-Amino-4-methylpentanoic acid

[61-90-5]

L-Leucine, when dried, contains not less than 98.5% of C\textsubscript{6}H\textsubscript{13}NO\textsubscript{2}.

**Description** — L-Leucine occurs as white crystals or crystal- line powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

**Identification** — Determine the infrared absorption spectrum of L-Leucine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [\( \alpha \)]\textsubscript{D}: +14.5 – +16.0° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** <2.54> Dissolve 1.0 g of L-Leucine in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

**Purity (1)** — Clarity and color of solution—Dissolve 0.5 g of L-Leucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of L-Leucine in 40 mL of water and 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.6 g of L-Leucine in 40 mL of water and 1 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Leucine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Leucine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Leucine according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Leucine in water by warming, after cooling, add water to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes; the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.47> Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** — Weigh accurately about 0.13 g of L-Leucine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 13.12 mg of C\textsubscript{6}H\textsubscript{13}NO\textsubscript{2}

**Containers and storage** — Containers—Well-closed contain-

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Levallorphan Tartrate

Description: Levallorphan Tartrate occurs as a white to pale yellow, crystalline powder. It is odorless. It is soluble in water and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Levallorphan Tartrate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Levallorphan Tartrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Levallorphan Tartrate (1 in 30) responds to the Qualitative Tests <1.09> (1) and (2) for tartrate.

Optical rotation <2.49> [α]D: −37.0° to −39.2° (after drying, 0.2 g, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.2 g of Levallorphan Tartrate in 20 mL of water: the pH of this solution is between 3.3 and 3.8.

Melting point <2.60> 174 – 178°C

Purity (1) Clarity and color of solution—Dissolve 0.2 g of Levallorphan Tartrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Levallorphan Tartrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Levallorphan Tartrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.63>. Spot 20 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia TS (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 4 hours).

Residue on ignition <2.44> Not more than 0.10% (1 g).

Assay Weigh accurately about 0.5 g of Levallorphan Tartrate, previously dried, dissolve in 30 mL of acetic acid (100), and titrate <2.30> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 43.35 mg of C19H25NO.C4H6O6

Containers and storage: Containers—Well-closed containers.

Levallorphan Tartrate Injection

Description: Levallorphan Tartrate Injection is a clear, colorless liquid. pH: 3.0 – 4.5

Identification: Take an exact volume of Levallorphan Tartrate Injection, equivalent to 3 mg of Levallorphan Tartrate according to the labeled amount, add 5 mL of water and 2 drops of dilute hydrochloric acid, and wash with five 15-mL portions of diethyl ether by a vigorous shaking. Take the water layer, evaporate the diethyl ether remained by warming on a water bath, and after cooling, add 0.01 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> : it exhibits a maximum between 277 nm and 281 nm.

Bacterial endotoxins <4.01> Less than 150 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay: Take exactly a volume of Levallorphan Tartrate Injection, equivalent to about 2 mg of levallorphan tartrate (C19H25NO.C4H6O6), add exactly 10 mL of the internal
Levodopa / Official Monographs

Levodopa  

\[
\text{C}_{8}\text{H}_{11}\text{NO}_{2} : 197.19
\]

3-Hydroxy-L-tyrosine  

\[\text{[59-92-7]}\]

Levodopa, when dried, contains not less than 98.5% of \(\text{C}_{8}\text{H}_{11}\text{NO}_{4}\).

Description  
Levodopa occurs as white or slightly grayish white crystals or crystalline powder. It is odorless. It is freely soluble in formic acid, slightly soluble in water, and practically insoluble in ethanol (95%). It dissolves in dilute hydrochloric acid. The pH of a saturated solution of Levodopa is between 5.0 and 6.5. Melting point: about 275°C (with decomposition).

Identification  
1. To 5 mL of a solution of Levodopa (1 in 1000) add 1 mL of ninhydrin TS, and heat for 3 minutes in a water bath: a purple color develops.

2. To 2 mL of a solution of Levodopa (1 in 5000) add 10 mL of 4-aminooantipyrine TS, and shake: a red color develops.

3. Dissolve 3 mg of Levodopa in 0.001 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance  
\(E_{2\text{80}}^{1\text{cm}} = 136 - 146\) (after drying, 30 mg, 0.001 mol/L hydrochloric acid TS, 1000 mL).

Optical rotation  
\([\alpha]_2\text{80} = -11.5 - -13.0^\circ\) (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 nm).

Purity  
(1) Clarity and color of solution—Dissolve 1.0 g of Levodopa in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of Levodopa in 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.3 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.16>—Dissolve 0.40 g of Levodopa in 1 mL of dilute hydrochloric acid and 30 mL of water, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Levodopa according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.17>—Dissolve 1.0 g of Levodopa in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

(6) Related substances—Dissolve 0.10 g of Levodopa in 10 mL of sodium sulfite TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add sodium sulfite TS to make exactly 25 mL. Pipet 1 mL of this solution, add sodium sulfite TS to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 5 \(\mu\)L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and methanol (10:5:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 90°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.
Loss on drying <2.4> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.43> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Levodopa, previously dried, dissolve in 3 mL of formic acid, add 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 19.72 mg of C9H11NO4

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Levofloxacin Hydrate

レボフロキサシン水和物

C18H20FN3O4·H2O: 370.38
(3S)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid hemihydrate

Levofloxacin Hydrate contains not less than 99.0% and not more than 101.0% of levofloxacin (C18H20FN3O4·H2O: 361.37), calculated on the anhydrous basis.

Description Levofloxacin Hydrate occurs as light yellowish white to yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

It gradually turns dark yellowish white on exposure to light.

Melting point: about 226°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Levofloxacin Hydrate in 0.1 mol/L hydrochloric acid solution (1 in 150,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Levofloxacin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D20: -92 to -99° (0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Levofloxacin Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Levofloxacin Hydrate in 10 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Pipet 1 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 1.2 with respect to levofloxacin obtained from the sample solution is not larger than 2/5 times the peak area of levofloxacin from the standard solution, and the area of each peak other than the peak of levofloxacin and other than the peak having the relative retention time of about 1.2 with respect to levofloxacin from the sample solution is not larger than 1/5 times the peak area of levofloxacin from the standard solution. Furthermore, the total area of the peaks other than the peak of levofloxacin and other than the peak having the relative retention time of about 1.2 with respect to levofloxacin from the sample solution is not larger than 3/10 times the peak area of levofloxacin from the standard solution.

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: Dissolve 1.76 g of L-valine, 7.71 g of ammonium acetate and 1.25 g of Copper (II) sulfate pentahydrate in water to make 1000 mL. To this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of levofloxacin is about 22 minutes.

Time span of measurement: About 2 times as long as the retention time of levofloxacin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of levofloxacin obtained from 10 µL of this solution is equivalent to 4 to 6% of that of levofloxacin from 10 µL of the standard solution.

System performance: Dissolve 10 mg of ofloxacin in 20 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add a mixture of water and methanol (1:1) to make 10 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, the resolution between the peak of levofloxacin and the peak having the relative retention time of about 1.2 with respect to levofloxacin is not less than 3.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operat-
ing conditions, the relative standard deviation of the peak area of levofloxacin is not more than 3.0%.

(3) Residual solvent Being specified separately.

Water $\leq 2.48$ 2.1 – 2.7% (0.5 g, volumetric titration, direct titration).

Residue on ignition $\leq 2.44$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Levofloxacin Hy-
drinate, dissolve in 100 mL of acetic acid (100), and titrate $\geq 2.50$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 36.14 \text{ mg of } \text{C}_{19}\text{H}_{24}\text{N}_{2}\text{O}_{4}\text{S}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

### Levomepromazine Maleate

レボメプロマジンマレイン酸塩

![](image)

C$_{19}$H$_{24}$N$_2$O$_6$·C$_4$H$_4$O$_4$·4H$_2$O 444.54

(2R)-3-(2-Methoxy-10H-phenothiazin-10-yl)-N,N,2-trimethylpropylamine monomaleate [7104-38-3]

Levomepromazine Maleate, when dried, contains not less than 98.0% of C$_{19}$H$_{24}$N$_2$O$_6$C$_4$H$_4$O$_4$.

**Description** Levomepromazine Maleate occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, very slightly soluble in water, and practically insoluble in diethyl ether.

Melting point: 184 – 190°C (with decomposition).

**Identification (1)** Dissolve 5 mg of Levomepromazine Maleate in 5 mL of sulfuric acid: a red-purple color develops, which slowly becomes deep red-purple. To this solution add 1 drop of potassium dichromate TS: a brownish yellow-red color is produced.

(2) To 0.2 g of Levomepromazine Maleate add 5 mL of sodium hydroxide TS 20 mL of diethyl ether, and shake well. Separate the diethyl ether layer, wash twice with 10-mL portions of water, add 0.5 g of anhydrous sodium sulfate, filter, evaporate the diethyl ether on a water bath, and dry the residue at 105°C for 2 hours: the residue melts $\geq 2.60$ between 128°C and 136°C.

(3) To 0.5 g of Levomepromazine Maleate add 5 mL of water and 2 mL of ammonia solution (28), extract with three 5-mL portions of chloroform, separate and evaporate the water layer to dryness. To the residue add 2 to 3 drops of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine all the diethyl ether extracts, evaporate the diethyl ether in a water bath at a temperature of about 35°C with the aid of a current of air: the residue melts $\leq 2.60$ between 128°C and 136°C.

**Optical rotation** $\leq 2.49$ $[\alpha]$: $-13.5 $ – $ -16.5 $° (after drying, 0.5 g, chloroform, 20 mL, 200 mm).

**Purity (1)** Clarity and color of solution—To 0.5 g of Levomepromazine Maleate add 10 mL of methanol, and dissolve by warming: the solution is clear, and colorless or pale yellow.

(2) Chloride $\leq 1.07$—Dissolve 0.5 g of Levomepromazine Maleate in 40 mL of methanol, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(3) Heavy metals $\leq 2.07$—Proceed with 2.0 g of Levomepromazine Maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Loss on drying** $\leq 2.41$ Not more than 0.5% (2 g, 105°C, 3 hours).

Residue on ignition $\leq 2.44$ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 1 g of Levomepromazine Maleate, previously dried, and dissolve in a mixture of 40 mL of acetic acid (100) and 20 mL of acetone for nonaqueous titration. Titrate $\leq 2.50$ with 0.1 mol/L perchloric acid VS until the color of the solution changes from red-purple through blue-purple to blue (indicator: 5 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 44.45 mg of C$_{19}$H$_{24}$N$_2$O$_6$C$_4$H$_4$O$_4$.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

### Levothyroxine Sodium Hydrate

レボチロキシンナトリウム水和物

C$_{15}$H$_{10}$I$_4$NNaO$_4$·xH$_2$O

Monosodium O-(4-hydroxy-3,5-diodophenyl)-3,5-diiodo-L-tyrosinate hydrate [25416-65-3]

Levothyroxine Sodium Hydrate contains not less than 97.0% of levothyroxine sodium (C$_{15}$H$_{10}$I$_4$NNaO$_4$: 798.85), calculated on the dried basis.

**Description** Levothyroxine Sodium Hydrate occurs as a pale yellowish white to light yellow-brown powder. It is odorless.

It is slightly soluble in ethanol (95), and practically insolu-
ble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

**Identification (1)** Heat 0.1 g of Levothyroxine Sodium Hydrate over a flame: a purple gas evolves.

(2) To 0.5 mg of Levothyroxine Sodium Hydrate add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and add 0.1 mL of sodium nitrite TS. Allow to stand in a dark place for 20 minutes, and add 1.5 mL of ammonia solution (28): a yellowish red color is produced.

(3) Determine the absorption spectrum of a solution of Levothyroxine Sodium Hydrate in dilute sodium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry  <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Moisten Levothyroxine Sodium Hydrate with sulfuric acid, and ignite: the residue responds to the Qualitative Tests <1.069> (1) and (2) for sodium salt.

**Optical rotation** <2.49> \[\text{[a]}_D^{	ext{20}} = -5 - 6^\circ \text{ (0.3 g), calculated on the dried basis, } \text{a mixture of ethanol (95) and sodium hydroxide TS (2:1), 10 mL, 100 mm.} \]

**Purity** (1) Clarity and color of solution—Dissolve 0.3 g of Levothyroxine Sodium Hydrate in 10 mL of a mixture of ethanol (95) and sodium hydroxide TS (2:1) by warming: the solution is clear and pale yellow to pale yellow-brown in color.

(2) Soluble halides—Dissolve 0.01 g of Levothyroxine Sodium Hydrate in 10 mL of water and 1 drop of dilute nitric acid, shake, filter, and add 0.1 mL of sodium nitrite TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.20 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of water and 1 drop of dilute nitric acid, and proceed as directed above.

(3) Related substances—Dissolve 20 mg of Levothyroxine Sodium Hydrate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (14:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (95) and ammonia solution (28) (14:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 \( \mu \)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile (95), water, and ammonia solution (28) and 2-butanol (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the red-purple spots other than the principal spot from the sample solution are not more intense than the corresponding spot from the standard solution.

**Loss on drying** <2.41> 7–11% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

**Assay** Weigh accurately about 25 mg of Levothyroxine Sodium Hydrate, and proceed as directed under Oxygen Flask Combustion Method <1.067>, using a mixture of 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of a freshly prepared sodium bisulfate solution (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate <2.50> the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS = 0.6657 mg of \( C_{15}H_{10}I_{4}NaO_{4} \).

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Levothyroxine Sodium Tablets**

レボチロキシンナトリウム錠

Levothyroxine Sodium Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of levothyroxine sodium (\( C_{15}H_{10}I_{4}NaO_{4} \): 798.85).

**Method of preparation** Prepare as directed under Tablets, with Levothyroxine Sodium Hydrate.

**Identification (1)** Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 0.5 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and filter. To the filtrate add 0.1 mL of sodium nitrite TS, and allow to stand in a dark place for 20 minutes. Add 1.5 mL of ammonia solution (28): a yellowish red color develops.

(2) To a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 1 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 10 mL of ethanol (95), shake, filter, and use the filtrate as the sample solution. Dissolve 0.01 g of levothyroxine sodium for thin-layer chromatography in 100 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 \( \mu \)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of t-butanol, t-amyl alcohol, water, and ammonia solution (28) and 2-butanol (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray a solution of 0.3 g of ninhydrin in 100 mL of a
mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same RF value.

**Purity** Soluble halides—Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 2.5 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 25 mL of water, warm to 40°C, shake for 5 minutes, add 3 drops of dilute nitric acid, and filter. To the filtrate add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 25 mL of water and 3 drops of dilute nitric acid, and proceed as directed above.

**Uniformity of dosage units** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Levothyroxine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 20 µL of the sample solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratio of the peak area of levothyroxine to that of the internal standard. Calculate the mean value from the ratios of each peak area of 10 samples: the deviation (%) of the mean value and the ratio of each peak area should be not more than 15%. When the deviation (%) is more than 15%, and 1 sample shows not more than 25%, order the peak area of 30 samples used in the 2 tests and the ratio of each peak area: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

**Internal standard solution**—A solution of ethinylestradiol in a mixture of acetonitrile and diluted phosphoric acid (1 in 10) (9:1) (3 in 40,000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: a constant wavelength between 220 nm and 230 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecylsilanized silica gel.

Column temperature: A constant temperature at about 25°C.

Mobile phase: A mixture of methanol, water and phosphoric acid (1340:660:1).

Flow rate: Adjust the flow rate so that the retention time of levothyroxine is about 9 minutes.

Selection of column: To 5 mL of a solution of levothyroxine sodium in 0.01 mol/L sodium hydroxide TS (1 in 200,000) add 1 mL of the internal standard solution. Proceed with 20 µL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of levothyroxine and the internal standard in this order with the resolution between these peaks being not less than 2.0.

**Identification** (1) Dissolve 40 mg of Lidocaine in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry.
and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Lidocaine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point $<2.60> 66 – 69°C$

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Lidocaine in 2 mL of dilute hydrochloric acid, and add water to make 10 mL: the solution is clear and colorless to light yellow.

(2) Chloride $<1.03>$—Dissolve 0.6 g of Lidocaine in 6 mL of dilute nitric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.041%).

(3) Sulfate $<1.14>$—Dissolve 0.6 g of Lidocaine in 5 mL of dilute hydrochloric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS, 5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.096%).

(4) Heavy metals $<1.07>$—Carbonize 2.0 g of Lidocaine by gentle ignition. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 0.1 ppm).

(5) Related substances—Dissolve 0.10 g of Lidocaine in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-butanol, water and formic acid (5:3:1:1) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $<2.41>$ Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition $<2.44>$ Not more than 0.1% (1 g).

Assay Dissolve about 0.5 g of Lidocaine, previously dried and accurately weighed, in 20 mL of acetic acid (100), and titrate $<2.50>$ with 0.1 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS) until the color of the solution changes from purple to blue-green through blue. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 23.43 mg of C$_{14}$H$_{22}$N$_{2}$O

Containers and storage Containers—Tight containers.

### Lidocaine Injection

#### Lidocaine Hydrochloride Injection

リドカイン注射液

Lidocaine Injection is an aqueous injection. It contains not less than 95.0% and not more than 105.0% of the labeled amount of lidocaine hydrochloride (C$_{14}$H$_{22}$N$_{2}$O.HCl: 270.80).

**Method of preparation** Prepare as directed under Injections, with Lidocaine and an equivalent amount of Hydrochloric Acid.

No preservative is added in the case of intravenous injections.

**Description** Lidocaine Injection is a colorless, clear liquid.

**pH:** 5.0 – 7.0

**Identification** To a volume of Lidocaine Injection, equivalent to 20 mg of lidocaine hydrochloride (C$_{14}$H$_{22}$N$_{2}$O.HCl) according to the labeled amount, add 1 mL of sodium hydroxide TS, and extract with 20 mL of hexane. To 10 mL of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, and shake vigorously. Determine the absorption spectrum of the water layer as directed under Ultraviolet-visible Spectrophotometry $<2.19>$: it exhibits a maximum between 261 nm and 265 nm.

**Bacterial endotoxins** $<4.01>$ Less than 1.0 EU/mg.

**Extractable volume** $<6.05>$ It meets the requirement.

**Foreign insoluble matter** $<6.06>$ Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** $<6.07>$ It meets the requirement.

**Sterility** $<4.06>$ Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** To an exactly measured volume of Lidocaine Injection, equivalent to about 0.1 g of lidocaine hydrochloride (C$_{14}$H$_{22}$N$_{2}$O.HCl), add exactly 10 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 85 mg of lidocaine for assay, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in 0.5 mL of 1 mol/L hydrochloric acid TS and a suitable volume of 0.001 mol/L hydrochloric acid TS, and add exactly 10 mL of the internal standard solution, then add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.07>$ according to the following conditions, and calculate the ratios, Q$_{5}$ and Q$_{10}$, of the peak area of lidocaine to that of the internal standard.

Amount (mg) of lidocaine hydrochloride (C$_{14}$H$_{22}$N$_{2}$O.HCl) = M$_{5}$ × Q$_{1}$ / Q$_{5}$ × 1.156

M$_{5}$: Amount (mg) of lidocaine for assay
Limaprost Alfadex

リマプロスト アルファデクス

\[\text{C}_{22}\text{H}_{36}\text{O}_{5}\cdot x\text{C}_{60}\text{H}_{36}\text{O}_{30} \]
\[(2E)-7-[[1R,2R,3R]-3-Hydroxy-2-[[1E,3S,5S]-3-hydroxy-5-methylnon-1-en-1-yl]-5-oxocyclpentyl]hept-2-enonic acid-\alpha\text{-cyclodextrin}
\[100459-01-6, \text{limaprost:alfadex} = 1:1; \text{clathrate compound}\]

Limaprost Alfadex is a \(\alpha\)-cyclodextrin clathrate compound of limaprost.

It contains not less than 2.8% and not more than 3.2% of limaprost (\(\text{C}_{22}\text{H}_{36}\text{O}_{5} = 380.52\)), calculated on the anhydrous basis.

**Description** Limaprost Alfadex occurs as a white powder.

It is freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in ethyl acetate.

It is hygroscopic.

**Identification** (1) Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and use the upper layer as the sample solution (1). Separately, to 20 mg of Limaprost Alfadex add 5 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution (2). Evaporate the solvent of the sample solutions (1) and (2) under reduced pressure, add 2 mL of sulfuric acid to each of the residue, and shake them for 5 minutes: the solution obtained from the sample solution (1) develops an orange-yellow color while the solution from the sample solution (2) does not develop any color.

(2) Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and evaporate the solvent of the upper layer under reduced pressure. Dissolve the residue in 2 mL of ethanol (95), 5 mL of 1,3-dinitrobenzene TS, add 5 mL of a solution of potassium hydroxide in ethanol (95) (17 in 100) while ice-cooling, and allow to stand in a dark place while ice-cooling for 20 minutes: a purple color develops.

(3) To 50 mg of Limaprost Alfadex add 1 mL of iodine TS, dissolve by heating in a water bath, and allow to stand: a dark blue precipitate is formed.

(4) Determine the absorption spectrum of a solution of Limaprost Alfadex in dilute ethanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it does not exhibit a maximum between 200 nm and 400 nm. To 10 mL of this solution add 1 mL of potassium hydroxide-ethanol TS, and allow to stand for 15 minutes. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum; both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** <2.499> \(\left[\alpha\right]_{D}^{25} = +125 - 135^\circ\) (0.1 g, calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

**Purity** Related substances—Perform the test immediately after preparation of the sample solution. Dissolve 0.10 g of Limaprost Alfadex in 2 mL of water, add 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 3 mL of the standard solution (1), add dilute ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 3 \(\mu\)L each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.07> according to the following operating conditions, and determine each peak area by the automatic integration method: the area of the peak of 17-epi-isomer, having the relative retention time of about 1.1 with respect to limaprost, and the area of the peak of 11-deoxy substance, having the relative retention time of about 2.1, are not larger than the peak area of limaprost from the standard solution (2), and the area of the peak other than the principal peak and the peaks mentioned above is not larger than 1/3 times the peak area of limaprost from the standard solution (2). The total area of the peaks other than limaprost from the samples solution is not larger than the peak area of limaprost from the standard solution (1).
3 μL of this solution is equivalent to 8 to 12% of that from 3 μL of the standard solution (1).

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution (1) under the above conditions, the relative standard deviation of the peak area of limaprost is not more than 2.0%.

Water <2.48> Not more than 6.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Limaprost Afladex, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of Limaprost RS, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 3 μL each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of limaprost to that of the internal standard.

Amount (mg) of limaprost \( (C_{22}H_{36}O_{5}) \) = \( M_S \times \frac{Q_T}{Q_S} \)

\( M_S \): Amount (mg) of Limaprost RS

Internal standard solution—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000).

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS, acetonitrile for liquid chromatography (9:5:2).

Flow rate: Adjust the flow rate so that the retention time of limaprost is about 12 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the internal standard and limaprost are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of limaprost to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding −10°C.

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**Lincomycin Hydrochloride Hydrate**

リンコマイシン塩酸塩水和物

\( C_{18}H_{31}NO_5\cdot HCl \cdot H_2O \): 461.01

Methyl 6,8-dideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-erythro-α-D-galacto-octopyranoside monohydrochloride monohydrate [7179-49-9]

Lincomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces lincolnensis* var. *lincolnensis*.

It contains not less than 825 μg (potency) per mg, calculated on the anhydrous basis. The potency of Lincomycin Hydrochloride Hydrate is expressed as mass (potency) of lincomycin (\( C_{18}H_{31}NO_5\cdot HCl \): 406.54).

**Description** Lincomycin Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in water and in methanol, sparingly soluble in ethanol (95), and very slightly soluble in acetone.

**Identification** (1) Determine the infrared absorption spectrum of Lincomycin Hydrochloride Hydrate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Lincomycin Hydrochloride RS; both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Lincomycin Hydrochloride Hydrate (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

**Optical rotation** <2.49> \([\alpha]_D^25°: +135 − +150^\circ\) (0.5 g, water, 25 mL, 100 mm).

**pH** <2.54> Dissolve 0.10 g of Lincomycin Hydrochloride Hydrate in 1 mL of water: 3.0 − 5.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Lincomycin Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Lincomycin Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 5 ppm).

(3) Lincomycin B—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of lincomycin and lincomycin B, having the relative retention time of about 0.5 with respect to lincomycin, by the automatic integration method: the peak area of lincomycin B is not more than 5.0% of the sum of the peak areas of lincomycin and linco-
Lincomycin Hydrochloride Injection

Lincomycin Hydrochloride Injection is an aqueous injection. It contains not less than 93.0% and not more than 107.0% of the labeled potency of lincomycin (C₁₈H₃₄N₂O₆S: 406.54).

**Method of preparation** Prepare as directed under Injections, with Lincomycin Hydrochloride Hydrate.

**Description** Lincomycin Hydrochloride Injection is a clear, colorless liquid.

**Identification** To a volume of Lincomycin Hydrochloride Injection, equivalent to 30 mg (potency) of Lincomycin Hydrochloride Hydrate according to the labeled amount, add 30 mL of water, and use this solution as the sample solution. Separately, dissolve 10 mg (potency) of Lincomycin Hydrochloride RS in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Dissolve 150 g of ammonium acetate in 800 mL of water, adjust the pH to 9.6 with ammonia solution (28), and add water to make 1000 mL. To 80 mL of this solution add 40 mL of 2-propanol and 90 mL of ethyl acetate, shake, develop the plate with the upper layer of this solution to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of potassium permanganate (1 in 1000) on the plate: the principal spots from the sample solution and standard solution show the same Rf value.

**pH** Less than 5.0 and not more than 5.5.

**Bacterial endotoxins** Less than 0.50 EU/mg (potency).

**Extractable volume** It meets the requirement.

**Foreign insoluble matter** Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** It meets the requirement.

**Sterility** Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Pipet a volume of Lincomycin Hydrochloride Injection, equivalent to about 0.3 g (potency) of Lincomycin Hydrochloride Hydrate, add the mobile phase to make exactly 30 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Lincomycin Hydrochloride RS, equivalent to 20 mg (potency), dissolve in the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Lincomycin Hydrochloride Hydrate.

Amount [mg (potency)] of lincomycin (C₁₈H₃₄N₂O₆S) = \(M_S \times \frac{A_T}{A_S} \times 15\)

**Containers and storage** Containers—Tight containers.
Liothyronine Sodium

リオチロニンナトリウム

C₁₅H₁₁I₃NNaO₄: 672.96

Monosodium O-(4-hydroxy-3-iodophenyl)-3,5-diido-L-tyrosinate

[55-06-1]

Liothyronine Sodium contains not less than 95.0% of C₁₅H₁₁I₃NNaO₄, calculated on the dried basis.

Identification (1) To 5 mL of a solution of Liothyronine Sodium in ethanol (95) (1 in 1000) add 1 mL of ninhydrin TS, and warm in a water bath for 5 minutes: a purple color develops.

(2) Heat 0.02 g of Liothyronine Sodium with a few drops of sulfuric acid over a flame: a purple gas is evolved.

(3) Determine the absorption spectrum of a solution of Liothyronine Sodium in ethanol (95) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Ignite 0.02 g of Liothyronine Sodium until thoroughly charred. After cooling, add 5 mL of water to the residue, shake, and filter: the filtrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> [α]D²₀: +18° to +22° (0.2 g, calculated on the dried basis, a mixture of ethanol (95) and 1 mol/L hydrochloric acid TS (4:1), 10 mL, 100 mm).

Purity (1) Soluble halide—To 10 mg of Liothyronine Sodium add 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes, and filter. Add water to the filtrate to make 10 mL, and mix with 3 drops of silver nitrate TS: the solution shows no more turbidity than the following control solution.

Control solution: To 0.35 mL of 0.01 mol/L hydrochloric acid VS add 1 drop of dilute nitric acid and water to make 10 mL, and add 3 drops of silver nitrate TS.

(2) Iodine and iodide—Dissolve 0.10 g of Liothyronine Sodium in 10 mL of dilute sodium hydroxide TS and 15 mL of water, add 5 mL of dilute sulfuric acid, and allow to stand for 10 minutes with occasional shaking. Filter the mixture into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodate (1 in 100) to the filtrate, mix for 30 seconds, and allow to stand: the chloroform layer has no more color than the following control solution.

Control solution: Weigh exactly 0.111 g of potassium iodide, and dissolve in water to make 1000 mL. Pipet 1 mL of this solution, add 10 mL of dilute hydroxide TS, 14 mL of water and 5 mL of dilute sulfuric acid, and mix. Filter the mixture into a Nessler tube, and perform the test with the filtrate in the same manner as for the sample.

(3) Related substances—Dissolve 0.15 g of Liothyronine Sodium in 5 mL of diluted ammonia TS (1 in 3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ammonia TS (1 in 3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>, Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of n-butanol, t-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 4.0% (0.2 g, 105°C, 2 hours).

Assay Weigh accurately about 25 mg of Liothyronine Sodium, and proceed as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 10 mL of a solution of sodium hydroxide (1 in 100) and 1 mL of a freshly prepared solution of sodium bisulfate (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water again. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate <2.50> the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS = 0.7477 mg of C₁₅H₁₁I₃NNaO₄

Containers and storage Containers—Tight containers.

Storage—Light-resistant.
Liothyronine Sodium Tablets

リオチロニンナトリウム錠

Liothyronine Sodium Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of liothyronine sodium (C₁₅H₁₁I₃NNaO₄; 672.96).

Method of preparation Prepare as directed under Tablets, with Liothyronine Sodium.

Identification (1) To a glass-stoppered centrifuge tube add a portion of finely powdered Liothyronine Sodium Tablets, equivalent to 0.1 mg of Liothyronine Sodium according to the labeled amount, add 30 mL of dilute sodium hydroxide TS, shake vigorously, and centrifuge. Transfer the supernatant liquid to a separator, add 10 mL of dilute hydrochloric acid, and extract with two 20-mL portions of ethyl acetate. Filter each extract successively through absorbent cotton previously overlaid with 8 g of anhydrous sodium sulfate. Evaporate the filtrate on a water bath to dryness with the aid of a current of nitrogen. Dissolve the residue in 0.5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 10 mg of liothyronine sodium for thin-layer chromatography in methanol to make 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of t-butanol, t-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same Rf value.

(2) The colored solution obtained in the Assay is blue in color.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Liothyronine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge for 5 minutes, and filter the supernatant liquid, if necessary. Pipet a definite volume of this solution, and add a volume of 0.01 mol/L sodium hydroxide TS to prepare a definite volume of a solution containing about 0.5 μg of liothyronine sodium (C₁₅H₁₁I₃NNaO₄) per mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 200 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratio of the peak area of the liothyronine to that of the internal standard. Calculate the mean value of the ratios of each peak area of 10 samples: the deviation (%) of each ratio of the peak area from the mean value should be not more than 15%. When the deviation (%) is more than 15%, and 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of each ratio of the peak area from the mean value of the 30 samples used in the two tests: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

Internal standard solution—A solution of propylparahydroxybenzoate in a mixture of methanol and diluted phosphoric acid (1 in 10) (9:1) (1 in 250,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 225 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Diluted methanol (57 in 100).
Flow rate: Adjust the flow rate so that the retention time of liothyronine is about 9 minutes.

System suitability—
System performance: To 5 mL of a solution of liothyronine sodium in 0.01 mol/L sodium hydroxide TS (1 in 2,000,000) add 1 mL of the internal standard solution, and use this solution as the solution for system suitability test. When the procedure is run with 200 μL of this solution under the above operating conditions, the internal standard and liothyronine are eluted in this order with the resolution between these peaks being not less than 2.0.
System repeatability: When the test is repeated 6 times with 200 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of liothyronine to that of the internal standard is not more than 1.0%.

Assay Weigh accurately not less than 20 Liothyronine Sodium Tablets, and finely powder. Place an accurately weighed portion of the powder, equivalent to about 50 μg of liothyronine sodium (C₁₅H₁₁I₃NNaO₄), in an agate mortar, add 1 g of powdered potassium carbonate, and mix well. Transfer the mixture cautiously to a porcelain crucible, and compact the contents by gently tapping the crucible on a hard surface. Ignite the combined mixture in a mixture of methanol and water, and dissolve in water to make exactly 20 mL. Measure exactly 5 mL of this solution, and add a solution of potassium carbonate (1 in 8) to make exactly 100 mL. Measure exactly 5 mL of the solution, and add a solution of potassium carbonate (1 in 8) to make exactly 20 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution into a solution for system suitability test.
Lisinopril Hydrate

C_{21}H_{31}N_{3}O_{5} \cdot 2H_{2}O: 441.52

(2S)-1\{(2S)-6-Amino-2\{(1S)-1-carboxy-3-phenylpropylamino\}hexanoyl\}pyrrolidine-2-carboxylic acid dihydrate
[83915-83-7]

Lisinopril Hydrate contains not less than 98.5% and not more than 101.0% of lisinopril (C_{21}H_{31}N_{3}O_{5}: 405.49), calculated on the anhydrous basis.

Description Lisinopril Hydrate occurs as a white crystalline powder, having a slight characteristic odor.

It is soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5). Melting point: about 160°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Lisinopril Hydrate in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry \(\lambda_{240}\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Lisinopril Hydrate as directed in the paste method under Infrared Spectrophotometry \(\lambda_{2.25}\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation \(\phi_{D}\) \(\theta^{25} - 43.0 \rightarrow -47.0^\circ\) (0.25 g calculated on the anhydrous basis, 0.25 mL/L zinc acetate buffer solution, pH 6.4, 25 mL, 100 mm).

Purity (1) Heavy metals \(\leq 0.07\%\)—Proceed with 2.0 g of Lisinopril Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve about 0.10 g of Lisinopril Hydrate in 50 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(\leq 0.01\%\) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.2 with respect to lisinopril, is not larger than 1/5 times the peak area of lisinopril from the standard solution, the area of the peak other than lisinopril and the peak mentioned above is not larger than 2/15 times the peak area of lisinopril from the standard solution, and the total area of the peaks other than lisinopril is not larger than the peak area of lisinopril from the standard solution.


Column: A stainless steel column 4.0 mm in inner diameter and 20 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase A: Diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2).

Mobile phase B: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>90 \rightarrow 50</td>
<td>10 \rightarrow 50</td>
</tr>
<tr>
<td>10 – 25</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Flow rate: About 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of lisinopril beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2.5 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that with 15 μL of the standard solution.

System performance: To 10 mg of Lisinopril Hydrate and 2 mL of a solution of anhydrous caffeine (1 in 1000) add water to make 200 mL. When the procedure is run with 15
μL of this solution under the above operating conditions, lisinopril and caffeine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

**Water** Not less than 8.0% and not more than 9.5% (0.3 g, volumetric titration, back titration).

**Residue on ignition** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.66 g of Lisinopril Hydrate, dissolve in 80 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 40.55 mg of C₂₁H₃₁N₃O₅

**Containers and storage** Well-closed containers.

# Lisinopril Tablets

リシノプリル錠

Lisinopril Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lisinopril (C₂₁H₃₁N₃O₅: 405.49).

**Method of preparation** Prepare as directed under Tablets, with Lisinopril Hydrate.

**Identification** To an amount of powdered Lisinopril Tablets, equivalent to 10 mg of lisinopril (C₂₁H₃₁N₃O₅), add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of lisinopril in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 30 μL of each sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, acetic acid (100), water and ethyl acetate (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 120°C: the principal spot with the sample solution and the spot with the standard solution show a red-purple color and their RF values are the same.

**Purity** Related substances—Powder not less than 20 Lisinopril Tablets. Take a portion of the powder, equivalent to about 25 mg of lisinopril (C₂₁H₃₁N₃O₅), add 25 mL of water, shake for 20 minutes, filter, and use the filtrate as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of lisinopril diketopiperazine, having the relative retention time of about 2.0 with respect to lisinopril, is not larger than 2/3 times the peak area of lisinopril from the standard solution.

**Operating conditions**— Proceed as directed in the operating conditions in the Purity (2) under Lisinopril Hydrate.

**System suitability**—

Test for required detectability: To exactly 2.5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that with 15 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Purity (2) under Lisinopril Hydrate.

**Uniformity of dosage units** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Lisinopril Tablets add exactly 5 mL each of the internal standard solution per every 1 mg of lisinopril (C₂₁H₃₁N₃O₅), shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Hereafter, proceed as directed in the Assay.

Amount (mg) of lisinopril (C₂₁H₃₁N₃O₅) = Mₛ × Qₛ/Qᵣ × C/10

Mₛ: Amount (mg) of lisinopril for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of lisinopril (C₂₁H₃₁N₃O₅) in 1 tablet

**Internal standard solution**—A solution of anhydrous caffeine (1 in 20,000).

**Dissolution** (50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate of a 5 mg tablet in 60 minutes and that of a 10-mg tablet in 90 minutes is not less than 80%, and that of a 20-mg tablet in 90 minutes is not less than 75%)

Start the test with 1 tablet of Lisinopril Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 5.6 μg of lisinopril (C₂₁H₃₁N₃O₅) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of lisinopril for assay, separately determined the water in the same manner as Lisinopril Hydrate, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A₁ and A₃, of lisinopril.

Dissolution rate (%) with respect to the labeled amount of lisinopril (C₂₁H₃₁N₃O₅)

Using the result for A₁ and A₃,

\[ Mₛ = \frac{A₁}{A₃} = \frac{V/V' \times 1/C \times 36}{A₁} \]
anhydrous basis

C: Labeled amount (mg) of lisinopril (C₂₁H₃₁N₃O₅) in 1 tablet

Operating conditions—

Detector, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Flow rate: Adjust the flow rate so that the retention time of lisinopril is about 7 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of lisinopril are not less than 1000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

Assay

Weigh accurately the mass of not less than 20 Lisinopril Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of lisinopril (C₂₁H₃₁N₃O₅), add exactly 25 mL of the internal standard solution, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of lisinopril for assay, separately determine the water in the same manner as Lisinopril Hydrate, add exactly 50 mL of the internal standard solution to dissolve, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Qₜ and Qₛ, of the peak area of lisinopril to that of the internal standard.

\[
\text{Amount (mg) of lisinopril (C₂₁H₃₁N₃O₅)} = Mₛ \times \frac{Qₜ}{Qₛ} \times 1/2
\]

Mₛ: Amount (mg) of lisinopril for assay, calculated on the anhydrous basis

Internal standard solution—A solution of anhydrous caffeine (1 in 20,000).

Operating conditions—


Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (19:1).

Flow rate: Adjust the flow rate so that the retention time of lisinopril is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lisinopril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of lisinopril to that of the internal standard is not more than 1.0%.

Containers and storage

Containers—Well-closed containers.

Lithium Carbonate

炭酸リチウム

Li₂CO₃: 73.89

Lithium Carbonate, when dried, contains not less than 99.5% of Li₂CO₃.

Description

Lithium Carbonate occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in water, slightly soluble in hot water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in dilute acetic acid.

The pH of a solution dissolved 1.0 g of Lithium Carbonate in 100 mL or water is between 10.9 and 11.5.

Identification (1) Perform the test as directed under Flame Coloration Test <1.04> (1) with Lithium Carbonate: a persistent red color appears.

(2) Dissolve 0.2 g of Lithium Carbonate in 3 mL of dilute hydrochloric acid, and add 4 mL of sodium hydroxide TS and 2 mL of disodium hydrogen phosphate TS: a white precipitate is produced. To the precipitate add 2 mL of dilute hydrochloric acid: it dissolves.

(3) A solution of Lithium Carbonate (1 in 100) responds to the Qualitative Tests <1.09> for carbonate.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Lithium Carbonate in 10 mL of water by warming: the solution is clear and colorless.

(2) Acetic acid-insoluble substances—Take 1.0 g of Lithium Carbonate, dissolve in 40 mL of dilute acetic acid, filter the insoluble substances using filter paper for quantitative analysis, wash with five 10-mL portions of water, and ignite the insoluble substances together with the filter paper to incinerate: the mass of the residue is not more than 1.5 mg.

(3) Chloride <1.03>—To 0.40 g of Lithium Carbonate add 10 mL of water and 7 mL of dilute nitric acid, and dissolve by heating to boil. After cooling, add 6 mL of dilute nitric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.022%).

(4) Sulfate <1.14>—To 0.40 g of Lithium Carbonate add 10 mL of water and 4 mL of dilute hydrochloric acid, and dissolve by heating to boil. After cooling, add 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).
(5) Heavy metals — To 4.0 g of Lithium Carbonate add 5 mL of water, gradually add 10 mL of hydrochloric acid while mixing, and dissolve. Evaporate the solution on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a slight red color, then add 2 mL of dilute acetic acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: Evaporate 10 mL of hydrochloric acid on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a pale red color, then add 2.0 mL of Standard Lead (3 in 20), and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 49.5 mg of magnesium sulfate heptahydrate, previously dried at 105°C for 2 hours and heated at 450°C for 3 hours, in water to make 1000 mL. To this solution add 3 mL of solution B obtained in (7), 0.2 mL of a solution of titanium yellow (1 in 1000) and water to make 20 mL, and proceed in the same manner.

(10) Magnesium — To 3.0 mL of solution A obtained in (7) add 0.2 mL of a solution of titan yellow (1 in 1000) and water to make 20 mL, then add 5 mL of sodium hydroxide (3 in 20), and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 49.5 mg of magnesium sulfate heptahydrate, previously dried at 105°C for 2 hours and heated at 450°C for 3 hours, in water to make 1000 mL. To this solution add 3 mL of solution B obtained in (7), 0.2 mL of a solution of titanium yellow (1 in 1000) and water to make 20 mL, and proceed in the same manner.

(11) Potassium — Dissolve 1.0 g of Lithium Carbonate in water to make 100 mL, and use this solution as the sample solution. To 5 mL of the sample solution add 1.0 mL of dilute acetic acid, shake, add 5 mL of a solution of sodium tetraphenylborate (1 in 30), shake immediately, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water to make 1000 mL. To 5 mL of this solution add 1.0 mL of dilute acetic acid, shake, and proceed in the same manner.

(12) Sodium — Weigh accurately about 0.8 g of Lithium Carbonate, dissolve in water to make exactly 100 mL, and use this solution as the sample stock solution. Measure exactly 25 mL of the sample stock solution, add water to make exactly 100 mL, and use this solution as the sample solution (1). Separately, weigh accurately 25.4 mg of sodium chloride, dissolve in water to make exactly 1000 mL, and use this solution as the standard solution. Measure exactly 25 mL of the sample stock solution, add exactly 20 mL of the standard solution, then add water to make exactly 100 mL, and use this solution as the sample solution (2). Determine emission intensities of sodium using a flame photometer with the sample solution (1) and the sample solution (2) under the following conditions. Adjust the wavelength dial to 589 nm, atomize the sample solution (2) into the flame, then adjust the sensitivity so that the emission intensity \( I_S \) shows 100 adjustment, and determine emission intensity \( I_T \) of the sample solution (1). Then, make the other conditions identical, change the wavelength dial to 580 nm, determine emission intensity \( I_B \) of the sample solution (1): the amount of sodium, calculated from the following equation, is not more than 0.05%.

\[
\text{Amount (~Na)} = \frac{(I_T - I_B)}{(I_S - I_T)} \times \frac{M'}{M} \times 100
\]

\( M \): Amount (mg) of the sample in 25 mL of the sample stock solution

\( M' \): Amount (mg) of sodium in 20 mL of the standard solution

(13) Arsenic — Prepare the test solution with 1.0 g of Lithium Carbonate, add 2 mL of water and 3 mL of hydrochloric acid, and perform the test (not more than 2 ppm).

Loss on drying — Not more than 0.5% (1 g, 105°C, 3 hours).
**Assay** Weigh accurately about 1 g of Lithium Carbonate, previously dried, add exactly 100 mL of water and 50 mL of 0.5 mol/L sulfuric acid VS, remove carbon dioxide by boiling gently, cool, and titrate $\geq 2.50$ the excess sulfuric acid with 1 mol/L sodium hydroxide VS until the color of the solution changes from red to yellow (indicator: 3 drops of methyl red TS). Perform a blank determination.

Each mL of 0.5 mol/L sulfuric acid VS $= 36.95$ mg of Li$_2$CO$_3$

**Containers and storage** Containers—Well-closed containers.

**Lorazepam**

ロラゼパム

Cl$_3$H$_9$ClN$_2$O$_2$: 321.16
(3RS)-7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2H-1,4-benzodiazepin-2-one [846-49-1]

Lorazepam, when dried, contains not less than 98.5% of C$_{15}$H$_{10}$Cl$_2$N$_2$O$_2$.

**Description** Lorazepam occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored by light.

**Identification** (1) To 0.02 g of Lorazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, and cool: the solution responds to the Qualitative Tests $<1.09>$ for primary aromatic amines.

(2) Determine the absorption spectrum of a solution of Lorazepam in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry $<2.24>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Lorazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Lorazepam as directed under Flame Coloration Test $<1.04>$ (2): a green color appears.

Absorbance $\geq 2.24$ $E_{1\text{cm}}^{1\text{cm}}$ (229 nm): 1080 – 1126 (after drying, 1 mg, ethanol (95), 200 mL).

**Purity** (1) Chloride $<1.07>$—To 1.0 g of Lorazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals $<1.07>$—Proceed with 1.0 g of Lorazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic $<1.11>$—Prepare the test solution with 1.0 g of Lorazepam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Lorazepam in 20 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 10 $\mu$L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, 1,4-dioxane and acetic acid (100) (91:5:4) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** $<2.47>$—Not more than 0.5% (1 g, in vacuum, 105°C, 3 hours).

**Residue on ignition** $<2.44>$—Not more than 0.3% (1 g).

**Assay** Weigh accurately about 0.4 g of Lorazepam, previously dried, dissolve in 50 mL of acetone, and titrate $\geq 2.50$ with 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS $= 32.12$ mg of Cl$_3$H$_9$ClN$_2$O$_2$

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Losartan Potassium**

ロサルタンカリウム

C$_{22}$H$_{22}$ClKN$_6$O: 461.00

Monopotassium 5-[4’-(2-butyl-4-chloro-5-hydroxymethyl-1H-imidazol-1-yl)methyl]biphenyl-2-yl]-1H-tetrazol-1-ide [124750-99-8]

Losartan Potassium contains not less than 98.5% and not more than 101.0% of C$_{22}$H$_{22}$ClKN$_6$O, calcu-
lated on the anhydrous basis.

**Description** Losartan Potassium occurs as a white crystalline powder. It is very soluble in water, and freely soluble in methanol and in ethanol (99.5).

**Identification (1)** Determine the absorption spectrum of a solution of Losartan Potassium in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Losartan Potassium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Losartan Potassium as directed in the potassium bromide powder exhibit similar intensities of absorption at the same wave numbers.

(3) Losartan Potassium responds to the Qualitative Tests <1.09> (1) for potassium salt.

(4) Perform the test with Losartan Potassium as directed under Flame Coloration Test <1.04> (2): a green color appears.

**Purity (1)** Heavy metals <1.07>—Proceed with 2.0 g of Losartan Potassium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 30 mg of Losartan Potassium in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peaks of solvent and losartan obtained from the sample solution is not larger than 1/10 times the peak area of losartan from the standard solution, and the total area of the peaks other than the peak of losartan from the sample solution is not larger than 3/10 times the peak area of losartan from the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadeclisilanzilated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Diluted phosphoric acid (1 in 1000).

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 25</td>
<td>75 → 10</td>
<td>25 → 90</td>
</tr>
<tr>
<td>25 – 35</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute.

Time span of measurement: 35 minutes after injection of the sample.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of losartan obtained from 10 μL of this solution is equivalent to 7 to 13% of that of losartan from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of losartan are not less than 10,000 and not more than 1.3, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 2.0%.

(3) Residual solvent—Being specified separately.

**Water** <2.48> Not more than 0.5% (0.25 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 25 mg each of Losartan Potassium and Losartan Potassium RS (separately, determine the water <2.48> in the same manner as Losartan Potassium), dissolve separately in methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of losartan in each solution.

\[
\text{Amount (mg) of losartan potassium (C}_{22}\text{H}_{22}\text{ClKN}_{6}\text{O}) = \frac{M_S}{M_S} \times \frac{A_T}{A_S}
\]

M_S: Amount (mg) of Losartan Potassium RS, calculated on the anhydrous basis.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadeclisilanzilated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of losartan is about 6 minutes.

**System suitability**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of losartan are not less than 5500 and not more than 1.4, respectively.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 1.0%.

Containers and storage Containers—Tight containers.

**Loxoprofen Sodium Hydrate**

ロキソプロフェンナトリウム水和物

C₁₅H₁₇NaO₃·2H₂O: 304.31

Loxoprofen Sodium Hydrate contains not less than 98.5% of loxoprofen sodium (C₁₅H₁₇NaO₃: 268.28), calculated on the anhydrous basis.

Description

Loxoprofen Sodium Hydrate occurs as white to yellowish white crystals or crystalline powder.

It is very soluble in water and in methanol, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Loxoprofen Sodium Hydrate (1 in 20) does not show optical rotation.

The pH of a solution of Loxoprofen Sodium Hydrate in freshly boiled and cooled water (1 in 20) is between 6.5 and 8.5.

Identification (1)

Determine the absorption spectrum of a solution of Loxoprofen Sodium Hydrate (1 in 55,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)

Determine the infrared absorption spectrum of Loxoprofen Sodium Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Loxoprofen Sodium Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1)

Clarity and color of solution—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of water: the solution is clear and colorless or pale yellow. The color is not darker than that of diluted Matching Fluid for Color A (1 in 2).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Loxoprofen Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane and acetic acid (100) (9:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.45> 11.0 – 13.0% (0.2 g, volumetric titration, direct titration).

Assay

Weigh accurately about 60 mg of Loxoprofen Sodium Hydrate, and dissolve in dilute methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, add diluted methanol (3 in 5) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Loxoprofen RS, previously dried in a desiccator (in vacuum, 60°C) for 3 hours, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, proceed in the same manner as directed for the preparation of the sample solution, and use so obtained solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of loxoprofen to that of the internal standard.

Amount (mg) of loxoprofen sodium (C₁₅H₁₇NaO₃) = \( M_s \times Q_1 / Q_2 \times 1.089 \)

\( M_s \): Amount (mg) of Loxoprofen RS

Internal standard solution—A solution of ethyl benzoate in dilute methanol (3 in 5) (7 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 222 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol, water, acetic acid (100) and triethylamine (600:400:1:1).

Flow rate: Adjust the flow rate so that the retention time of loxoprofen is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, loxoprofen and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 5 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of loxoprofen to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
L-Lysine Acetate

L-リシン酢酸塩

\[
\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2 \cdot \text{C}_2\text{H}_4\text{O}_2
\]

(2S)-2,6-Diaminohexanoic acid monoacetate

[57282-49-2]

L-Lysine Acetate, when dried, contains not less than 98.5% and not more than 101.0%. C\textsubscript{6}H\textsubscript{14}N\textsubscript{2}O\textsubscript{2}, C\textsubscript{2}H\textsubscript{4}O\textsubscript{2}.

**Description** L-Lysine Acetate occurs as white crystals or crystalline powder. It has a characteristic odor and a slightly acid taste.

It is very soluble in water, freely soluble in formic acid, and practically insoluble in ethanol (99.5).

**Identification (1)** Determine the infrared absorption spectrum of L-Lysine Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of L-Lysine Acetate (1 in 20) responds to the Qualitative Tests (1.09) (2) for acetate.

**Optical rotation** <2.49° [α]D +8.5 -10.0° (after drying, 2.5 g, water, 25 mL, 100 mm).

**pH** <2.54 Dissolve 1.0 g of L-Lysine Acetate in 10 mL of water: the pH of the solution is between 6.5 and 7.5.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of L-Lysine Acetate in 10 mL of water: the solution is colorless and clear.

(2) Chloride <1.0%—Perform the test with 0.5 g of L-Lysine Acetate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.4%—Perform the test with 0.6 g of L-Lysine Acetate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.0%—Perform the test with 0.25 g of L-Lysine Acetate. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07%—Proced with 1.0 g of L-Lysine Acetate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <1.10%—Proced with the test solution with 1.0 g of L-Lysine Acetate according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Weigh accurately about 0.5 g of L-Lysine Acetate, dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately 2.5 mol amounts of L-aspartic acid, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine and L-arginine, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions. Based on the peak heights of the amino acids obtained from the sample solution and standard solution, determine the mass of the amino acids other than lysine contained in 1 mL of the sample solution, and calculate the mass percent: the amount of each amino acids other than lysine is not more than 0.1%.

**Operating conditions**—

Detector: A visible spectrophotometer (wavelength: 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (Na type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table, and to each phase add 0.1 mL of capric acid.

<table>
<thead>
<tr>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
<th>Mobile phase C</th>
<th>Mobile phase D</th>
<th>Mobile phase E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid monohydrate</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>12.80 g</td>
<td>6.10 g</td>
</tr>
<tr>
<td>Trisodium citrate dihydrate</td>
<td>6.19 g</td>
<td>7.74 g</td>
<td>13.31 g</td>
<td>26.67 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>3.74 g</td>
<td>54.35 g</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol (99.5)</td>
<td>130 mL</td>
<td>20 mL</td>
<td>4 mL</td>
<td>100 mL</td>
</tr>
<tr>
<td>Thiodiglycol</td>
<td>5 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td>—</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5 mL</td>
</tr>
<tr>
<td>Lauramomacrogol solution (1 in 4)</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
</tr>
<tr>
<td>Water</td>
<td>Appropriate amount</td>
<td>Appropriate amount</td>
<td>Appropriate amount</td>
<td>Appropriate amount</td>
</tr>
<tr>
<td>Total volume</td>
<td>1000 mL</td>
<td>1000 mL</td>
<td>1000 mL</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Changing mobile phases: Proceed with 20 μL of the standard solution under the above operating conditions: aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine and arginine are eluted in this order. Switchover the mobile phases A, B, C, D and E in sequence so that the resolution between the peaks of isoleucine and leucine is not less than 1.2.

Reaction reagents: Dissolve 204 g of lithium acetate dihydrate in water, and add 123 mL of acetic acid (100), 401 mL
of 1-methoxy-2-propanol, and water to make 1000 mL, gas with water for 10 minutes, and use this solution as the solution (I). Separately, to 979 mL of 1-methoxy-2-propanol add 39 g of ninhydrin, gas with nitrogen for 5 minutes, add 81 mg of sodium borohydride, gas the solution with nitrogen for 30 minutes, and use this solution as solution (II). To 1 volume of the solution (I) add 1 volume of the solution (II). Prepare before use.

Mobile phase flow rate: 0.20 mL per minute.

Reaction reagent flow rate: 0.24 mL per minute.

System suitability—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and alanine is not less than 1.2.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak height of each amino acid in the standard solution is not more than 5.0%, and the relative standard deviation of the retention time is not more than 1.0%.

Loss on drying ⟨2.41⟩ Not more than 0.3% (1 g, 80°C, 3 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of L-Lysine Acetate, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 10.31 mg of C₆H₁₄N₂O₂.HCl

Containers and storage Containers—Tight containers.

**L-Lysine Hydrochloride**

**L-Lysine Hydrochloride**

C₆H₁₄N₂O₂·HCl: 182.65

(2S)-2,6-Diaminohexanoic acid monohydrochloride [657-27-2]

L-Lysine Hydrochloride, when dried, contains not less than 98.5% of C₆H₁₄N₂O₂·HCl.

Description L-Lysine Hydrochloride occurs as a white powder. It is odorless, and has a slight, characteristic taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95).

Identification (1) Determine the infrared absorption spectrum of L-Lysine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve L-Lysine Hydrochloride in water, evaporate the water to dryness at 60°C, and repeat the test with the residue.

(2) A solution of L-Lysine Hydrochloride (1 in 10) responds to the Qualitative Tests ⟨1.09⟩ for chloride.

Optical rotation ⟨2.49⟩ [α]D: +19.0° – +21.5° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH ⟨2.54⟩ Dissolve 1.0 g of L-Lysine Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Lysine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate ⟨1.14⟩—Perform the test with 0.6 g of L-Lysine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(3) Ammonium ⟨1.02⟩—Perform the test with 0.25 g of L-Lysine Hydrochloride. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals ⟨1.07⟩—Proceed with 2.0 g of L-Lysine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic ⟨1.11⟩—Prepare the test solution with 1.0 g of L-Lysine Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.10 g of L-Lysine Hydrochloride in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨2.03⟩. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol and ammonia water (28:72) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50) and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨2.41⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of L-Lysine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 9.132 mg of C₆H₁₄N₂O₂·HCl

Containers and storage Containers—Tight containers.
**Lysozyme Hydrochloride**

リゾチーム塩酸塩

**Description**

Lysozyme Hydrochloride occurs as white, crystals, or crystalline or amorphous powder. It is freely soluble in water, and practically insoluble in ethanol (99.5). It is hygroscopic.

The pH of a solution of Lysozyme Hydrochloride (3 in 200) is between 3.0 and 5.0.

**Identification (1)**

To 5 mL of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 500) add 1 mL of ninhydrin TS, and heat for 10 minutes: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)**

Clarity of solution—To 5 mL of a solution of Lysozyme Hydrochloride (3 in 200) add, if necessary, dilute hydrochloric acid to adjust the pH to 3: the solution is clear.

**Residue on ignition**

Not more than 2.0% (0.1 g, 105°C, 2 hours).

**Nitrogen**

Perform the test as directed under Nitrogen Determination (1.08): the amount of nitrogen (N: 14.01) is between 16.8% and 16.4%, calculated on the dried basis.

**Assay**

Weigh accurately an amount of Lysozyme Hydrochloride, equivalent to about 25 mg (potency), dissolve in phosphate buffer solution, pH 6.2 to make exactly 100 mL. Pipet 2 mL of this solution, add phosphate buffer solution, pH 6.2 to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Lysozyme RS (separately determine its loss on drying in the same manner as Lysozyme Hydrochloride), equivalent to about 25 mg (potency), and dissolve in phosphate buffer solution, pH 6.2 to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, add phosphate buffer solution, pH 6.2 to make exactly 50 mL, and use these solutions as the standard solution (1) and the solution (2), respectively. Keep the sample solution and the standard solutions in an ice-bath. Pipet 4 mL of substrate solution for lysozyme hydrochloride, previously warmed in a water bath of 35°C for about 5 minutes, add exactly 100 µL of the sample solution, previously warmed in a water bath of 35°C for about 3 minutes, and allow to stand at 35°C for exactly 10 minutes, then add exactly 0.5 mL of 1 mol/L hydrochloric acid TS, and immediately shake. Determine the absorbance under Ultraviolet-visible Spectrophotometry (2.24), A_t, of this solution at 640 nm, using water as the blank. Determine the absorbances, A_S1 and A_S2, of the solutions obtained with the standard solution (1) and the standard solution (2) in the same manner as the sample solution.

Amount [mg (potency)] of lysozyme per mg, calculated on the dried basis = \( M_S/2M_T \times [(A_S1 - A_t)/(A_S1 - A_S2) + 1] \)

\( M_S \): Amount (mg) of Lysozyme RS, calculated on the dried basis.

\( M_T \): Amount (mg) of the sample, calculated on the dried basis.

**Containers and storage**

Containers—Tight containers.

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**Macrogol 400**

**Polyethylene Glycol 400**

マクロゴール 400

Macrogol 400 is a polymer of ethylene oxide and water, represented by the formula \( \text{HOCH}_2(\text{CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH} \), in which the value of \( n \) ranges from 7 to 9.

**Description**

Macrogol 400 occurs as a clear, colorless and viscous liquid. It has no odor or a slight, characteristic odor. It is miscible with water, with methanol, with ethanol (95) and with pyridine.

It is soluble in diethyl ether.

It is slightly hygroscopic.

Congealing point: 4 – 8°C

Specific gravity: \( d_{20}^\rho \): 1.110 – 1.140

**Identification**

Dissolve 50 mg of Macrogol 400 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid n-hydrate (1 in 10): a yellow-green precipitate is formed.

pH (2.54)

Dissolve 1.0 g of Macrogol 400 in 20 mL of water: the pH of this solution is between 4.0 and 7.0.

**Purity (1)**

Acidity—Dissolve 5.0 g of Macrogol 400 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(2) Ethylene glycol and diethylene glycol—Dissolve 4.0 g of Macrogol 400 in water to make exactly 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg each of ethylene glycol and diethylene glycol, dissolve...
in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 µL each of the sample solution and standard solution as directed under Gas Chromatography §2.029 according to the following conditions: Determine the peak heights, \( H_{T_a} \) and \( H_{S_a} \), of ethylene glycol of each solution, and the peak heights, \( H_{T_b} \) and \( H_{S_b} \), of diethylene glycol, and calculate the amount of ethylene glycol and diethylene glycol: the sum of the contents of ethylene glycol and diethylene glycol is not more than 0.25%.

Amount (mg) of ethylene glycol
\[
M_{S_a} = M_a \times \frac{H_{T_a}}{H_{S_a}} \times \frac{1}{1/10}
\]

Amount (mg) of diethylene glycol
\[
M_{S_b} = M_b \times \frac{H_{T_b}}{H_{S_b}} \times \frac{1}{1/10}
\]

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column about 3 mm in inside diameter and about 1.5 m in length, packed with siliceous earth for gas chromatography, 150 to 180 µm in particle diameter, coated with D-sorbitol at the ratio of 12%.

Column temperature: A constant temperature of about 165°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of diethylene glycol is about 8 minutes.

Selection of column: Proceed with 2 µL of the standard solution under the above operating conditions, and calculate the resolution. Use a column clearly dividing peaks of ethylene glycol and diethylene glycol in this order.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of diethylene glycol obtained from 2 µL of the standard solution composes about 80% of the full scale.

Average molecular mass—Add 42 g of phthalic anhydride to 300 mL of freshly distilled pyridine, exactly measured, in a 1-L light-resistant glass-stoppered bottle. Shake the bottle vigorously to dissolve the solid, and allow to stand for 16 hours or more. Pipet 25 mL of this solution into an about 200-mL glass-stoppered pressure bottle. Add about 1.5 g of Macrogol 400, accurately weighed, stopper the bottle, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate \(<2.5d\) with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

Average molecular mass
\[
M = \frac{(M \times 4000)/(a - b)}{M}
\]

M: Amount (g) of sample
a: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination
b: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample

Average molecular mass is between 380 and 420.

Water \(<2.48\) Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition \(<2.44\) Not more than 0.1% (1 g).

Containers and storage—Containers—Tight containers.

Macrogol 1500

Polyethylene Glycol 1500

Macrogol 1500 is a mixture containing equal amounts of lower and higher polymers of ethylene oxide and water, represented by the formula \( HOCH_{2}(CH_{2}OCH_{2})_{n}CH_{2}OH \), in which the value of \( n \) is 5 or 6 for the lower polymers and from 28 to 36 for the higher.

Description—Macrogol 1500 occurs as a white, smooth petrolatum-like solid. It is odorless or has a faint, characteristic odor.

It is very soluble in water, in pyridine and in diphenyl ether, freely soluble in methanol, sparingly soluble in ethanol (95), very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Congealing point: 37 – 41°C

Identification—Dissolve 50 mg of Macrogol 1500 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid \( n \)-hydrate (1 in 10): a yellow-green precipitate is formed.

pH \(<2.5\)—Dissolve 1.0 g of Macrogol 1500 in 20 mL of water: the pH of the solution is between 4.0 and 7.0.

Purity—(1) Clarity and color of solution—Dissolve 5.0 g of Macrogol 1500 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 1500 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(3) Ethylene glycol and diethylene glycol—Place 50.0 g of Macrogol 1500 in a distilling flask, add 75 mL of diphenyl ether, warm to dissolve if necessary, distil slowly under a reduced pressure of 0.13 to 0.27 kPa and take 25 mL of the distillate in a 100-mL container with 1-mL graduation. To the distillate add exactly 20 mL of water, shake vigorously, cool in ice water, congeal the diphenyl ether, and filter into a 25-mL volumetric flask. Wash the residue with 5.0 mL of ice-cold water, combine the washings with the filtrate, warm to room temperature, and add water to make 25 mL. Transfer this solution to a glass-stoppered flask, shake with 25.0 mL of freshly distilled acetonitrile, and use this solution as the sample solution. Separately, to 62.5 mg of diethylene glycol add a mixture of water and freshly distilled acetonitrile (1:1) to make exactly 25 mL, and use this solution as the standard solution. Take exactly 10 mL each of the sam-
**Macrogol 4000**

**Polyethylene Glycol 4000**

Macrogol 4000 is a polymer of ethylene oxide and water, represented by the formula \( \text{HOCH}_2(\text{CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH} \), in which the value of \( n \) ranges from 59 to 84.

**Description** Macrogol 4000 is a white, paraffin-like solid, occurring as flakes or powder. It is odorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in methanol and in pyridine, and practically insoluble in ethanol (99.5) and in diethyl ether.

**Congealing point:** 53 – 57°C

**Identification** Dissolve 50 mg of Macrogol 4000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid \( n \)-hydrate (1 in 10): a yellow-green precipitate is formed.

**pH** **<2.45** Dissolve 1.0 g of Macrogol 4000 in 20 mL of water: the pH of this solution is between 4.0 and 7.5.

**Purity** (1) Clarity and color of solution—A solution of 5.0 g of Macrogol 4000 in 50 mL of water is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 4000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

**Average molecular mass** Weigh accurately about 12.5 g of Macrogol 4000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate **<2.50** with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

Average molecular mass = \( (M \times 4000)/(a - b) \)

M: Amount (g) of sample
a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination
b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample

Average molecular mass is between 2600 and 3800.

**Water** **<2.48** Not more than 1.0% (2 g, volumetric titration, direct titration).

**Residue on ignition** **<2.44** Not more than 0.1% (1 g).

**Containers and storage** Containers—Well-closed containers.

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**Macrogol 6000**

**Polyethylene Glycol 6000**

Macrogol 6000 is a polymer of ethylene oxide and water, represented by the formula \( \text{HOCH}_2(\text{CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH} \), in which the value of \( n \) ranges from 165 to 210.

**Description** Macrogol 6000 is a white, paraffin-like solid, occurring as flakes or powder. It is odorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in pyridine, and practically insoluble in methanol, in ethanol (95), in ethanol (99.5) and in diethyl ether.

**Congealing point:** 56 – 61°C

**Identification** Dissolve 50 mg of Macrogol 6000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid \( n \)-hydrate (1 in 10): a yellow-green precipitate is formed.

**pH** **<2.54** Dissolve 1.0 g of Macrogol 6000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

**Purity** (1) Clarity and color of solution—A solution of 5.0 g of Macrogol 6000 in 50 mL of water is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 6000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

**Average molecular mass** Weigh accurately about 12.5 g of Macrogol 6000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dis-
solve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate to 2.50° with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination in the same manner.

Average molecular mass = \( M \times 4000 \)/\( (a - b) \)

- **M**: Amount (g) of sample
  - **a**: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination
  - **b**: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample

Average molecular mass is between 7300 and 9300.

**Water** <2.48> Not more than 1.0% (2 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Containers and storage** Containers—Well-closed containers.

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**Macrogol 20000**

**Polyethylene Glycol 20000**

マクロゴール 20000

Macrogol 20000 is a polymer of ethylene oxide and water, represented by the formula HOCH\(_2\)(CH\(_2\)OCH\(_2\))\(_n\)CH\(_2\)OH, in which the value of \( n \) lies between 340 and 570.

**Description** Macrogol 20000 occurs as white, paraffin-like flakes or powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water and in pyridine, and practically insoluble in methanol, in ethanol (95), in dehydrated diethyl ether, in petroleum benzine and in macrogol 400.

**Congealing point**: 56 – 64°C

**Identification** Dissolve 50 mg of Macrogol 20000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid \( n \)-hydrate (1 in 10): a yellow-green precipitate is formed.

**pH** <2.54> Dissolve 1.0 g of Macrogol 20000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

**Purity** (1) Clarity and color of solution—Dissolve 5.0 g of Macrogol 20000 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 20000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

**Average molecular mass** Weigh accurately about 15 g of Macrogol 20000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of 98 ± 2°C, to the same depth as the mixture in the bottle. Maintain the temperature of the bath at 98 ± 2°C for 60 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate to 2.50° with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

Average molecular mass = \( M \times 4000 \)/\( (a - b) \)

- **M**: Amount (g) of sample
  - **a**: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination
  - **b**: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample

Average molecular mass is between 15000 and 25000.

**Water** <2.48> Not more than 1.0% (2 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Containers and storage** Containers—Well-closed containers.

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**Macrogol Ointment**

**Polyethylene Glycol Ointment**

マクロゴール軟膏

To make 1000 g

| Macrogol 4000 | 500 g |
| Macrogol 400 | 500 g |

Melt Macrogol 4000 and Macrogol 400 by warming on a water bath at 65°C, and mix well until it congeals. Less than 100 g of Macrogol 4000 or Macrogol 400 may be replaced by an equal amount of Macrogol 400 or Macrogol 4000 to prepare 1000 g of a proper soft ointment.

**Description** Macrogol Ointment is white in color. It has a faint, characteristic odor.

**Identification** Dissolve 50 mg of Macrogol Ointment in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, filter if necessary, and add 1 mL of a solution of phosphomolybdic acid \( n \)-hydrate (1 in 10) to the filtrate: a yellow-green precipitate is formed.
Magneisum Carbonate

Magnesium Carbonate is a basic hydrated magnesium carbonate, or a normal hydrated magnesium carbonate. Magnesium Carbonate contains not less than 40.0% and not more than 44.0% of magnesium oxide (MgO: 40.30).

"Heavy magnesium carbonate" may be used as commonly used name for Magnesium Carbonate which shows the height of the precipitate below the 12.0-mL graduation line in the Precipitation test.

**Identification**
1. Dissolve 1 g of Magnesium Carbonate in 10 mL of dilute hydrochloric acid, boil, then cool, neutralize with sodium hydroxide TS, and filter, if necessary: the solution responds to the Qualitative Tests <1.09> for magnesium salt.
2. Magnesium Carbonate responds to the Qualitative Tests <1.09> (1) for carbonate.

**Purity**
1. Soluble salts—To 2.0 g of Magnesium Carbonate add 40 mL of 1-propanol and 40 mL of water, heat to boil with constant stirring, cool, and filter. Wash the residue with water, combine the washings with the filtrate, and add water to make exactly 100 mL. Evaporate 50 mL of the solution on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue does not exceed 10.0 mg.
2. Heavy metals <1.07>—Moisten 1.0 g of Magnesium Carbonate with 4 mL of water, dissolve by addition of 10 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid, 1 drop of ammonia TS, filter, if necessary, wash the filter paper with water, combine the washings with the filtrate, and add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 10 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 30 ppm).
3. Iron <1.10>—Prepare the test solution with 0.10 g of Magnesium Carbonate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 200 ppm).
4. Arsenic <1.11>—Prepare the test solution with 0.40 g of Magnesium Carbonate, previously moistened with 1.5 mL of water, add 3.5 mL of dilute hydrochloric acid, and perform the test (not more than 5 ppm).
5. Calcium oxide—Weigh accurately about 0.6 g of Magnesium Carbonate, and dissolve in 35 mL of water and 6 mL of dilute hydrochloric acid. Add 250 mL of water and 5 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2′,2′-nitrotrisethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes form red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

\[ \text{Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 0.5608 \text{ mg of CaO} \]

The content of calcium oxide (CaO: 56.08) is not more than 0.6%.

(6) Acid-insoluble substances—Mix 5.0 g of Magnesium Carbonate and 75 mL of water, add 10 mL of hydrochloric acid dropwise while stirring, boil for 5 minutes, and cool. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the residue is not more than 2.5 mg.

**Precipitation test**
Transfer 1.0 g of Magnesium Carbonate, previously sifted through a No. 100 (150 μm) sieve to a glass-stoppered measuring cylinder with a 50-mL graduation line at 150 mm from the bottom, and add water to make 50 mL. Shake vigorously for exactly 1 minute, allow to stand for 15 minutes, and measure the height of the precipitate (in graduation in ml).

**Assay**
Weigh accurately about 0.4 g of Magnesium Carbonate, dissolve in 10 mL of water and 3.5 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of the solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Prepare a blank determination, and make any necessary correction. From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS corresponding to the content of calcium oxide (CaO) obtained in the Purity (5).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

\[ \text{Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 2.015 \text{ mg of MgO} \]

Each mg of calcium oxide (CaO)
\[ = 0.36 \text{ mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} \]

**Containers and storage**
Containers—Well-closed containers.

**Calcium oxide**
Weigh accurately about 0.6 g of Magnesium Carbonate, and dissolve in 35 mL of water and 6 ml of dilute hydrochloric acid. Add 250 mL of water and 5 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2′,2′-nitrotrisethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes form red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

\[ \text{Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 0.5608 \text{ mg of CaO} \]

The content of calcium oxide (CaO: 56.08) is not more than 0.6%.

(6) Acid-insoluble substances—Mix 5.0 g of Magnesium Carbonate and 75 mL of water, add 10 mL of hydrochloric acid dropwise while stirring, boil for 5 minutes, and cool. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the residue is not more than 2.5 mg.

**Precipitation test**
Transfer 1.0 g of Magnesium Carbonate, previously sifted through a No. 100 (150 μm) sieve to a glass-stoppered measuring cylinder with a 50-mL graduation line at 150 mm from the bottom, and add water to make 50 mL. Shake vigorously for exactly 1 minute, allow to stand for 15 minutes, and measure the height of the precipitate (in graduation in ml).

**Assay**
Weigh accurately about 0.4 g of Magnesium Carbonate, dissolve in 10 mL of water and 3.5 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of the solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Prepare a blank determination, and make any necessary correction. From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS corresponding to the content of calcium oxide (CaO) obtained in the Purity (5).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

\[ \text{Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 2.015 \text{ mg of MgO} \]

Each mg of calcium oxide (CaO)
\[ = 0.36 \text{ mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} \]
Magnesium Oxide

酸化マグネシウム

MgO: 40.30

Magnesium Oxide, when ignited, contains not less than 96.0% of MgO.

When 5 g of Magnesium Oxide has a volume not more than 30 mL, it may be labeled heavy magnesium oxide.

Description Magnesium Oxide occurs as a white powder or granules. It is odorless. It is practically insoluble in water, in ethanol (95) and in diethyl ether. It dissolves in dilute hydrochloric acid. It absorbs moisture and carbon dioxide in air.

Identification A solution of Magnesium Oxide in dilute hydrochloric acid (1 in 50) responds to the Qualitative Tests for magnesium salt.

Purity (1) Alkali and soluble salts—Transfer 2.0 g of Magnesium Oxide to a beaker, add 100 mL of water, cover the beaker with a watch-glass, heat on a water bath for 5 minutes, and filter immediately. After cooling, to 50 mL of the filtrate add 2 drops of methyl red TS and 2.0 mL of 0.05 mol/L sulfuric acid VS: a red color develops. Evaporate 25 mL of the remaining filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 10 mg.

(2) Carbonate—Boil 0.10 g of Magnesium Oxide with 5 mL of water, cool, and add 5 mL of acetic acid (31): almost no effervescence occurs.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Magnesium Oxide in 20 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, add 1 drop of phenolphthalein TS, neutralize with ammonia TS, add 2 mL of dilute acetic acid, and filter, if necessary. Wash the filter paper with water, add water to the combined washing and the filtrate to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 20 mL of dilute hydrochloric acid add 1 drop of phenolphthalein TS, neutralize with ammonia TS, and add 2 mL of dilute acetic acid, 4.0 mL of Standard Lead Solution and water to make 50 mL (not more than 40 ppm).

(4) Iron <1.10>—Prepare the test solution with 40 mg of Magnesium Oxide according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(5) Calcium oxide—Weigh accurately about 0.25 g of Magnesium Oxide, previously ignited, dissolve in 6 mL of dilute hydrochloric acid by heating. Cool, add 300 mL of water and 3 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2’,2”-nitrilotriethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide VS, allow to stand for 5 minutes, and titrate 2.50 mL with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determina-

The figures are in mm.

A: Distilling flask of about 300-mL capacity.
B: Steam generator of about 1000-mL capacity, containing a few boiling lips to prevent bumping
C: Condenser
D: Receiver; 200-mL volumetric flask
E: Steam-introducing tube having an internal diameter of about 8 mm
F, G: Rubber tube with a clamp
H: Thermometer

The mass of calcium oxide (CaO: 56.08) is not more than 1.5%.

(6) Arsenic <1.11>—Dissolve 0.20 g of Magnesium Oxide in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 10 ppm).

(7) Acid-insoluble substances—Mix 2.0 g of Magnesium Oxide with 75 mL of water, add 12 mL of hydrochloric acid dropwise, while shaking, and boil for 5 minutes. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the ignited residue does not more than 2.0 mg.

(8) Fluoride—(i) Apparatus: Use a hard glass apparatus as illustrated in the figure. Ground-glass joints may be used.

(ii) Procedure: Transfer 5.0 g of Natural Aluminum Silicate to the distilling flask A with the aid of 20 mL of water, add about 1 g of glass wool and 50 mL of diluted purified sulfuric acid (1 in 2), and connect A to the distillation apparatus, previously washed with steam streamed through the steam introducing tube E. Connect the condenser C with the receiver D containing 10 mL of 0.01 mol/L sodium hydroxide VS and 10 mL of water so that the lower end of C is immersed in the solution. Heat A gradually until the temperature of the solution in A reaches 130°C, then open the rub-
ber tube \( F \), close the rubber tube \( G \), boil water in the steam generator \( B \) vigorously, and introduce the generated steam into \( F \). Simultaneously, heat \( A \), and maintain the temperature of the solution in \( A \) between 135°C and 145°C. Adjust the distilling rate to about 10 mL per minute. Collect about 170 mL of the distillate, then stop the distillation, wash \( C \) with a small quantity of water, combine the washings with the distillate, add water to make exactly 200 mL, and use this solution as the test solution. Perform the test with the test solution as directed in the procedure of determination for fluoride under Oxygen Flask Combustion Method \(<1.06>\). 

No corrective solution is used in this procedure. The content of fluoride (\( F \)) is not more than 0.08%.

Amount (mg) of fluoride (\( F \): 19.00) in the test solution

\[ \text{Amount (mg) of fluoride in } 5 \text{ mL of the standard solution} = \left( \text{Amount (mg) of fluoride in } 5 \text{ mL of the test solution} \right) \times \frac{A_1}{A_3} \times \frac{200}{V} \]

\textbf{Loss on ignition} \(<2.43>\) Not more than 10% (\(0.25 \text{ g, } 900^\circ C\), constant mass).

\textbf{Assay} Ignite Magnesium Oxide to constant mass at \( 900^\circ C \), weigh accurately about 0.2 g of the residue, dissolve in 10 mL of water and 4.0 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of potassium chloride buffer solution, \( \text{pH } 10.7 \), and titrate \(<2.50>\) with 0.05 mol/L disodium dihydrogen ethylenediamine tetracacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetracacetate VS consumed, deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetracacetate VS corresponding to the content of calcium oxide (\( \text{CaO} \)) obtained in the Purity (5).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetracacetate VS

\[ = 2.015 \text{ mg of MgO} \]

Each mg of calcium oxide (\( \text{CaO} \))

\[ = 0.36 \text{ mL of } 0.05 \text{ mol/L disodium dihydrogen ethylenediamine tetracacetate VS} \]

\textbf{Containers and storage} Containers—Tight containers.

**Magnesium Silicate**

ケイ酸マグネシウム

Magnesium Silicate contains not less than 45.0% of silicon dioxide (\( \text{SiO}_2 \): 60.08) and not less than 20.0% of magnesium oxide (\( \text{MgO} \): 40.30), and the ratio of percentage (%) of magnesium oxide to silicon dioxide is not less than 2.2 and not more than 2.5.

\textbf{Description} Magnesium Silicate occurs as a white, fine powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

\textbf{Identification (1)} Mix 0.5 g of Magnesium Silicate with 10 mL of dilute hydrochloric acid, filter, and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests \(<1.09>\) for magnesium salt.

(2) Prepare a bead by fusing ammonium sodium hydrogen phosphate tetrahydrate on a platinum loop. Place the bead in contact with Magnesium Silicate, and fuse again: an infusible matter appears in the bead, which changes to an opaque bead with a web-like structure upon cooling.

\textbf{Purity (1)} Soluble salts—Add 150 mL of water to 10.0 g of Magnesium Silicate, heat on a water bath for 60 minutes with occasional shaking, then cool, dilute with water to 150 mL, and centrifuge. Dilute 75 mL of the resultant transparent liquid with water to 100 mL, and use this solution as the sample solution. Evaporate 25 mL of the sample solution on a water bath to dryness, and ignite the residue at 700°C for 2 hours: the mass of the ignited residue is not more than 0.02 g.

(2) Alkalinity—To 20 mL of the sample solution obtained in (1) add 2 drops of phenolphthalein TS and 1.0 mL of 0.1 mol/L hydrochloric acid VS: no color develops.

(3) Chloride \(<1.07>\)—Take 10 mL of the sample solution obtained in (1), add 6 mL of dilute nitric acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.75 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(4) Sulfate \(<1.14>\)—To the residue obtained in (1) add about 3 mL of dilute hydrochloric acid, and heat on a water bath for 10 minutes. Add 30 mL of water, filter, wash the residue on the filter with water, combine the washings with the filtrate, and dilute to 50 mL with water. To 4 mL of the solution add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(5) Heavy metals \(<1.07>\)—To 1.0 g of Magnesium Silicate add 20 mL of water and 3 mL of hydrochloric acid, and boil for 2 minutes. Filter, and wash the residue on the filter with two 5-mL portions of water. Evaporate the combined filtrate and washings on a water bath to dryness, add 2 mL of dilute acetic acid to the residue, warm until solution is complete, filter, if necessary, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 30 ppm).

(6) Arsenic \(<1.11>\)—To 0.4 g of Magnesium Silicate add 5 mL of dilute hydrochloric acid, heat gently to boiling while shaking well, cool rapidly, and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue, and repeat the extraction in the same manner. Concentrate the combined extracts on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 5 ppm).

\textbf{Loss on ignition} \(<2.43>\) Not more than 34% (\(0.5 \text{ g, } 850^\circ C\), 3 hours).

\textbf{Acid-consuming capacity} \(<6.0>\) Place about 0.2 g of Magnesium Silicate, accurately weighed, in a glass-stoppered flask, add exactly 30 mL of 0.1 mol/L hydrochloric acid VS and 20 mL of water, shake at 37 ± 2°C for 1 hour, and cool. Pipet 25 mL of the supernatant liquid, and titrate \(<2.50>\) the excess hydrochloric acid, while stirring well, with...
0.1 mol/L sodium hydroxide VS until the pH becomes 3.5.
1 g of Magnesium Silicate, calculated on the anhydrous basis by making allowance for the observed loss on ignition determined as directed in the preceding Loss on ignition, consumes not less than 140 mL and not more than 160 mL of 0.1 mol/L hydrochloric acid VS.

Assay (1) Silicon dioxide—Weigh accurately about 0.7 g of Magnesium Silicate, add 10 mL of 0.5 mol/L sulfuric acid TS, evaporate on a water bath to dryness, add 25 mL of water to the residue, and heat on a water bath for 15 minutes with occasional stirring. Filter the supernatant liquid through filter paper for assay, add 25 mL of hot water to the residue, stir, and decant the supernatant liquid on the filter paper to filter. Wash the residue in the same manner with two 25-mL portions of hot water, transfer the residue onto paper to filter. Wash the residue, stir, and decant the supernatant liquid on the filter paper to filter. Wash the residue with water, and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool, and weigh the residue as a (g). Moisten the residue with water, and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool, and weigh the residue as b (g).

Content (%) of silicon dioxide (SiO₂) = (a - b)/M × 100
M: Mass (g) of the sample

(2) Magnesium oxide—Weigh accurately about 0.3 g of Magnesium Silicate, transfer to a 50-mL conical flask, add 10 mL of 0.5 mol/L sulfuric acid VS, and heat on a water bath for 15 minutes. Cool, transfer to a 100-mL volumetric flask, wash the conical flask with water, add the washings to the volumetric flask, dilute with water to 100 mL, and filter. Pipet 50 mL of the filtrate, shake with 50 mL of water and 5 mL of 2,2',2''-nitrodiethanol (1 in 2), add 2.0 mL of ammonia TS and 10 mL of 10 mol/L sodium-ammonium chloride buffer solution, pH 10.7, and titrate with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.015 mg of MgO

(3) Ratio of percentage (%) of magnesium oxide (MgO) to silicon dioxide (SiO₂) = Calculate the quotient from the percentages obtained in (1) and (2).

Containers and storage Containers—Well-closed containers.

Magnesium Stearate

ステアリン酸マグネシウム

Magnesium Stearate consists chiefly magnesium salts of stearic acid (C₁₈H₃₆O₂: 284.48) and palmitic acid (C₁₆H₃₂O₂: 256.42).

It contains, when dried, not less than 4.0% and not more than 5.0% of magnesium (Mg: 24.31).

Description Magnesium Stearate occurs as a white, light, bulky powder.

It is smooth to the touch and sticky to the skin. It has no odor or a faint, characteristic odor.

It is practically insoluble in water and in ethanol (95).

Identification (1) Mix 5.0 g of Magnesium Stearate with 50 mL of peroxide-free diethyl ether, 20 mL of dilute nitric acid, and 20 mL of water in a round-bottom flask, and heat to dissolve completely under a reflux condenser. After cooling, transfer the contents of the flask to a separator, shake, allow the layers to separate, and transfer the aqueous layer to a flask. Extract the diethyl ether layer with two 4-mL portions of water, and combine these extracts to the main aqueous extract. After washing the combined aqueous extract with 15 mL of peroxide-free diethyl ether, transfer to a 50-mL volumetric flask, add water to make exactly 50 mL, mix, and use this solution as the sample solution: the sample solution responds to the Qualitative Tests <1.09> for magnesium.

(2) The retention times of the peaks corresponding to methyl stearate and methyl palmitate in the chromatogram of the sample solution correspond to those of methyl stearate and methyl palmitate in the chromatogram of the system suitability solution, as obtained in the Purity (5).

Purity (1) Acidity or alkalinity—Heat 1.0 g of Magnesium Stearate in 20 mL of freshly boiled and cooled water on a water bath for 1 minute while shaking, and filter after cooling. To 10 mL of the filtrate add 0.05 mL of bromothymol blue TS, and add exactly 0.05 mL of 0.1 mol/L hydrochloric acid VS or 0.1 mol/L sodium hydroxide VS: the color of the solution changes.

Chloride <1.03>—Perform the test with 10.0 mL of the sample solution obtained in Identification (1). Prepare the control solution with 1.40 mL of 0.02 mol/L hydrochloric acid VS (not more than 0.10%).

Sulfate <1.10>—Perform the test with 10.0 mL of the sample solution obtained in Identification (1). Prepare the control solution with 10.2 mL of 0.01 mol/L sulfuric acid VS (not more than 1.0%).

Heavy metals <1.07>—Heat 1.0 g of Magnesium Stearate weakly first, then incinerate at about 500 ± 25°C. After cooling, add 2 mL of hydrochloric acid, evaporate on a water bath to dryness, add 20 mL of water and 2 mL of dilute acetic acid to the residue, and heat for 2 minutes. After cooling, filter this solution through a filter paper, wash the filter paper with 15 mL of water, and combine the washing with the filtrate. To the filtrate add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

Relative content of stearic acid and palmitic acid—Transfer exactly 0.01 g of Magnesium Stearate to a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and reflux for about 10 minutes to dissolve the solids. Add 4.0 mL of heptane through the condenser, and reflux for about 10 minutes. After cooling, add 20 mL of saturated sodium chloride solution, shake, and allow the layers to separate. Transfer the heptane layer through about 0.1 g of anhydrous sodium sulfate, previously washed with heptane, to another
flask. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with heptane to volume, mix, and use this solution as the sample solution. Perform the test with 1 μL of the sample solution as directed under Gas chromatography according to the following conditions, and determine the area, A, of the methyl stearate peak and the total of the areas, B, of all of fatty acid ester peaks. Calculate the percentage of stearic acid in the fatty acid fraction of Magnesium Stearate by the following formula.

\[
\text{Content(\%)} = \frac{A}{B} \times 100
\]

Similarly, calculate the percentage of palmitic acid in Magnesium Stearate. The methyl stearate peak, and the total of the methyl stearate and methyl palmitate peaks are not less than 40% and not less than 90% of the total area of all fatty acid ester peaks, respectively, in the chromatogram.

**Operating conditions—**

Detector: A hydrogen flame-ionization detector maintained at a constant temperature of about 260°C.

Sample injection port: A splitless injection system maintained at a constant temperature of about 220°C.

Column: A fused silica capillary column 0.32 mm in inside diameter and 30 m in length, the inside coated with a 0.5-μm layer of polyethylene glycol 15000-diepoxide for gas chromatography.

Column temperature: Maintain at 70°C for 2 minutes after injection, then program to increase the temperature at the rate of 5°C per minute to 240°C and to maintain this temperature for 5 minutes.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of methyl stearate is about 32 minutes.

Split ratio: Splitless.

Time span of measurement: About 1.5 time as long as the retention time of methyl stearate beginning after the solvent peak.

**System suitability—**

Test for required detection: Place exactly 50 mg each of stearic acid for gas chromatography and palmitic acid for gas chromatography, each previously dried in a desiccator (silica gel) for 4 hours, in a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and proceed in the same manner as directed for the preparation of the sample solution, and use the solution so obtained as the solution for system suitability test. To exactly 1 mL of the solution add heptane to make exactly 10 mL. Confirm that the peak area of methyl stearate obtained from 1 μL of this solution is equivalent to 5 to 15% of that from 1 μL of the solution for system suitability test.

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, methyl palmitate and methyl stearate are eluted in this order, with the relative retention time of methyl palmitate to methyl stearate being about 0.86, and with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of methyl palmitate and methyl stearate are not more than 6.0%, respectively, and the relative standard deviation of the ratios of the peak area of methyl palmitate to methyl stearate is not more than 1.0%.

**Loss on drying** Not more than 6.0% (2 g, 105°C, constant mass).

**Microbial limit** The acceptance criteria of TAMC and TYMC are 10^2 CFU/g and 5 x 10^1 CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.

**Assay** Transfer about 0.5 g of previously dried Magnesium Stearate, accurately weighed, to a 250-mL flask, add 50 mL of a mixture of 1-butanol and ethanol (99.5) (1:1), 5 mL of ammonia solution (28), 3 mL of ammonium chloride buffer solution, pH 10, 30.0 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and 1 to 2 drops of eriochrome black T TS, and mix. Heat at 45°C to 50°C to make the solution clear, and after cooling, titrate with disodium dihydrogen ethylenediamine tetraacetate with 0.1 mol/L zinc sulfate VS until the solution changes from blue to purple in color. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.431 mg of Mg

**Containers and storage** Containers—Tight containers.

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**Magnesium Sulfate Hydrate**

**MgSO₄·7H₂O: 246.47**

Magnesium Sulfate Hydrate, when ignited, contains not less than 99.0% of magnesium sulfate (MgSO₄·120.37).

**Description** Magnesium Sulfate Hydrate occurs as colorless or white crystals. It has a cooling, saline, bitter taste. It is very soluble in water, and practically insoluble in ethanol (95%). It dissolves in dilute hydrochloric acid.

**Identification** A solution of Magnesium Sulfate Hydrate (1 in 40) responds to the Qualitative Tests for magnesium and for sulfate.

pH 2.54—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the pH of this solution is between 5.0 and 8.2.

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the solution is clear and colorless.

2. Chloride—Perform the test with 1.0 g of Magnesium Sulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

3. Heavy metals—Proceed with 2.0 g of Magnesium Sulfate Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

4. Zinc—Dissolve 2.0 g of Magnesium Sulfate Hydrate in 20 mL of water, and add 1 mL of acetic acid and 5 drops of potassium hexacyanoferrate (II) TS: no turbidity is produced.
(5) Calcium—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 5.0 mL of dilute hydrochloric acid, add water to make 100 mL, and use this solution as the sample solution. Separately, dissolve 1.0 g of Magnesium Sulfate Hydrate in 2.0 mL of Standard Calcium Solution and 5.0 mL of dilute hydrochloric acid, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry \(2.23\) according to the following conditions, and determine the absorbances, \(A_T\) and \(A_S\), of both solutions: \(A_T\) is not bigger than \(A_S - 0.02\%\).

- Gas: Combustible gas—Acetylene or hydrogen.
- Lamp: Calcium hollow-cathod lamp.
- Wavelength: 422.7 nm.

(6) Arsenic \(1.11\)—Prepare the test solution with 1.0 g of Magnesium Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

**Loss on ignition** \(2.43\) 45.0 – 52.0% (1 g, after drying at 105°C for 2 hours, ignite at 450°C for 3 hours).

**Assay** Weigh accurately about 0.6 g of Magnesium Sulfate Hydrate, previously ignited at 450°C for 3 hours after drying at 105°C for 2 hours, and dissolve in 2 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonium-ammonium chloride buffer solution, pH 10.7, and titrate \(2.50\) with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 6.018 \text{ mg of MgSO}_4
\]

**Containers and storage** Containers—Well-closed containers.

**Magnesium Sulfate Injection**

硫酸マグネシウム注射液

Magnesium Sulfate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of magnesium sulfate hydrate (\(\text{MgSO}_4\cdot7\text{H}_2\text{O}\): 246.47).

**Method of preparation** Prepare as directed under Injections, with Magnesium Sulfate Hydrate.

**Description** Magnesium Sulfate Injection is a clear, colorless liquid.

**Identification** Measure a volume of Magnesium Sulfate Injection, equivalent to 0.5 g of Magnesium Sulfate Hydrate according to the labeled amount, and add water to make 20 mL: the solution responds to the Qualitative Tests \(1.09\) for magnesium salt and for sulfate.

**pH** \(2.54\) 5.5 – 7.0 When the labeled concentration exceeds 5%, prepare a solution of 5% with water, and perform the test.

**Bacterial endotoxins** \(<4.07\) Less than 0.09 EU/mg.

**Extractable volume** \(6.05\) It meets the requirement.

**Foreign insoluble matter** \(6.06\) Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** \(6.07\) It meets the requirement.

**Sterility** \(<4.06\) Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Measure exactly a volume of Magnesium Sulfate Injection, equivalent to about 0.3 g of magnesium sulfate hydrate (\(\text{MgSO}_4\cdot7\text{H}_2\text{O}\)), and add water to make 75 mL. Then add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and proceed as directed in the Assay under Magnesium Sulfate Hydrate.

\[
\text{Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 12.32 \text{ mg of MgSO}_4\cdot7\text{H}_2\text{O}
\]

**Containers and storage** Containers—Well-closed containers.

**Magnesium Sulfate Mixture**

硫酸マグネシウム水

Magnesium Sulfate Mixture contains not less than 13.5 w/v% and not more than 16.5 w/v% of magnesium sulfate hydrate (\(\text{MgSO}_4\cdot7\text{H}_2\text{O}\): 246.47).

**Method of preparation**

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium Sulfate Hydrate</td>
<td>150 g</td>
</tr>
<tr>
<td>Bitter Tincture</td>
<td>20 mL</td>
</tr>
<tr>
<td>Dilute Hydrochloric Acid</td>
<td>5 mL</td>
</tr>
<tr>
<td>Purified Water or Purified</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td>Water in Containers</td>
<td>To make 1000 mL</td>
</tr>
</tbody>
</table>

Prepare before use, with the above ingredients.

**Description** Magnesium Sulfate Mixture is a light yellowish clear liquid. It has a bitter and acid taste.

**Identification** (1) Magnesium Sulfate Mixture responds to the Qualitative Tests \(1.09\) for magnesium salt.

(2) Magnesium Sulfate Mixture responds to the Qualitative Tests \(1.09\) (2) for chloride.

**Assay** Pipet 10 mL of Magnesium Sulfate Mixture, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add 50 mL of water and 5 mL of pH 10.7 ammonium-ammonium chloride buffer solution, and titrate \(2.50\) with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

\[
\text{Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 12.32 \text{ mg of MgSO}_4\cdot7\text{H}_2\text{O}
\]

**Containers and storage** Containers—Tight containers.
Maltose Hydrate

マルトース水和物

C_{12}H_{22}O_{11}.H_2O: 360.31
α-D-Glucopyranosyl-(1→4)-β-D-glucopyranose monohydrate

Maltose Hydrate, when dried, contains not less than 98.0% of C_{12}H_{22}O_{11}.H_2O.

Description

Maltose Hydrate occurs as white crystals or crystalline powder.

It has a sweet taste.

It is freely soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification

(1) Dissolve 0.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of ammonia TS, and heat for 5 minutes on a water bath: an orange color develops.

(2) Add 2 to 3 drops of a solution of Maltose Hydrate (1 in 50) to 5 mL of boiling Fehling TS: a red precipitate is formed.

Optical rotation

$\lbrack \alpha \rbrack_{D}^n = +126^\circ - +131^\circ$ Weigh accurately about 10 g of Maltose Hydrate, previously dried, dissolve in 0.2 mL of ammonia TS and water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

pH

$<2.54$ The pH of a solution of Maltose Hydrate (1 in 10) is between 4.5 and 6.5.

Purity

(1) Clarity and color of solution—Put 10 g of Maltose Hydrate in 30 mL of water in a Nessler tube, warm at 60°C in a water bath to dissolve, and after cooling, add water to make 50 mL: the solution is clear, and has no more color than the following control solution.

Control solution: Add water to a mixture of 1.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS to make 10.0 mL. To 1.0 mL of this solution add water to make 50 mL.

(2) Chloride $<1.05$—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Sulfate $<1.14$—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(4) Heavy metals $<1.07$—Proceed with 5.0 g of Maltose Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).

(5) Arsenic $<1.12$—Dissolve 1.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, heat on a water bath for 5 minutes, then heat to concentrate to 5 mL, and use this solution as the test solution after cooling. Perform the test (not more than 1.3 ppm).

(6) Dextrin, soluble starch and sulfite—Dissolve 1.0 g of Maltose Hydrate in 10 mL of water, and add 1 drop of iodine TS: a yellow color appears, and the color changes to a blue by adding 1 drop of starch TS.

(7) Nitrogen—Weigh accurately about 2 g of Maltose Hydrate, and perform the test as directed under Nitrogen Determination $<1.05>$ using 10 mL of sulfuric acid for the decomposition and 45 mL of a solution of sodium hydroxide (2 in 5) for the addition: the amount of nitrogen (N: 14.01) is not more than 0.01%.

(8) Related substances—Dissolve 0.5 g of Maltose Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following operating conditions. Determine the peak areas from both solutions by the automatic integration method: the total area of the peaks which appear before the peak of maltose from the standard solution is not larger than 1.5 times the peak area of maltose from the sample solution, and the total area of the peaks which appear after the peak of maltose from the sample solution is not larger than 1/2 times the peak area of maltose from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the sensitivity so that the peak height of maltose obtained from 20 μL of the standard solution is about 30 mm.

Time span of measurement: About 2 times as long as the retention time of maltose.

Loss on drying $<2.41$ Not more than 0.5% (1 g, 80°C, 4 hours).

Residue on ignition $<2.44$ Not more than 0.1% (1 g).

Assay

Weigh accurately about 0.1 g each of Maltose Hydrate and Maltose RS, previously dried, dissolve in exactly 10 mL each of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following operating conditions, and calculate the ratios, $Q_1$ and $Q_2$, of the peak area of maltose to that of the internal standard.

Amount (mg) of C_{12}H_{22}O_{11}.H_2O

$= M_S \times Q_2/Q_1$

$M_S$: Amount (mg) of Maltose RS

Internal standard solution—A solution of ethylene glycol (1 in 50).

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column about 8 mm in inside diameter and about 55 cm in length, packed with gel-type strong acid cation-exchange resin for liquid chromatography (degree of cross-linking: 8%) (10 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Water.

Flow rate: Adjust the flow rate so that the retention time
of maltose is about 18 minutes.

Selection of column: Dissolve 0.25 g of maltose, 0.25 g of glucose and 0.4 g of ethylene glycol in water to make 100 mL. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of maltose, glucose and ethylene glycol in this order with the resolution of between the peaks of maltose and glucose being not less than 4.

Containers and storage Containers—Tight containers.

Freeze-dried Mamushi Antivenom, Equine

乾燥まむしウマ抗毒素

Freeze-dried Mamushi Antivenom, Equine, is a preparation for injection which is dissolved before use. It contains Agkistrodon Halys antivenin in immunoglobulin of horse origin. It conforms to the requirements of Freeze-dried Mamushi Antivenom, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Mamushi Antivenom, Equine, becomes a colorless or light yellow-brown, clear liquid, or a slightly white-turbid liquid on addition of solvent.

Manidipine Hydrochloride

マンジピン塩酸塩

\[
\text{Manidipine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of } C_{35}H_{38}N_4O_6 \cdot 2HCl. \\
\text{Description Manidipine Hydrochloride occurs as white to pale yellow crystals or crystalline powder. It is freely soluble in dimethylsulfoxide, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water. A solution of Manidipine Hydrochloride in dimethylsulfoxide (1 in 100) shows no optical rotation. Manidipine Hydrochloride turns slightly brown-yellowish white on exposure to light. Melting point: about 207°C (with decomposition). Identification (1) Determine the absorption spectrum of a solution of Manidipine Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Manidipine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. (2) Determine the infrared absorption spectrum of Manidipine Hydrochloride as directed in the potassium chloride disc method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Manidipine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. (3) Add 10 mL of water to 0.1 g of Manidipine Hydrochloride, shake vigorously, and filter. Add 1 drop of ammonia TS to 3 mL of the filtrate, allow to stand 5 minutes, and filter. The filtrate responds to the Qualitative Tests <1.09> (2) for chlorides. Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Manidipine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm). (2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Manidipine Hydrochloride according to Method 4, and perform the test (not more than 1 ppm). (3) Related substances—Dissolve 20 mg of Manidipine Hydrochloride in 200 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the area of the peaks other than manidipine obtained from the sample solution is not larger than 1/5 times the manidipine peak area from the standard solution. Furthermore, the total of the areas of all peaks other than manidipine is not larger than 7/10 times the peak area of manidipine from the standard solution. Operating conditions—Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay. Time span of measurement: About 3.5 times as long as the retention time of manidipine, beginning after the solvent peak. System suitability—Test for required detectability: Pipet 10 mL of the standard solution, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. Confirm that the peak area of manidipine obtained from 20 μL of this solution is equivalent to 8 to 12% of that from 20 μL of the standard solution. System performance: Dissolve 50 mg of Manidipine Hydrochloride in a mixture of water and acetonitrile (1:1) to make 50 mL. To 10 mL of this solution add 5 mL of a solution of butyl benzoate in acetonitrile (7 in 5000) and the mixture of water and acetonitrile (1:1) to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, manidipine and butyl benzoate
are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of manidipine is not more than 2.0%.

**Loss on drying**<sup>2.41</sup> Not more than 1.5% (1 g, 105°C, 4 hours).

**Residue on ignition**<sup>2.44</sup> Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.1 g of Manidipine Hydrochloride, previously dried, and dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, and dissolve in the mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography<sup>2.01</sup> according to the following conditions, and calculate the ratios, Q<sub>1</sub> and Q<sub>0</sub>, of the peak area of manidipine to that of the internal standard.

\[
M_S = \frac{M_S}{Q_1/Q_0} \times 4
\]

where

- \(M_S\): Amount (mg) of Manidipine Hydrochloride RS
- \(Q_1\): Area of manidipine
- \(Q_0\): Area of the internal standard

\(M_S\) calculated from the standard solution show the same R<sub>f</sub> value.

**Uniformity of dosage units**<sup>6.02</sup> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Manidipine Hydrochloride Tablets, add exactly 1 mL of the internal standard solution per 1 mg of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl), disintegrate by adding a mixture of water and acetonitrile (1:1) to make 50 mL and adjust to pH 4.6 with diluted potassium hydroxide TS (1 in 10). To 490 mL of this mixture add 5 mL of the internal standard solution and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

\[
M_S = \frac{M_S}{Q_1/Q_0} \times V/250
\]

where

- \(M_S\): Amount (mg) of Manidipine Hydrochloride RS
- \(Q_1\): Area of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl)
- \(Q_0\): Area of the internal standard
- \(V\): Volume of the filtrate

**Manidipine Hydrochloride Tablets**

Manidipine Hydrochloride Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl: 683.62).

**Method of preparation** Prepare as directed under Tablets, with Manidipine Hydrochloride.

**Identification** To a quantity of powdered Manidipine Hydrochloride Tablets, equivalent to 10 mg of Manidipine Hydrochloride according to the labeled amount, add 5 mL of methanol, shake vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Manidipine Hydrochloride RS in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography<sup>2.03</sup>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diethylamine (200:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same \(R_f\) value.

**Containers and storage** Containers—Light-resistant.

\(M_S\) calculated from the standard solution show the same \(R_f\) value.

**Manidipine Hydrochloride Tablets**

マニジピン塩酸塩

Manidipine Hydrochloride Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl: 683.62).

**Method of preparation** Prepare as directed under Tablets, with Manidipine Hydrochloride.

**Identification** To a quantity of powdered Manidipine Hydrochloride Tablets, equivalent to 10 mg of Manidipine Hydrochloride according to the labeled amount, add 5 mL of methanol, shake vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Manidipine Hydrochloride RS in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography<sup>2.03</sup>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diethylamine (200:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same \(R_f\) value.

**Uniformity of dosage units**<sup>6.02</sup> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Manidipine Hydrochloride Tablets, add exactly 1 mL of the internal standard solution per 1 mg of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl), disintegrate by adding a mixture of water and acetonitrile (1:1) to make 50 mL so that each mL contains about 0.1 mg of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl), shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

\[
M_S = \frac{M_S}{Q_1/Q_0} \times V/250
\]

where

- \(M_S\): Amount (mg) of Manidipine Hydrochloride RS
- \(Q_1\): Area of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl)
- \(Q_0\): Area of the internal standard
- \(V\): Volume of the filtrate

**Manidipine Hydrochloride Tablets**

マニジピン塩酸塩

Manidipine Hydrochloride Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl: 683.62).

**Method of preparation** Prepare as directed under Tablets, with Manidipine Hydrochloride.

**Identification** To a quantity of powdered Manidipine Hydrochloride Tablets, equivalent to 10 mg of Manidipine Hydrochloride according to the labeled amount, add 5 mL of methanol, shake vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Manidipine Hydrochloride RS in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography<sup>2.03</sup>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diethylamine (200:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same \(R_f\) value.

**Uniformity of dosage units**<sup>6.02</sup> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Manidipine Hydrochloride Tablets, add exactly 1 mL of the internal standard solution per 1 mg of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl), disintegrate by adding a mixture of water and acetonitrile (1:1) to make 50 mL so that each mL contains about 0.1 mg of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl), shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

\[
M_S = \frac{M_S}{Q_1/Q_0} \times V/250
\]

where

- \(M_S\): Amount (mg) of Manidipine Hydrochloride RS
- \(Q_1\): Area of manidipine hydrochloride (C<sub>35</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>·2HCl)
- \(Q_0\): Area of the internal standard
- \(V\): Volume of the filtrate

**Manidipine Hydrochloride Tablets**

マニジピン塩酸塩
first 10 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, and add the dissolution medium to make exactly $V$ mL so that each mL contains about 5.6 $\mu$g of manidipine hydrochloride ($C_{35}H_{38}N_4O_6 \cdot 2HCl$) according to the labeled amount. Pipet 2 mL of this solution, add exactly 2 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of methanol, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography. Weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of methanol, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography ($<2.0\%$) according to the following conditions, and determine the manidipine peak areas, $A_1$ and $A_2$, of both solutions.

Dissolution rate (%) with respect to the labeled amount of manidipine hydrochloride ($C_{35}H_{38}N_4O_6 \cdot 2HCl$)

$$M_\text{S} = \frac{M_\text{S} \times A_1}{A_2} \times \frac{V_1}{V_2} \times \frac{1}{C} \times 18$$

$M_\text{S}$: Amount (mg) of Manidipine Hydrochloride RS

C: Labeled amount (mg) of manidipine hydrochloride ($C_{35}H_{38}N_4O_6 \cdot 2HCl$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and a solution of potassium dihydrogen phosphate (681 in 10,000) (3:2).

Flow rate: Adjust the flow rate so that the retention time of manidipine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of manidipine are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of manidipine is not more than 2.0%.

Assay Conduct this procedure using light-resistant vessels. Weigh accurately not less than 20 Manidipine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6 \cdot 2HCl$), add exactly 10 mL of the internal standard solution, add a mixture of water and acetonitrile (1:1) to make 100 mL, shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 $\mu$m. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, and dissolve in the mixture of water and acetonitrile (1:1) to make 50 mL. Pipet 20 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, and use this solution as the standard solution.

Then, proceed as directed in the Assay under Manidipine Hydrochloride.

Amount (mg) of manidipine hydrochloride ($C_{35}H_{38}N_4O_6 \cdot 2HCl$)

$$M_\text{S} = \frac{M_\text{S} \times Q_1/Q_2 \times 2/5}{M_\text{S}}$$

$M_\text{S}$: Amount (mg) of Manidipine Hydrochloride RS

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 10,000).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

d-Mannitol

D-Mannitol, when dried, contains not less than 98.0% of $C_6H_{14}O_6$.

Description D-Mannitol occurs as white crystals or powder. It is odorless, and has a sweet taste with a cold sensation.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) To 5 drops of a saturated solution of d-Mannitol add 1 mL of iron (III) chloride TS and 5 drops of a solution of sodium hydroxide (1 in 5): a yellow precipitate is produced. Shake this solution vigorously: a clear solution is produced. On addition of a solution of sodium hydroxide (1 in 5), no precipitate is produced.

(2) Determine the infrared absorption spectrum of d-Mannitol as directed in the potassium bromide disk method under Infrared Spectrophotometry ($<2.25\%$), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 1 g of d-Mannitol in 3 mL of warm water, then allow to stand at 5°C for 24 hours or until crystals appear, and filter. Wash the crystals so obtained with a few amount of cold water, dry at 105°C for 4 hours, and perform the test with the crystals.

Optical rotation $<2.49$ [α]$^\text{D}$: +137 ~ +145° Weigh accurately about 1.0 g of d-Mannitol, previously dried, dissolve in 80 mL of a solution of hexammonium heptamolybdate tetrahydrate (1 in 20), and add diluted sulfuric acid (1 in 35) to make exactly 100 mL. Measure the optical rotation of this solution in a 100-mm cell.

Melting point $<2.60\%$ 166 ~ 169°C

Purity (1) Clarity and color of solution—Dissolve 2.0 g of d-Mannitol in 10 mL of water by warming: the solution is clear and colorless.
Acidity—Dissolve 5.0 g of d-Mannitol in 50 mL of freshly boiled and cooled water, and add 1 drop of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS; a red color develops.

Chloride <4.03>—Perform the test with 2.0 g of d-Mannitol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

Sulfate <1.14>—Perform the test with 2.0 g of d-Mannitol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

Heavy metals <1.07>—Proceed with 5.0 g of d-Mannitol according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

Nickel—Dissolve 0.5 g of d-Mannitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color develops.

Arsenic <1.11>—Prepare the test solution with 1.5 g of d-Mannitol according to Method 1, and perform the test (not more than 1.3 ppm).

Sugars—To 5.0 g of d-Mannitol add 15 mL of water and 4.0 mL of dilute hydrochloric acid, and heat under a reflux condenser in a water bath for 3 hours. After cooling, neutralize with sodium hydroxide TS (indicator: 2 drops of methyl orange TS), and add water to make 50 mL. Pipet 10 mL of this solution into a flask, boil gently with 10 mL of water and 40 mL of Fehling’s TS for 3 minutes, and allow to stand to precipitate copper (I) oxide. Filter the supernatant liquid through a glass filter (G4), wash the precipitate with hot water until the last washing no longer shows an alkaline reaction, and filter the washings through the glass filter described above. Dissolve the precipitate in 20 mL of iron (III) sulfate TS in the flask, filter through the glass filter described above, and wash the filter with water. Combine the washings and the filtrate, heat to 80°C, and titrate <2.50> with 0.02 mol/L potassium permanganate VS: the consumed volume is not more than 1.0 mL.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of d-Mannitol, previously dried, and dissolve in water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. After cooling, add 2.5 g of potassium iodide, stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS = 1.822 mg of C₆H₁₂O₆

Containers and storage Containers—Tight containers.

D-Mannitol Injection

D-Mannite Injection

D-マンニトール注射液

D-Mannitol Injection is an aqueous solution for injection. It contains not less than 95.0% and not more than 105.0% of the labeled amount of d-mannitol (C₆H₁₂O₆; 182.17).

Method of preparation Prepare as directed under Injections, with d-Mannitol. No preservative is added.

Description D-Mannitol Injection is a clear, colorless liquid. It has a sweet taste. It may precipitate crystals.

Identification Concentrate d-Mannitol Injection on a water bath to make a saturated solution. Proceed with 5 drops of this solution as directed in the Identification (1) under d-Mannitol.

pH <2.54> 4.5 – 7.0

Bacterial endotoxins <4.01> Less than 0.50 EU/mL.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of d-Mannitol Injection, equivalent to about 5 g of d-mannitol (C₆H₁₂O₆), and add water to make exactly 250 mL. To exactly 10 mL of this solution add water to make exactly 100 mL. Measure exactly 10 mL of this solution into an iodine flask, and proceed as directed in the Assay under d-Mannitol.

Each mL of 0.1 mol/L sodium thiosulfate VS = 1.822 mg of C₆H₁₂O₆

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.
Maprotiline Hydrochloride

マプロチリン塩酸塩

C₂₀H₂₃N•HCl: 313.86
3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-N-methylpropylamine monohydrochloride
[10347-81-6]

Maprotiline Hydrochloride, when dried, contains not less than 99.0% of C₂₀H₂₃N•HCl.

Description Maprotiline Hydrochloride occurs as a white crystalline powder.
It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water.
Melting point: about 244°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Maprotiline Hydrochloride in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Maprotiline Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Maprotiline Hydrochloride with ethanol (99.5), filter, dry the crystals so obtained, and perform the test with the crystals.

(3) To 5 mL of a solution of Maprotiline Hydrochloride (1 in 200) add 2 mL of ammonia TS, heat on a water bath for 5 minutes, cool, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Maprotiline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Maprotiline Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of 2-butanol, diluted ammonia solution (28) (1 in 3) and ethyl acetate (14:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 2 and they are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Maprotiline Hydrochloride, previously dried, dissolve in 180 mL of acetic acid (100), add 8 mL of a solution of bismuth nitrate pentahydrate in acetic acid (100) (1 in 50), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.39 mg of C₂₀H₂₃N•HCl

Containers and storage Containers—Well-closed containers.

Freeze-dried Live Attenuated Measles Vaccine

乾燥弱毒麻しんワクチン

Freeze-dried Live Attenuated Measles Vaccine is a preparation for injection which is dissolved before use.
It contains live attenuated measles virus.
It conforms to the requirements of Freeze-dried Live Attenuated Measles Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Live Attenuated Measles Vaccine becomes a colorless, yellowish or reddish clear liquid on addition of solvent.

Meclofenoxate Hydrochloride

メクロフェノキサート塩酸塩

C₁₂H₁₆ClNO₃•HCl: 294.17
2-(Dimethylamino)ethyl (4-chlorophenoxy)acetate monohydrochloride
[3685-84-5]

Meclofenoxate Hydrochloride contains not less than 98.0% of C₁₂H₁₆ClNO₃•HCl, calculated on the anhydrous basis.

Description Meclofenoxate Hydrochloride occurs as white crystals or crystalline powder. It has a faint, characteristic odor and a bitter taste.
It is freely soluble in water and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.
The pH of a solution of Meclofenoxate Hydrochloride (1 in 20) is between 3.5 and 4.5.

Identification (1) To 10 mg of Meclofenoxate Hydrochloro-
Mecobalamin

メコバラミン

C₅₃H₉₁CoN₁₃O₁₄P: 1344.38

Mecobalamin contains not less than 98.0% of C₅₃H₉₁CoN₁₃O₁₄P, calculated on the anhydrous basis.

Description Mecobalamin occurs as dark red crystals or crystalline powder.
It is sparingly soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetone and in acetonitrile.
It is affected by light.

Identification (1) Conduct this procedure without exposure to light, using light-resistant vessels. Determine the absorption spectrum of a solution of Mecobalamin in hydrochloric acid-potassium chloride buffer solution, pH 2.0 (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Mecobalamin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Mecobalamin in phosphate buffer solution, pH 7.0 (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Mecobalamin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Mecobalamin with 50 mg of potassium bisulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.
Purity (1) Clarity and color of solution—Dissolve 20 mg of Mecobalamin in 10 mL of water: the solution is clear and red color.

(2) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography according to the following conditions. Determine the peak area of Mecobalamin and others of the sample solution by the automatic integration method: each area of the peaks other than Mecobalamin is not more than 0.5% of the peak area of Mecobalamin, and the total area of the peaks other than Mecobalamin is not more than 2.0%.

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of Mecobalamin.

System suitability—
Test for required detection: To exactly 1 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add the mobile phase to make exactly 10 mL, and confirm that the peak area of Mecobalamin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of Mecobalamin is not more than 3.0%.

Water <2.48> Not more than 12% (0.1 g, volumetric titration, direct titration).

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 50 mg of Mecobalamin and Mecobalamin RS (separately, determine the water in the same manner as Mecobalamin), dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A₁ and A₅, of Mecobalamin in each solution.

\[ M₅ = \frac{A₅}{A₁} \]

M₅: Amount (mg) of Mecobalamin RS, calculated on the anhydrous basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 266 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To 200 mL of acetonitrile add 800 mL of 0.02 mol/L phosphate buffer solution, pH 3.5, then add 3.76 g of sodium 1-hexane sulfonate to dissolve.

Flow rate: Adjust the flow rate so that the retention time of Mecobalamin is about 12 minutes.

System suitability—
System performance: Dissolve 5 mg each of cyanocobalamin and hydroxocobalamin acetate in the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cyanocobalamin and hydroxocobalamin are eluted in this order with the resolution between these peaks being not less than 3. And when the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of Mecobalamin is not less than 6000.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of Mecobalamin is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Medazepam

C₁₆H₁₂ClIN₂: 270.76
7-Chloro-1-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepine [2898-12-6]

Medazepam, when dried, contains not less than 98.5% and not more than 101.0% of C₁₆H₁₂ClIN₂.

Description Medazepam occurs as white to light yellow crystals or crystalline powder.

It is freely soluble in methanol, in ethanol (99.5), in acetic acid (100) and in diethyl ether, and practically insoluble in water.

It gradually turns yellow on exposure to light.

Identification (1) Dissolve 10 mg of Medazepam in 3 mL of citric acid-acetic acid TS: a deep orange color develops. Heat in a water bath for 3 minutes: the color changes to dark red.

(2) Determine the absorption spectrum of a solution of Medazepam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Medazepam as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave.
numbers.

(4) Perform the test with Medazepam as directed under Flame Coloration Test \(<1.04\) (2); a green color appears.

**Melting point** \(<2.60\> 101 - 104°C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Medazepam in 10 mL of methanol; the solution is clear and light yellow to yellow in color.

(2) Chloride \(<1.03\>—Dissolve 1.5 g of Medazepam in 50 mL of diethyl ether, add 46 mL of water and 4 mL of sodium carbonate TS, shake, and collect the water layer. Wash the water layer with two 20-mL portions of diethyl ether, and filter. To 20 mL of the filtrate add dilute nitric acid to neutralize, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Heavy metals \(<1.07\>—Proceed with 1.0 g of Medazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic \(<1.11\>—Prepare the test solution with 1.0 g of Medazepam according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.25 g of Medazepam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.60\>$. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and ammonia solution (28:60:40:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.41\> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

**Residue on ignition** \(<2.44\> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Medazepam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate \(<2.50\> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 27.08 \text{ mg of C}_{16}H_{15}ClN_2
\]

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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### Medicinal Carbon

**Description** Medicinal Carbon occurs as a black, odorless and tasteless powder.

**Identification** Place 0.5 g of Medicinal Carbon in a test tube, and heat by direct application of flame with the aid of a current of air: it burns without any flame. Pass the evolved gas through calcium hydroxide TS: a white turbidity is produced.

**Purity (1)** Acidity or alkalinity—Boil 3.0 g of Medicinal Carbon with 60 mL of water for 5 minutes, allow to cool, dilute to 60 mL with water, and filter: the filtrate is colorless and neutral.

(2) Chloride \(<1.03\>—Take 4.0 mL of the filtrate obtained in (1) in a Nessler tube, add 6 mL of dilute nitric acid and sufficient water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142%).

(3) Sulfate \(<1.14\>—Take 5 mL of the filtrate obtained in (1) in a Nessler tube, add 1 mL of dilute hydrochloric acid and sufficient water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.192%).

(4) Sulfide—Boil 0.5 g of Medicinal Carbon with a mixture of 15 mL of dilute hydrochloric acid and 10 mL of water: lead (II) acetate paper does not become brown when held in the evolved gas within 5 minutes.

(5) Cyanogen compounds—Place a mixture of 5 g of Medicinal Carbon, 2 g of tartaric acid and 50 mL of water in a distilling flask connected to a condenser provided with a tightly fitting adapter, the end of which dips below the surface of a mixture of 2 mL of sodium hydroxide TS and 10 mL of water, contained in a small flask surrounded by ice. Heat the mixture in the distilling flask to boiling, and distill to 25 mL. Dilute the distillate with water to 50 mL. To 25 mL of the diluted distillate add 1 mL of a solution of iron (II) sulfate heptahydrate (1 in 20), heat the mixture almost to boiling, cool, and filter. To the filtrate add 1 mL of hydrochloric acid and 0.5 mL of dilute iron (III) chloride TS: no blue color is produced.

(6) Acid soluble substances—To about 1 g of Medicinal Carbon, accurately weighed, add 20 mL of water and 5 mL of hydrochloric acid, boil for 5 minutes, filter, wash the residue with 10 mL of hot water, and add the washings to the filtrate. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite the residue strongly: the mass of the residue is not more than 3.0%.

(7) Heavy metals \(<1.07\>—Proceed with 0.5 g of Medicinal Carbon according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(8) Zinc—Ignite 0.5 g of Medicinal Carbon to ash, add 5 mL of dilute nitric acid to the residue, boil gently for 5 minutes, filter, wash with 10 mL of water, and combine the washings and the filtrate. Add 3 mL of ammonia TS to the solution, filter again, wash with water, combine the washings and the filtrate, add another washing to make 25 mL,
add 1 drop of sodium sulfide TS, and allow to stand for 3 minutes: the liquid produces no turbidity.

(9) Arsenic <1.11>-Prepare the test solution with 1.0 g of Medicinal Carbon according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 15.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 4.0% (1 g).

Adsorptive power (1) Add 1.0 g of Medicinal Carbon, previously dried, to 100 mL of water containing 120 mg of quinine sulfate, shake the mixture vigorously for 5 minutes, filter immediately, and reject the first 20 mL of the filtrate. Add 5 drops of iodine TS to 10 mL of the subsequent filtrate: no turbidity is produced.

(2) Dissolve 250 mg of methylene blue trihydrate, exactly weighed, in water to make exactly 250 mL. Measure two 50-mL portions of this solution into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Filter the contents of each flask, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrate into two 250-mL volumetric flasks. To each volumetric flask add 50 mL of a solution of sodium acetate trihydrate (1 in 10), then add exactly 35 mL of 0.05 mol/L iodine metric flask add 50 mL of a solution of sodium acetate trihydrate: no turbidity is produced.

Add 5 drops of iodine TS to 10 mL of the subsequent filtrate. Pipet 25-mL portions of the remaining filtrate into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Dilute each mixture to exactly 250 mL with water, allow to stand for 10 minutes, and filter immediately, and reject the first 20 mL of the filtrate. Add 5 drops of iodine TS to 10 mL of the subsequent filtrate: no turbidity is produced.

Residue on ignition <2.44> Not more than 4.0% (1 g).

Adsorptive power (1) Add 1.0 g of Medicinal Carbon, previously dried, to 100 mL of water containing 120 mg of quinine sulfate, shake the mixture vigorously for 5 minutes, filter immediately, and reject the first 20 mL of the filtrate. Add 5 drops of iodine TS to 10 mL of the subsequent filtrate: no turbidity is produced.

(2) Dissolve 250 mg of methylene blue trihydrate, exactly weighed, in water to make exactly 250 mL. Measure two 50-mL portions of this solution into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Filter the contents of each flask, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrate into two 250-mL volumetric flasks. To each volumetric flask add 50 mL of a solution of sodium acetate trihydrate (1 in 10), then add exactly 35 mL of 0.05 mol/L iodine VS with swirling. Allow them to stand for 50 minutes, shaking vigorously from time to time. Dilute each mixture to exactly 250 mL with water, allow to stand for 10 minutes, and filter each solution at a temperature not exceeding 20°C, rejecting the first 30 mL of each filtrate. Titrate <2.50> the excess iodine in a 100-mL aliquot of each filtrate with 0.1 mol/L sodium thiosulfate VS: a red color develops.

Add 5 drops of iodine TS to 10 mL of the subsequent filtrate. Pipet 25-mL portions of the remaining filtrate into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Dilute each mixture to exactly 250 mL with water, allow to stand for 10 minutes, and filter each solution at a temperature not exceeding 20°C, rejecting the first 30 mL of each filtrate. Titrate <2.50> the excess iodine in a 100-mL aliquot of each filtrate with 0.1 mol/L sodium thiosulfate VS: a red color develops.

Add 5 drops of iodine TS to 10 mL of the subsequent filtrate. Pipet 25-mL portions of the remaining filtrate into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Filter the contents of each flask, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrate into two 250-mL volumetric flasks. To each volumetric flask add 50 mL of a solution of sodium acetate trihydrate (1 in 10), then add exactly 35 mL of 0.05 mol/L iodine VS with swirling. Allow them to stand for 50 minutes, shaking vigorously from time to time. Dilute each mixture to exactly 250 mL with water, allow to stand for 10 minutes, and filter each solution at a temperature not exceeding 20°C, rejecting the first 30 mL of each filtrate. Titrate <2.50> the excess iodine in a 100-mL aliquot of each filtrate with 0.1 mol/L sodium thiosulfate VS: a red color develops.

Add 5 drops of iodine TS to 10 mL of the subsequent filtrate. Pipet 25-mL portions of the remaining filtrate into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Filter the contents of each flask, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrate into two 250-mL volumetric flasks. To each volumetric flask add 50 mL of a solution of sodium acetate trihydrate (1 in 10), then add exactly 35 mL of 0.05 mol/L iodine VS with swirling. Allow them to stand for 50 minutes, shaking vigorously from time to time. Dilute each mixture to exactly 250 mL with water, allow to stand for 10 minutes, and filter each solution at a temperature not exceeding 20°C, rejecting the first 30 mL of each filtrate. Titrate <2.50> the excess iodine in a 100-mL aliquot of each filtrate with 0.1 mol/L sodium thiosulfate VS: a red color develops.

Water-insoluble substances—Wash thoroughly the dried substances obtained in (3) with 200 mL of water, and dry at 105°C for 4 hours: the mass of the residue is not more than 0.15%.

(5) Alkali carbonates—To the washings obtained in (4) add 3 drops of methyl orange TS and 2 mL of 0.05 mol/L sulfuric acid VS: a red color develops.

Loss on drying Not more than 5.0% in the case of the powder, and not more than 10.0% in the case of the granules.

Weigh accurately about 0.5 g of Medicinal Soap in a tared beaker, add 10 g of sea sand (No. 1), previously dried at 105°C for 1 hour, and again weigh the beaker. Add 10 mL of ethanol (95), evaporate on a water bath to dryness with thorough stirring, and dry at 105°C for 3 hours.

Containers and storage Containers—Well-closed containers.

Medicinal Soap薬用石ケン

Medicinal Soap occurs as white to light yellow powder or granules. It has a characteristic odor free from rancidity.

Medicinal Soap is sparingly soluble in water, and slightly soluble in ethanol (95).

A solution of Medicinal Soap (1 in 100) is alkaline.

Fatty acid Dissolve 25 g of Medicinal Soap in 300 mL of hot water, add 60 mL of dilute sulfuric acid slowly, and warm in a water bath for 20 minutes. After cooling, filter off the precipitate, and wash with warm water until the washing no longer shows acidity to methyl orange TS. Transfer the precipitate to a small beaker, and heat on a water bath to complete separation of water and transparent fatty acids. Filter the fatty acid into a small beaker while warm, dry at 100°C for 20 minutes, and perform the test with this material as directed under Fats and Fatty Oils <1.13>. The congealing point of the fatty acid is between 18°C and 28°C. The acid value is 185 – 205. The iodine value is 82 – 92.

Purity (1) Acidity or alkalinity—Dissolve 5.0 g of Medicinal Soap in 85 mL of neutralized ethanol by warming on a water bath, filter while hot through absorbent cotton, and wash the filter and the residue with three 5-mL portions of hot neutralized ethanol. Combine the filtrate and the washings, add hot neutralized ethanol to make exactly 100 mL, and perform the following tests quickly using this as the sample solution at 70°C.

(i) Add 3 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS to 40 mL of the sample solution: a red color develops.

(ii) Add 3 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid VS to 40 mL of the sample solution: no red color develops.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Medicinal Soap according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Ethanol-insoluble substances—Weigh accurately about 2 g of Medicinal Soap, dissolve by warming in 100 mL of neutralized ethanol, filter the solution through a glass filter (G4), wash the residue with hot neutralized ethanol, and dry at 105°C for 4 hours: the mass of the residue is not more than 1.0%.

(4) Water-insoluble substances—Wash thoroughly the dried substances obtained in (3) with 200 mL of water, and dry at 105°C for 4 hours: the mass of the residue is not more than 0.15%.

(5) Alkali carbonates—To the washings obtained in (4) add 3 drops of methyl orange TS and 2 mL of 0.05 mol/L sulfuric acid VS: a red color develops.

Containers and storage Containers—Well-closed containers.

Mefenamic Acidメフェナム酸

Mefenamic Acid, when dried, contains not less than 99.0% of C₁₅H₁₅NO₂.

Description Mefenamic Acid occurs as a white to light yellow powder. It is odorless and tasteless at first, but leaves a slightly bitter aftertaste.
It is sparingly soluble in diethyl ether, slightly soluble in methanol, in ethanol (95) and in chloroform, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 225°C (with decomposition).

Identification (1)
Dissolve 10 mg of Mefenamic Acid in 1 mL of methanol by warming, cool, add 1 mL of a solution of p-nitrobenzene diazonium fluoroborate (1 in 1000) and 1 mL of sodium hydroxide TS, and mix thoroughly: an orange-red color is produced.

(2) Dissolve 10 mg of Mefenamic Acid in 2 mL of sulfuric acid, and heat: the solution shows a yellow color and a green fluorescence.

(3) Dissolve 7 mg of Mefenamic Acid in a solution of hydrochloric acid in methanol (1 in 1000) to make 500 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2,24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1)
Chloride <1.03—To 1.0 g of Mefenamic Acid add 20 mL of sodium hydroxide TS, and dissolve by warming. Cool, add 2 mL of acetic acid (100) and water to make 100 mL, and mix well. Remove the produced precipitate by filtration, discard the first 10 mL of the filtrate, and to subsequent 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 0.5 mL of acetic acid (100), 6 mL of nitric acid and water to make 50 mL (not more than 0.071%).

(2) Heavy metals <1.07—Proceed with 2.0 g of Mefenamic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11—Prepare the test solution with 1.0 g of Mefenamic Acid according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Mefenamic Acid, in 5 mL of a mixture of chloroform and methanol (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (3:1) to make exactly 200 mL, pipet 10 mL of this solution, add a mixture of chloroform and methanol (3:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2,03>. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-methyl-1-propanol and ammonia solution (28) (3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm) the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2,41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2,44> Not more than 0.1% (1 g).

Assay
Weigh accurately about 0.5 g of Mefenamic Acid, previously dried, and dissolve in 100 mL of ethanol (95), previously neutralized to phenol red TS with 0.1 mol/L sodium hydroxide VS, by warming gently. Cool, and titrate <2,56> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through yellow-red to red-purple (indicator: 2 to 3 drops of phenol red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 0.4280 g of C_{17}H_{16}F_{6}N_{2}O.HCl.

Containers and storage
Containers—Well-closed containers.

Mefloquine Hydrochloride
メフロキン塩酸塩

C_{17}H_{16}F_{6}N_{2}O.HCl: 414.77
(1RS)-[2,8-Bis(trifluoromethyl)quinolin-4-yl][2SR]-piperidin-2-yl)methanol monohydrochloride [51773-92-3]

Mefloquine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C_{17}H_{16}F_{6}N_{2}O.HCl.

Description
Mefloquine Hydrochloride occurs as white crystals or a white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and slightly soluble in water.

It dissolves in sulfuric acid.

A solution of Mefloquine Hydrochloride in methanol (1 in 20) shows no optical rotation.

Melting point: about 260°C (with decomposition).

Identification (1)
Dissolve 2 mg of Mefloquine Hydrochloride in 1 mL of sulfuric acid: the solution shows a blue fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Mefloquine Hydrochloride in methanol (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry (2,24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Mefloquine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry (2,25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 5 mL of a solution of Mefloquine Hydrochloride (1 in 1000) add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is formed, and the separated precipitate dissolves on the addition of an excess amount of
ammonia TS.

**Purity** (1) Heavy metals <1.0>:—Proceed with 1.0 g of Mefloquine Hydrochloride according to Method 2 using a quartz crucible, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.1>—To 1.0 g of Mefloquine Hydrochloride add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, gradually heat, and incinerate by ignition at 800°C. If a carbonized residue still retains, moisten the residue with a little amount of nitric acid, and ignite again to incinerate. After cooling, to the residue add 3 mL of hydrochloric acid, warm on a water bath to dissolve, and perform the test using this solution as the test solution (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Mefloquine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than mefloquine and the peak eluted first from the sample solution is not larger than the peak area of mefloquine from the standard solution, and the total area of the peaks other than the peak of mefloquine and the peak eluted first from the sample solution is not larger than 2.5 times the peak area of mefloquine from the standard solution.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 282 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 14) (24:1).
Flow rate: Adjust the flow rate so that the retention time of mefloquine is about 10 minutes.
Time span of measurement: About 3 times as long as the retention time of mefloquine.

**System suitability**—
Test for required detectability: To exactly 10 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of mefloquine obtained with 10 μL of this solution is equivalent to 40 to 60% of that with 10 μL of the standard solution.
System performance: Dissolve 10 mg of mefloquine hydrochloride and 5 mg of diprophylline in 50 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, diprophylline and mefloquine are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mefloquine is not more than 2.0%.

(4) Residual solvent—Being specified separately.

**Loss on drying** <2.4>—Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.4>—Not more than 0.1% (1 g, platinum crucible).

**Assay** Weigh accurately about 0.5 g of Mefloquine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.3D> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 41.48 mg of C17H16F6N2O.HCl

**Containers and storage**—Well-closed containers.

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**Mefruside**

メフルシド

![Image of Mefruside](image)

C_{13}H_{19}ClN_{2}O_{5}S_{2}: 382.88
4-Chloro-N-methyl-2-[(2RS)-2-methyltetrahydrofuran-2- ylmethyl]-3-sulfamoylbenzenesulfonamide [7195-27-9]

Mefruside, when dried, contains not less than 98.5% of C_{13}H_{19}ClN_{2}O_{5}S_{2}.

**Description** Mefruside occurs as a white crystalline powder.

It is very soluble in dimethylformamide, freely soluble in acetone, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

A solution of Mefruside in dimethylformamide (1 in 10) has no optical rotation.

**Identification** (1) Determine the absorption spectrum of a solution of Mefruside in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mefruside, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Mefruside as directed under Flame Coloration Test <1.04> (2): a green color appears.

**Melting point** <2.6D> 149 – 152°C
Purity (1) Heavy metals \(<1.0\) — Dissolve 1.0 g of Mefruside in 30 mL of acetone, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Arsenic \(<1.1\) — Prepare the test solution with 1.0 g of Mefruside according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.20 g of Mefruside in 10 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone \((5:2)\) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm) the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying \(<2.4\) — Not more than 0.5% \((1\ g,\ 105^\circ\ C,\ 2\ hours)\).

Residue on ignition \(<2.44\) — Not more than 0.1% \((1\ g)\).

Assay Weigh a quantity of powdered Mefruside, previously dried, dissolve in 80 mL of \(N, N\)-dimethylformamide, and titrate \(<2.50\) with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination with a solution prepared by adding 13 mL of water to 80 mL of \(N, N\)-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS

\[
= 38.29 \text{ mg of } C_{13}H_{19}ClN_2O_5S_2
\]

Containers and storage Containers—Well-closed containers.

Mefruside Tablets

メフルシド錠

Mefruside Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mefruside \((C_{13}H_{19}ClN_2O_5S_2)\) 382.88.

Method of preparation Prepare as directed under Tablets, with Mefruside.

Identification (1) Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.3 g of Mefruside according to the labeled amount, shake with 15 mL of heated methanol for 20 minutes, and filter. Add 25 mL of water to the filtrate, and allow to stand while ice-cooling for 30 minutes. Filter the white precipitate formed, wash with water, and dry at 105°C for 2 hours: the precipitate melts \(<2.50\) between 149°C and 152°C.

(2) Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.01 g of Mefruside according to the labeled amount, shake with 70 mL of methanol strongly for 15 minutes, add methanol to make 100 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry \(<2.24\); it exhibits maxima between 274 nm and 278 nm, and between 283 nm and 287 nm.

Uniformity of dosage units \(<6.02\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Mefruside Tablets add 40 mL of methanol, disintegrate the tablet using ultrasonic waves with occasional stirring, then further treat with ultrasonic waves for 10 minutes, and add methanol to make exactly \(V\) mL of a solution containing about 0.5 mg of mefruside \((C_{13}H_{19}ClN_2O_5S_2)\) per mL. Centrifuge the solution, pipet 5 mL of the supernatant liquid, add methanol to make exactly 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of mefruside } (C_{13}H_{19}ClN_2O_5S_2) = M_5 \times A_1/A_5 \times V/125
\]

\(M_5\) Amount (mg) of mefruside for assay

Dissolution \(<6.10\) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Mefruside Tablets is not less than 85%.

Start the test with 1 tablet of Mefruside Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a filter paper for quantitative analysis \((5C)\). Discard the first 5 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains about 28 \(\mu\)g of mefruside \((C_{13}H_{19}ClN_2O_5S_2)\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of mefruside for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_t\) and \(A_s\) of the sample solution and standard solution at 285 nm in a layer of 5 cm in length as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), using water as the blank.

Dissolution rate (%) with respect to the labeled amount of mefruside \((C_{13}H_{19}ClN_2O_5S_2)\)

\[
= M_5 \times A_1/A_5 \times V/V \times 1/C \times 36
\]

\(M_5\) Amount (mg) of mefruside for assay

C: Labeled amount (mg) of mefruside \((C_{13}H_{19}ClN_2O_5S_2)\) in 1 tablet

Assay Weigh accurately not less than 20 Mefruside Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 65 mg of mefruside \((C_{13}H_{19}ClN_2O_5S_2)\), shake with 70 mL of methanol for 15 minutes, then add methanol to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, take exactly 10 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 65 mg of mefruside for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution,
add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, \( A_2 \) and \( A_3 \), of the sample solution and standard solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry \( <2.4> \).

\[
\text{Amount (mg) of mefruside (C}_{15}\text{H}_{24}\text{ClN}_2\text{O}_{5}\text{S}_2) = M_s \times A_1/A_3
\]

\( M_s \): Amount (mg) of mefruside for assay

Containers and storage  Containers—Tight containers.

## Meglumine

メグルミン

![Meglumine structure](image)

\( \text{C}_{17}\text{H}_{17}\text{NO}_5 \): 195.21

1-Deoxy-1-methylamino-\( \alpha \)-glucitol

[6284-40-8]

Meglumine, when dried, contains not less than 99.0% of \( \text{C}_{17}\text{H}_{17}\text{NO}_5 \).

**Description** Meglumine occurs as a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in water, and slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Meglumine (1 in 10) is between 11.0 and 12.0.

**Identification**

1. To 1 mL of a solution of Meglumine (1 in 10) add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS: a deep red color develops.

2. To 2 mL of a solution of Meglumine (1 in 10) add 1 drop of methyl red TS, and add 0.5 mL of dilute sodium hydroxide TS and 0.5 g of boric acid after neutralizing with 0.5 mol/L sulfuric acid TS: a deep red color develops.

3. Dissolve 0.5 g of Meglumine in 1 mL of diluted hydrochloric acid (1 in 3), and add 10 mL of ethanol (99.5): a white precipitate is produced. Then, rubbing the inside wall of the container with a glass rod, cool with ice and produce more precipitate. Filter the precipitate by suction through a glass filter (G3), wash the precipitate with a small volume of ethanol (99.5), and dry at 105°C for 1 hour: the residue thus obtained melts \( <2.60> \) between 149°C and 152°C.

**Optical rotation** \( <2.40> \) \([\alpha]_D^{20} = -16.0 - 17.0°\) (after drying, 1 g, water, 10 mL, 100 mm).

**Melting point** \( <2.60> \) 128 - 131°C

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Meglumine in 10 mL of water: the solution is clear and colorless.

2. Chloride \( <1.03> \)—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 10 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).

3. Sulfate \( <1.14> \)—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 5 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

4. Heavy metals \( <1.07> \)—Proceed with 2.0 g of Meglumine according to Method 4, and prepare the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

5. Arsenic \( <1.11> \)—Prepare the test solution with 2.0 g of Meglumine according to Method 3, and perform the test (not more than 1 ppm).

6. Reducing substances—To 5 mL of a solution of Meglumine (1 in 20) add 5 mL of Fehling’s TS, and boil for 2 minutes: no red-brown precipitate is produced.

**Loss on drying** \( <2.44> \) Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** \( <2.44> \) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Meglumine, previously dried, dissolve in 25 mL of water, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of methyl red TS).

\[
\text{Each mL of 0.1 mol/L hydrochloric acid VS} \quad = 19.52 \text{mg of C}_{17}\text{H}_{17}\text{NO}_5
\]

Containers and storage  Containers—Tight containers.

## Meglumine Iotalamate Injection

イオタラム酸メグルミン注射液

Meglumine Iotalamate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of iotalamic acid (\( \text{C}_{11}\text{H}_{9}\text{I}_3\text{N}_2\text{O}_4 \): 613.91).

**Method of preparation**

1. Iotalamic Acid 227.59 g

Meglumine 72.41 g

Water for Injection or Sterile Water

---

**To make** 1000 mL

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iotalamic Acid</td>
<td>227.59 g</td>
</tr>
<tr>
<td>Meglumine</td>
<td>72.41 g</td>
</tr>
<tr>
<td>Water for Injection or Sterile Water</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

2. Iotalamic Acid 455 g

Meglumine 145 g

Water for Injection or Sterile Water

---

**To make** 1000 mL

Prepare as directed under Injections, with the above ingredients (1) or (2).

**Description** Meglumine Iotalamate Injection is a clear, colorless to pale yellow, slightly viscous liquid. It gradually changes in color by light.

**Identification**

1. To 1 mL of Meglumine Iotalamate Injection add 1 mL of potassium naphthoquinone sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.
(2) To a volume of Meglumine Iotalamate Injection, equivalent to 1 g of Iotalamic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid while shaking: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash the precipitate with two 10-mL portions of water, and dry at 105°C for 4 hours. Proceed with the precipitate so obtained as directed in the Identification (2) under Iotalamic Acid.

**Optical rotation** <2.49>

Method of preparation (1) \( \alpha_D^{20} = -1.67 - 1.93^\circ \) (100 mm).

Method of preparation (2) \( \alpha_D^{20} = -3.35 - 3.86^\circ \) (100 mm).

**pH** <2.54> 6.5 - 7.7

**Purity (1)** Primary aromatic amines—To a volume of Meglumine Iotalamate Injection, equivalent to 0.20 g of Iotalamic Acid according to the labeled amount, add 15 mL of water, shake, add 4 mL of a solution of sodium nitrite (1 in 100) under ice-cooling, and proceed as directed in the Purity (2) under Iotalamic Acid: the absorbance is not more than 0.17.

(2) Iodine and iodide—Take a volume of Meglumine Iotalamate Injection, equivalent to 1.5 g of Iotalamic Acid according to the labeled amount, and proceed as directed in the Purity (2) under Sodium Iotalamate Injection.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** To an exactly measured volume of Meglumine Iotalamate Injection, equivalent to about 4 g of iotalamic acid (C\(_{11}\)H\(_9\)I\(_3\)N\(_2\)O\(_4\)), add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of iotalamic acid for assay, previously dried at 105°C for 4 hours, dissolve in 100 mL of water and 1 mL of sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of iotalamic acid to that of the internal standard.

\[
\frac{\text{Amount (mg) of iotalamic acid (C}_{11}\text{H}_{9}\text{I}_{3}\text{N}_{2}\text{O}_{4})}{M_S} = M_S \times \frac{Q_1}{Q_3} \times 10
\]

\( M_S \): Amount (mg) of iotalamic acid for assay

**Internal standard solution**—A solution of L-tryptophan in the mobile phase (3 in 2500).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 3.9 g of phosphoric acid and 2.8 mL of triethylamine in water to make 2000 mL. To this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of iotalamic acid is about 6 minutes.

**System suitability**—

System performance: When the procedure is run with 10 \( \mu \)L of the standard solution under the above operating conditions, iotalamic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of iotalamic acid to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

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**Meglumine Sodium Amidotrizoate Injection**

アミドトリゾ酸ナトリウムメグルミン注射液

Meglumine Sodium Amidotrizoate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of amidotrizoic acid (C\(_{11}\)H\(_9\)I\(_3\)N\(_2\)O\(_4\)): 613.91.

**Method of preparation**

(1)

| Amidotrizoic Acid (anhydrous) | 471.78 g |
| Sodium Hydroxide | 5.03 g |
| Meglumine | 125.46 g |

Water for Injection in Containers a sufficient quantity

For Injection (in Containers) 1000 mL

(2)

| Amidotorizoic Acid (anhydrous) | 597.30 g |
| Sodium Hydroxide | 6.29 g |
| Meglumine | 159.24 g |

Water for Injection in Containers a sufficient quantity

For Injection (in Containers) 1000 mL

Prepare as directed under Injections, with the above ingredients (1) or (2).

**Description** Meglumine Sodium Amidotrizoate Injection is a clear, colorless to pale yellow, slightly viscous liquid.
It gradually changes in color by light.

**Identification** (1) To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 1 g of Amidotrizoic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate so obtained as directed in the Identification (2) under Amidotrizoic Acid.

(2) To 1 mL of Meglumine Sodium Amidotrizoate Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(3) Meglumine Sodium Amidotrizoate Injection responds to the Qualitative Tests 2.4(1) for sodium salt.

**Optical rotation** 2.49

Method of preparation (1) \( \alpha_D^{210} = -2.91\) – \(-3.36°\) (100 mm).

Method of preparation (2) \( \alpha_D^{20} = -3.69\) – \(-4.27°\) (100 mm).

**pH** 2.54 – 6.0 – 7.7

**Purity** (1) Primary aromatic amines—To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 0.20 g of Amidotrizoic Acid according to the labeled amount, add 6 mL of water, mix, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, and shake. Proceed as directed in the Purity (2) under Amidotrizoic Acid: the absorbance is not more than 0.19.

(2) Iodine and iodide—To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 0.25 g of Amidotrizoic Acid according to the labeled amount, add water to make 20 mL, add 5 mL of dilute nitric acid, shake well, and filter by suction through a glass filter (G4). Add 5 mL of chloroform to the filtrate, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of hydrogen peroxide (30), and shake vigorously: the chloroform layer has no more color than the following control solution. Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. Add 20 mL of water to 0.10 mL of this solution, add 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of hydrogen peroxide (30), and shake vigorously.

**Extractable volume** 6.05

It meets the requirement.

**Foreign insoluble matter** 6.06

Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** 6.07

It meets the requirement.

**Sterility** 4.05

Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** To an exactly measured volume of Meglumine Sodium Amidotrizoate Injection, equivalent to about 0.5 g of amidotrizoic acid (C\(_{11}\)H\(_9\)I\(_3\)N\(_2\)O\(_4\)), add water to make exactly 200 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of amidotrizoic acid for assay (previously determine the loss on drying 2.47 in the same manner as Amidotrizoic Acid), dissolve in a solution of meglumine (3 in 1000) to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the standard solution. Perform the test with 5 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, \(Q_3\) and \(Q_2\), of the peak area of amidotrizoic acid to that of the internal standard.

\[
\text{Amount (mg) of amidotrizoic acid (C}_{11}\text{H}_{15}\text{I}_{3}\text{N}_{2}\text{O}_{4}) = M_5 \times \frac{Q_2}{Q_3} \times 2
\]

\(M_5\): Amount (mg) of amidotrizoic acid for assay, calculated on the dried basis

**Internal standard solution**—Dissolve 0.06 g of acetylizico acid in a solution of meglumine (3 in 1000) to make 100 mL.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.7 g of tetrabutylammonium phosphate and 7.0 g of dipotassium hydrogenphosphate in 750 mL of water, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), add water to make 800 mL, then add 210 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of amidotrizoic acid is about 5 minutes.

**System suitability**—

System performance: When the procedure is run with 5 \(\mu\)L of the standard solution under the above operating conditions, amidotrizoic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of amidotrizoic acid to that of the internal standard is not more than 1.0%. 

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

**Meglumine Sodium Iodamide Injection**

ヨーダミドナトリウムメグルミン注射液

Meglumine Sodium Iodamide Injection is an aqueous solution for injection.

It contains not less than 59.7 w/v% and not more than 65.9 w/v% of iodamide (C\(_{12}\)H\(_11\)I\(_3\)N\(_2\)O\(_4\): 627.94).
Method of preparation

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodamide</td>
<td>627.9 g</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>6.0 g</td>
</tr>
<tr>
<td>Meglumine</td>
<td>165.9 g</td>
</tr>
</tbody>
</table>

To make 1000 mL, prepare as directed under Injections, with the above ingredients.

Description

Meglumine Sodium Iodamide Injection is a clear, colorless to pale yellow, slightly viscous liquid. It gradually changes in color by light.

Identification (1) To 2 mL of Meglumine Sodium Iodamide Injection add 25 mL of water, and add 3 mL of dilute hydrochloric acid with thorough stirring: a white precipitate is formed. Filter the precipitate by suction through a glass filter (G3), and wash with two 10-mL portions of water. Transfer the precipitate to a suitable flask, add 100 mL of water, dissolve by heating, and gently boil until the volume becomes about 30 mL. After cooling, collect the separated crystals by filtration, dry at 105°C for 1 hour, and proceed as directed in the Identification (1) and (2) under Iodamide.

(2) Determine the infrared absorption spectrum of the dried crystals obtained in (1) as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3390 cm⁻¹, 1369 cm⁻¹, 1296 cm⁻¹, 1210 cm⁻¹ and 1194 cm⁻¹.

(3) To 1 mL of Meglumine Sodium Iodamide Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color is produced.

(4) Meglumine Sodium Iodamide Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> αD: -3.84 – -4.42° (100 mm).

pH <2.54> 6.5 – 7.5

Purity (1) Primary aromatic amines—Mix 0.30 mL of Meglumine Sodium Iodamide Injection and 6 mL of water, then add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake well, and proceed as directed in the Purity (2) under Iodamide: the absorbance is not more than 0.22.

(2) Iodine and iodide—To 0.40 mL of Meglumine Sodium Iodamide Injection add water to make 20 mL, then add 5 mL of dilute nitric acid, shake well, filter by suction through a glass filter (G3). To the filtrate add 5 mL of chloroform, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of a strong hydrogen peroxide solution, and shake vigorously: the chloroform layer has no color.

Extractable volume <6.05> It meets the requirement.

Pyrogen <4.04> Dilute Meglumine Sodium Iodamide Injection with isotonic sodium chloride solution so as to contain 0.30 mL of Meglumine Sodium Iodamide Injection per mL according to the labeled amount, and perform the test: it meets the requirements.

Assay

To an exactly measured 8 mL of Meglumine Sodium Iodamide Injection add sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution into a saponification flask, add 30 mL of sodium hydroxide TS and 1 g of zinc powder, and proceed as directed in the Assay under Iodamide.

Each mL of 0.1 mol/L silver nitrate VS = 20.93 mg of CsH1I1N2O4.

Containers and storage

Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Melphalan

メルファラン

C13H18Cl2N2O2: 305.20
4-Bis(2-chloroethyl)amino-L-phenylalanine [148-82-3]

Melphalan contains not less than 93.0% of C13H18Cl2N2O2, calculated on the dried basis.

Description

Melphalan occurs as a white, to light yellowish white, crystalline powder. It is slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether. It dissolves in dilute hydrochloric acid and in dilute sodium hydroxide TS.

It is gradually colored by light.

Optical rotation [α]D: about -32° (0.5 g, calculated on the dried basis, methanol, 100 mL, 100 mm).

Identification (1) To 20 mg of Melphalan add 50 mL of methanol, dissolve by warming, add 1 mL of a solution of 4-(4-nitrobenzyl)pyridine in acetone (1 in 20), and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of warmed methanol and add 2 drops of ammonia solution (28): a purple color develops.

(2) Dissolve 0.1 g of Melphalan in 10 mL of dilute sodium hydroxide TS, and heat on a water bath for 10 minutes. After cooling, add dilute nitric acid to acidify, and filter: the filtrate responds to the Qualitative Tests <1.09> for chloride.

(3) Determine the absorption spectrum of a solution of Melphalan in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Ionisable chloride—Weigh accurately about 0.5 g of Melphalan, dissolve in 80 mL of dilute nitric acid...
(1 in 40), stir for 2 minutes, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration): the consumed volume is not more than 1.0 mL to 0.50 g of Melphalan.

(2) Heavy metals: Proceed with 1.0 g of Melphalan according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic: Prepare the test solution with 1.0 g of Melphalan according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying**<sup>2.41</sup> Not more than 7.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 105°C, 2 hours).

**Residue on ignition**<sup>2.44</sup> Not more than 0.3% (1 g).

**Assay**
Weigh accurately about 0.25 g of Melphalan, add 20 mL of a solution of potassium hydroxide (1 in 5), and heat under a reflux condenser on a water bath for 2 hours. After cooling, add 75 mL of water and 5 mL of nitric acid, cool, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration). Make any necessary correction by using the results obtained in the Purity (1).

Each mL of 0.1 mol/L silver nitrate VS is equivalent to 15.26 mg of C<sub>13</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>

**Containers and storage**
Containers—Tight containers.
Storage—Light-resistant.

**Menatetrenone**

メナテトレノン

C<sub>31</sub>H<sub>40</sub>O<sub>2</sub>: 444.65
2-Methyl-3-[(2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl]-1,4-naphthoquinone

[863-61-6]

Menatetrenone contains not less than 98.0% of C<sub>31</sub>H<sub>40</sub>O<sub>2</sub>, calculated on the dehydrated basis.

**Description**
Menatetrenone occurs as yellow, crystals, crystalline powder, waxy mass or oily material.

It is very soluble in hexane, soluble in ethanol (99.5), sparingly soluble in 2-propanol, slightly soluble in methanol, and practically insoluble in water.

It decomposes and the color becomes more intense by exposure to daylight, using a light-resistant vessel. Dissolve 0.1 g of Menatetrenone in 100 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add hexane to make exactly 50 mL. Conform that the peak area of menatetrenone obtained from the sample solution is not larger than the peak area of menatetrenone from the sample solution is not more than 1.0%.

**Operating conditions**
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of menatetrenone beginning after the solvent peak.

**System suitability**
Test for required detection: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 50 mL. Confirm that the peak area of menatetrenone obtained from 20 µL of this solution is equivalent to 7 to 13% of that from 20 µL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of menatetrenone is not more than 1.0%.

**Water**<sup>2.48</sup> Not more than 0.5% (0.5 g, volumetric titration, direct titration).

Menatetrenone contains not less than 98.0% of C<sub>31</sub>H<sub>40</sub>O<sub>2</sub>, calculated on the dehydrated basis.

**Description**
Menatetrenone occurs as yellow, crystals, crystalline powder, waxy mass or oily material.

It is very soluble in hexane, soluble in ethanol (99.5), sparingly soluble in 2-propanol, slightly soluble in methanol, and practically insoluble in water.

It decomposes and the color becomes more intense by exposure to daylight, using a light-resistant vessel. Dissolve 0.1 g of Menatetrenone in 100 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add hexane to make exactly 50 mL. Conform that the peak area of menatetrenone obtained from the sample solution is not larger than the peak area of menatetrenone from the sample solution is not more than 1.0%.

**Operating conditions**
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of menatetrenone beginning after the solvent peak.

**System suitability**
Test for required detection: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 50 mL. Confirm that the peak area of menatetrenone obtained from 20 µL of this solution is equivalent to 7 to 13% of that from 20 µL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of menatetrenone is not more than 1.0%.

**Water**<sup>2.48</sup> Not more than 0.5% (0.5 g, volumetric titration, direct titration).
Residue on ignition \(<2.44\) Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to daylight, using a light-resistant vessel. Weigh accurately about 0.1 g each of Menatetrenone and Menatetrenone RS (separately, determine the water \(<2.48\) in the same manner as Menatetrenone), dissolve each in 50 mL of 2-propanol, and add ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of these solutions, and add ethanol (99.5) to make exactly 100 mL. Pipet 2 mL each of these solutions, add exactly 4 mL each of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07\) according to the following conditions, and calculate the ratios, \(Q_1\) and \(Q_2\), of the peak area of menatetrenone to that of the internal standard.

\[
\text{Amount (mg) of } C_{10}H_{20}O = M_S \times Q_1/Q_2
\]

\(M_S\): Amount (mg) of Menatetrenone RS, calculated on the dehydrated basis

**Internal standard solution**—A solution of phytonadione in 2-propanol (1 in 20,000).

**Operating conditions**—
- **Detector**: An ultraviolet absorption photometer (wavelength: 270 nm).
- **Column**: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
- **Column temperature**: A constant temperature of about 40°C.
- **Mobile phase**: Methanol.
- **Flow rate**: Adjust the flow rate so that the retention time of menatetrenone is about 7 minutes.

**System suitability**—
- **System performance**: When the procedure is run with 20 \(\mu\)L of the standard solution under the above operating conditions, menatetrenone and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
- **System repeatability**: When the test is repeated 6 times with 20 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of menatetrenone to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

**Storage**—Light-resistant.

**dl-Menthol**

dl-メントール

\(C_{10}H_{20}O: 156.27\)

\((1S,2SR,5RS)-5\)-Methyl-2-(1-methylethyl)cyclohexanol

**Description** dl-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

It is very soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It sublimes gradually at room temperature.

**Identification (1)** Triturate dl-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

(2) Shake 1 g of dl-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer possesses no aroma of menthol is separated.

**Congealing point \(<2.42\)** 27–28°C

**Optical rotation \(<2.49\)** \([\alpha]_D^20 = -2.0 - +2.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

**Purity (1)** Non-volatile residue—Volatilize 2.0 g of dl-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

(2) Thymol—Add 0.20 g of dl-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

(3) Nitromethane or nitroethane—To 0.5 g of dl-Menthol placed in a flask add 2 mL of a solution of sodium hydroxide (1 in 2) and 1 mL of strong hydrogen peroxide, connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, then add 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of \(N,N\)-diethyl-N’-1-naphthylethlenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

**Assay** Weigh accurately about 2 g of dl-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash down the condenser with 20 mL of water, and titrate \(<2.50\) with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.
Menthol on a water bath, and dry the residue at 105°C.

Melting point

ethanol (95), 25 mL, 100 mm).

Assay

purple color immediately develops.

water bath for 2 hours. Wash the condenser with 20 mL of

anhydride (8:1), connect a reflux condenser, and heat on a

exactly 20 mL of a mixture of dehydrated pyridine and acetic

acid (1 in 100), allow to stand for 2 minutes, and then add 1

drops of nitric acid: no green to blue-green color immedi-

ately develops.

Mepenzolate Bromide according to Method 2, and perform

heavy metals—Prepare the test solution with 1.0 g of

Mepenzolate Bromide in exactly measured 10 mL of methanol, and use

this solution as the sample solution. Pipet 1 mL of the sam-

ple solution, add methanol to make exactly 200 mL, and use

water, and titrate <2.5D with 1 mol/L sodium hydroxide VS

(indicator: 5 drops of phenolphthalein TS). Perform a blank
determination and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS

= 156.3 mg of C10H20O

Containers and storage

Containers—Tight containers.

Storage—In a cold place.

C10H20O: 156.27

(1R,2S,5S)-5-Methyl-2-(1-methylethyl)cyclohexanol [2216-51-5]

l-Menthol contains not less than 98.0% of C10H20O.

Description

l-Menthol occurs as colorless crystals. It has a

characteristic and refreshing odor and a burning taste, fol-

lowed by a cool taste.

It is very soluble in ethanol (95) and in diethyl ether, and

very slightly soluble in water.

It sublimes gradually at room temperature.

Identification (1) Triturate l-Menthol with an equal

amount of camphor, chloral hydrate or thymol: the mixture

liquefies.

(2) Shake 1 g of l-Menthol with 20 mL of sulfuric acid: the mixture

becomes turbid with a yellow-red color. Allow to

stand for 3 hours: a clear, oily layer which possesses no aro-

ma of menthol is separated.

Optical rotation <2.49° [α]D20 = −45.0° −51.0° (2.5 g,

ethanol (95), 25 mL, 100 mm).

Melting point <2.60° 42 – 44°C

Purity (1) Non-volatile residue—Volatilize 2.0 g of l-

Menthol on a water bath, and dry the residue at 105°C for

2 hours: the residue weighs not more than 1.0 mg.

(2) Thymol—Add 0.20 g of l-Menthol to a cold mixture

of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2

drops of nitric acid: no green to blue-green color immedi-

ately develops.

(3) Nitromethane or nitroethane—To 0.5 g of l-Menthol

placed in a flask add 2 mL of sodium hydroxide solution (1

in 2) and 1 mL of strong hydrogen peroxide, connect a reflux

condenser to the flask, and boil the mixture gently for 10

minutes. After cooling, add water to make exactly 20 mL,

and filter. Take 1 mL of the filtrate in a Nessler tube, add

water to make 10 mL, neutralize with dilute hydrochloric

acid, add another 1 mL of dilute hydrochloric acid, and

cool. To the mixture add 1 mL of a solution of sulfanilic

acid (1 in 100), allow to stand for 2 minutes, and then add 1

mL of a solution of N,N-diethyl-N'-1-naphthylethylendi-

mine oxalate (1 in 1000) and water to make 25 mL: no red-

purple color immediately develops.

Assay

Weigh accurately about 2 g of l-Menthol, add ex-

actly 20 mL of a mixture of dehydrated pyridine and acetic

anhydride (8:1), connect a reflux condenser, and heat on a

water bath for 2 hours. Wash the condenser with 20 mL of

water, and titrate <2.5D with 1 mol/L sodium hydroxide VS

(indicator: 5 drops of phenolphthalein TS). Perform a blank
determination and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS

= 156.3 mg of C10H20O

Containers and storage

Containers—Tight containers.

Storage—In a cold place.

Mepenzolate Bromide

メペンゾラート臭化物

C21H26BrNO3: 420.34

(3RS)-3-[(Hydroxy)(diphenyl)acetoxy]-1,1-
dimethylpiperidinium bromide [76-90-4]

Mepenzolate Bromide, when dried, contains not less

than 98.5% of C21H26BrNO3.

Description

Mepenzolate Bromide is white to pale yellow

crystals or crystalline powder. It is odorless, and has a bitter

taste.

It is very soluble in formic acid, freely soluble in metha-

ol, soluble in hot water, slightly soluble in water and in

ethanol (95), very slightly soluble in acetic anhydride, and

practically insoluble in diethyl ether.

Melting point: about 230°C (with decomposition).

Identification (1) To 30 mg of Mepenzolate Bromide add

10 drops of sulfuric acid: a red color develops.

(2) Dissolve 10 mg of Mepenzolate Bromide in 20 mL of

water and 5 mL of dilute hydrochloric acid, and to 5 mL of

this solution add 1 mL of Dragendorff’s TS: an orange pre-

cipitate is produced.

(3) Determine the absorption spectrum of a solution of

Mepenzolate Bromide in 0.01 mol/L hydrochloric acid TS (1

in 2000) as directed under Ultraviolet-visible Spectropho-

tometry <2.2D>, and compare the spectrum with the Refer-

ence Spectrum: both spectra exhibit similar intensities of ab-

sorption at the same wavelengths.

(4) Dissolve 0.5 g of Mepenzolate Bromide in 50 mL of

water and 3 mL of nitric acid by heating. This solution re-

sponds to the Qualitative Tests <1.0D> for Bromide.

Purity (1) Heavy Metals <1.07>—Proceed with 1.0 g of

Mepenzolate Bromide according to Method 2, and perform

the test. Prepare the control solution with 2.0 mL of Stan-

dard Lead Solution (not less than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g

of Mepenzolate Bromide according to Method 3, and per-

form the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.40 g of Mepenzolate

Bromide in exactly measured 10 mL of methanol, and use

this solution as the sample solution. Pipet 1 mL of the sam-

ple solution, add methanol to make exactly 200 mL, and use
this solution as the standard solution (1). Separately, dissolve 40 mg of benzophenone in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution, standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, methanol, water and acetic acid (100:3:3:2:1) to a distance of about 10 cm, and air-dry the plate and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than either the principal spot or the spot corresponding to benzophenone from the sample solution are not more intense than the spot from standard solution (1), and the spot corresponding to benzophenone from the sample solution is not more intense than the spot from standard solution (2). Spray evenly Dragendorff’s TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from standard solution (1).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Mepenzolate Bromide, previously dried, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 42.03 mg of C_{21}H_{30}BrNO_{3}

Containers and storage Containers—Tight containers.

Mepitiostane

Mepitiostane contains not less than 96.0% and not more than 102.0% of C_{25}H_{40}O_{2}S, calculated on the anhydrous basis.

Description Mepitiostane occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in triethylamine, in chloroform, in diethyl ether and in cyclohexane, soluble in diethylene glycol dimethyl ether and in petroleum ether, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is hydrolyzed in moist air.

Identification (1) Dissolve 1 mg of Mepitiostane in 1 mL of methanol, and add 0.5 mL of palladium (II) chloride TS: an orange precipitate is formed. To this suspension add 1 mL of water and 2 mL of chloroform, shake well, and allow to stand: an orange color develops in the chloroform layer.

(2) Dissolve 0.1 g of Mepitiostane in 2 mL of diethylene glycol dimethyl ether, shake with 1 mL of 1 mol/L hydrochloric acid TS, and filter. To the filtrate add 1.5 mL of 2,4-dinitrophenylhydrazine-diethylene glycol dimethyl ether TS and 1.5 mL of diluted ethanol (95) (2 in 3): an orange-yellow precipitate is formed. Filter the precipitate, recrystallize from ethanol (99.5), and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the crystals melt <2.60> between 144°C and 149°C.

(3) Determine the infrared absorption spectrum of Mepitiostane as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_{D}^{20} = +20° - +23° (0.1 g, chloroform, 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Mepitiostane in 4 mL of petroleum ether: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Mepitiostane according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Mepitiostane in exactly 5 mL of a mixture of acetone and triethylamine (1000:1), and use this solution as the sample solution. Separately, dissolve 10 mg of Epitostanol RS in a mixture of acetone and triethylamine (1000:1) to make exactly 10 mL. Pipet 1 mL and 3 mL of this solution, to each add a mixture of acetone and triethylamine (1000:1) to make exactly 25 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, heat between 120°C and 130°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution showing the same Rf value as the standard solutions are not more intense than the spot from the standard solution (2), and the remaining spots other than the principal spot are not more intense than the spot from the standard solution (1).

Water <2.48> Not more than 0.7% (0.3 g, volumetric titration, back titration).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.3 g of Mepitiostane, and dissolve in cyclohexane to make exactly 10 mL. Pipet 2 mL
Mepivacaine Hydrochloride

メピバカイン塩酸塩

C₁₅H₂₂N₂O.HCl: 282.81
(2RS)-N-(2,6-Dimethylphenyl)-1-methylpiperidine-2-
 carboxamide monohydrochloride
[1722-62-9]

Mepivacaine Hydrochloride, when dried, contains
not less than 98.5% and not more than 101.0% of
C₁₅H₂₂N₂O.HCl.

Description Mepivacaine Hydrochloride occurs as white
crystals or crystalline powder.
It is freely soluble in water and in methanol, soluble in
acetic acid (100), and sparingly soluble in ethanol (99.5).
A solution of Mepivacaine Hydrochloride (1 in 10) shows
no optical rotation.
Melting point: about 256°C (with decomposition).

Identification (1) Determine the absorption spectrum of a
solution of Mepivacaine Hydrochloride (1 in 2500) as di-
rected under Ultraviolet-visible Spectrophotometry <2.24>,
and compare the spectrum with the Reference Spectrum:
both spectra exhibit similar intensities of absorption at the
same wavelengths.
(2) Determine the infrared absorption spectrum of
Mepivacaine Hydrochloride as directed in the potassium
chloride disk method under Infrared Spectrophotometry <2.25>,
and compare the spectrum with the Reference Spectrum:
both spectra exhibit similar intensities of absorption at the
same wave numbers.
(3) A solution of Mepivacaine Hydrochloride (1 in 50)
responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 0.2 g of Mepivacaine Hydrochloride in
10 mL of water: the pH of this solution is between 4.0 and
5.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of
Mepivacaine Hydrochloride in 10 mL of water: the solu-
tion is clear and colorless.
(2) Sulfate <1.14>—Perform the test with 0.5 g of
Mepivacaine Hydrochloride. Prepare the control solution
with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more
than 0.038%).
(3) Heavy metals <1.07>—Produce with 2.0 g of
Mepivacaine Hydrochloride according to Method 1, and per-
form the test. Prepare the control solution with 2.0 mL of
Standard Lead Solution (not more than 10 ppm).
(4) Related substances—Dissolve 0.10 g of Mepivacaine
Hydrochloride in 5 mL of methanol, and use this solution as
the sample solution. Pipet 1 mL of the sample solution, and
add methanol to make exactly 20 mL. Pipet 2 mL of this so-
lution, add methanol to make exactly 50 mL, and use this sol-
solution as the standard solution. Perform the test with these
solutions as directed under Thin-layer Chromatography
<2.03>. Spot 10 μL each of the sample solution and standard

of this solution, add 10 mL of ethanol (99.5), mix with ex-
actly 2 mL each of 0.01 mol/L hydrochloric acid TS and the
internal standard solution, add ethanol (99.5) to make 20
mL, allow to stand at ordinary temperature for 30 minutes,
and use this solution as the sample solution. Separately,
weigh accurately about 45 mg of Epitiostanol RS, dissolve in
exactly 2 mL of the internal standard solution, add ethanol
(99.5) to make 20 mL, and use this solution as the standard
solution. Perform the test with 10 μL each of the sample
solution and standard solution as directed under Liquid
Chromatography <2.01> according to the following condi-
tions, and calculate the ratios, Q₁ and Q₂, of the peak area
of epitiostanol to that of the internal standard, respectively.

\[ Q₁ = \frac{M_S}{M₅} \times \frac{Q₂}{Q₅} \times 5 \times 1.320 \]

\[ M_S: \text{Amount (mg) of Epitiostanol RS, calculated on the anhydrous basis} \]

\[ \text{Internal standard solution—A solution of n-octylbenzene in ethanol (99.5) (1 in 300).} \]

Operating conditions—
Detector: An ultraviolet absorption photometer (wave-
length: 265 nm).
Column: A stainless steel column 4.0 mm in inside diame-
ter and 15 cm in length, packed with octadecylsilanized silica
gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about
25°C.
Mobile phase: A mixture of methanol and water (20:3).
Flow rate: Adjust the flow rate so that the retention time
of epitiostanol is about 6 minutes.

System suitability—
System performance: When the procedure is run with 10
μL of the standard solution under the above operating con-
ditions, epitiostanol and the internal standard are eluted in
this order with the resolution between these peaks being not
less than 4.
System repeatability: When the test is repeated 6 times
with 10 μL of the standard solution under the above operat-
ing conditions, the relative standard deviation of the ratios
of the peak area of epitiostanol to that of the internal stand-
ard is not more than 1.0%.

Containers and storage—Containers—Hermetic containers.
Storage—Light-resistant, under Nitrogen atmosphere, and
in a cold place.
solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, methanol and ammonia solution (28) (100:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly bismuth nitrate-potassium iodide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Mepivacaine Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100) and add 70 mL of acetic anhydride. Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 28.28 mg of C₁₅H₂₂N₂O.HCl

**Containers and storage** Containers—Tight containers.

### Mepivacaine Hydrochloride Injection

メピバカイン塩酸塩注射液

Mepivacaine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of mepivacaine hydrochloride (C₁₅H₂₂N₂O.HCl: 282.81).

**Method of preparation** Prepare as directed under Injections, with Mepivacaine Hydrochloride.

**Description** Mepivacaine Hydrochloride Injection is a clear, colorless liquid.

**Identification** To a volume of Mepivacaine Hydrochloride Injection, equivalent to about 20 mg of Mepivacaine Hydrochloride according to the labeled amount, add 1 mL of sodium hydrochloride TS, and extract with 20 mL of hexane. To 8 mL of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, shake vigorously, and determine the absorption spectrum of the water layer separated as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 261 nm and 265 nm, and between 270 nm and 273 nm.

**pH** Being specified separately.

**Bacterial endotoxins** <4.01> Less than 0.6 EU/mg.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.08> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Pipet a volume of Mepivacaine Hydrochloride Injection, equivalent to about 40 mg of mepivacaine hydrochloride (C₁₅H₂₂N₂O.HCl), add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of mepivacaine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in 0.001 mol/L hydrochloric acid TS, add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₈, of the peak area of mepivacaine to that of the internal standard.

\[
\text{Amount (mg) of mepivacaine hydrochloride (C₁₅H₂₂N₂O.HCl)} = M_S \times \frac{Q_1}{Q_8}
\]

Mₘₜ: Amount (mg) of mepivacaine hydrochloride for assay

**Internal standard solution**—A solution of benzophenone in methanol (1 in 4000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0, and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of mepivacaine is about 6 minutes.

**System suitability**—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, mepivacaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of mepivacaine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.
Mequitazine
メキタジン

C₂₀H₂₂N₂S: 322.47
10-{(3RS)-1-Azabicyclo[2.2.2]oct-3-ylmethyl}-10H-phenothiazine
[29216-28-2]

Mequitazine, when dried, contains not less than 98.5% of C₂₀H₂₂N₂S.

Description  Mequitazine occurs as white crystals or crystalline powder.
It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and practically insoluble in water.
It is gradually colored by light.
A solution of Mequitazine in methanol (1 in 50) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Mequitazine in ethanol (95) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mequitazine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 146 - 150°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Mequitazine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 50 mg of Mequitazine in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, then pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.06>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of ethyl acetate, methanol and diethylamine (7:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 3 and they are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay  Weigh accurately about 0.25 g of Mequitazine, dissolve in 50 mL of acetic acid (100), titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.25 mg of C₂₀H₂₂N₂S

Containers and storage  Containers—Light-resistant.

Mercaptopurine Hydrate
メルカプトプリン水和物

C₉H₇N₃S.H₂O: 170.19
1,7-Dihydro-6H-purine-6-thione monohydrate [6112-76-7]

Mercaptopurine Hydrate contains not less than 98.0% of mercaptopurine (C₅H₄N₄S: 152.18), calculated on the anhydrous basis.

Description  Mercaptopurine Hydrate occurs as light yellow to yellow crystals or crystalline powder. It is odorless.
It is practically insoluble in water, in acetic acid and in diethyl ether.
It dissolves in sodium hydroxide TS and in ammonia TS.

Identification (1) Dissolve 0.6 g of Mercaptopurine Hydrate in 6 mL of sodium hydroxide solution (3 in 100), and add slowly 0.5 mL of iodomethane with vigorous stirring. Stir well for 10 minutes, cool in an ice bath, and adjust the pH with acetic acid (31) to about 5. Collect the separated crystals by filtration, recrystallize from water, and dry at 120°C for 30 minutes: the crystals melt <2.60> between 218°C and 222°C (with decomposition).

(2) Determine the absorption spectrum of a solution of Mercaptopurine Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity of solution—Dissolve 0.20 g of Mercaptopurine Hydrate in 10 mL of ammonia TS: the solution is clear.

(2) Sulfate <1.14>—Dissolve 50 mg of Mercaptopurine Hydrate in 10 mL of dilute hydrochloric acid, add 5 drops of barium chloride TS, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Mercaptopurine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(4) Hypoxanthine—Dissolve 50 mg of Mercaptopterine Hydrate in exactly 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), and use this solution as the sample solution. Separately, dissolve 5.0 mg of hypoxanthine in a solution of ammonia solution (28) in methanol (1 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, n-butyl formate and ammonia solution (28) (8:6:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution observed at the same place as that from the standard solution, is not larger and not more intense than that from the standard solution.

(5) Phosphorus—Take 0.20 g of Mercaptopterine Hydrate in a crucible, add 2 mL of diluted sulfuric acid (3 in 7), then heat gently, slowly adding dropwise several 0.5-mL portions of nitric acid, until the liquid becomes colorless. Continue to heat until most of the liquid has evaporated, cool, and dissolve the residue in 10 mL of water. Transfer the solution to a 25-mL volumetric flask, wash the crucible with two 4-mL portions of water, combine the washings with the solution in the volumetric flask, and use this solution as the sample solution. Separately, dissolve 0.4396 g of potassium dihydrogenphosphate in water to make exactly 200 mL. To 2.0 mL of this solution add water to make exactly 100 mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 16 mL of water, and use this solution as the standard solution. To the sample solution and standard solution add 1 mL of diluted sulfuric acid (3 in 7), 0.5 mL of nitric acid, 0.75 mL of hexammonium heptamolybdate TS, 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS and water to make 25 mL, and allow to stand for 5 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbance of the subsequent solution of the sample solution at 750 nm is not larger than that of the subsequent solution of the standard solution.

Water <2.48> 10.0 – 12.0% (0.2 g, volumetric titration, back titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Mercaptopterine Hydrate, dissolve in 90 mL of N,N-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination with a mixture of 90 mL of N,N-dimethylformamide and 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 15.22 mg of C₃H₇N₃S

Containers and storage Containers—Well-closed containers.

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**Mercurochrome**

**Merbromin**

マーキュロクロム

Mercurochrome is a sodium salt of a mixture of brominated and mercurized fluoresceins.

When dried, it contains not less than 18.0% and not more than 22.4% of bromine (Br: 79.90), and not less than 22.4% and not more than 26.7% of mercury (Hg: 200.59).

**Description** Mercurochrome occurs as blue-green to greenish red-brown scales or granules. It is odorless.

It is freely soluble in water, but sometimes leaves a small amount of insoluble matter. It is practically insoluble in ethanol (95) and in diethyl ether.

**Identification** (1) A solution of Mercurochrome (1 in 2000) shows a red color and a yellow-green fluorescence.

(2) To 5 mL of a solution of Mercurochrome (1 in 250) add 3 drops of dilute sulfuric acid: a reddish orange precipitate is produced.

(3) Heat 0.1 g of Mercurochrome with small crystals of iodine in a test tube: red crystals are sublimed on the upper part of the tube. If yellow crystals are produced, scratch with a glass rod: the color of the crystals changes to red.

(4) Place 0.1 g of Mercurochrome in a porcelain crucible, add 1 mL of a solution of sodium hydroxide (1 in 6), evaporate to dryness with stirring, and ignite. Dissolve the residue in 5 mL of water, acidify with hydrochloric acid, and shake with 3 drops of chlorine TS and 2 mL of chloroform: a yellowish brown color develops in the chloroform layer.

**Purity** (1) Dyestuff—Dissolve 0.40 g of Mercurochrome in 20 mL of water, add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

(2) Soluble halides—Dissolve 5.0 g of Mercurochrome in 80 mL of water, add 10 mL of dilute nitric acid and water to make 100 mL, shake, and filter. Transfer 40 mL of the filtrate to a Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes protected from direct sunlight: no turbidity is produced, or even if produced, it is not more than that of the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, and proceed as directed above.

(3) Soluble mercury salts—To 5 mL of the filtrate obtained in (1) add 5 mL of water, and use this solution as the sample solution. Dissolve 40 mg of mercury (II) chloride, weighed accurately, in water to make 1000 mL, and add 3 mL of dilute sulfuric acid to 20 mL of this solution. To 5 mL of the solution add 5 mL of water, and use this as the control solution. Add 1 drop each of sodium sulfide TS to these solutions, and compare: the sample solution has no more color than the control solution.

(4) Insoluble mercury compounds—Dissolve 2.5 g of Mercurochrome in 50 mL of water, allow to stand for 24 hours, centrifuge, and wash the precipitate with small por-
tions of water until the last washing becomes colorless. Transfer the precipitate to a glass-stoppered flask, add exactly 5 mL of 0.05 mol/L iodine VS, allow to stand for 1 hour with frequent agitation, add 4.3 mL of 0.1 mol/L sodium thiosulfate VS dropwise with shaking, and add 1 mL of starch TS: a blue color develops.

**Loss on drying** <2.4%> Not more than 5.0% (1 g, 105°C, 5 hours).

**Assay (1)** Mercuric—Weigh accurately about 0.6 g of Mercurochrome, previously powdered and dried, transfer to an iodine flask, dissolve in 50 mL of water, add 8 mL of acetic acid (31), 20 mL of chloroform and exactly 30 mL of 0.05 mol/L iodine VS, stopper tightly, and allow to stand for 1 hour with frequent, vigorous shaking. Titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS with vigorous shaking (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

(2) Bromine—Weigh accurately about 0.5 g of Mercurochrome, previously powdered and dried, in a porcelain crucible, add 2 g of potassium nitrate, 3 g of potassium carbonate and 3 g of anhydrous sodium carbonate, mix well, cover the surface of the mixture with 3 g of a mixture of equal amounts of potassium carbonate and anhydrous sodium carbonate, and ignite almost to fusion. Cool, dissolve the ignited mixture in 80 mL of warm water, acidify with nitric acid, and add exactly 25 mL of 0.1 mol/L silver nitrate VS. Shake well, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 7.990 mg of Br

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Mercurochrome Solution**

**Merbromin Solution**

メルブロミン液

Mercurochrome Solution contains not less than 0.42 w/v% and not more than 0.56 w/v% of mercury (Hg: 200.59).

**Method of preparation**

<table>
<thead>
<tr>
<th>Mercurochrome</th>
<th>20 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare by mixing the above ingredients.

**Description** Mercurochrome Solution is a dark red liquid.

**Identification (1)** To 1 mL of Mercurochrome Solution add 40 mL of water: the resulting solution shows a red color and a yellow-green fluorescence.

(2) Dilute 1 mL of Mercurochrome Solution with 4 mL of water, and add 3 drops of dilute sulfuric acid: a red-orange precipitate is produced.

(3) Evaporate 5 mL of Mercurochrome Solution to dryness, and proceed with the residue as directed in the Identification (3) under Mercurochrome.

(4) To 5 mL of Mercurochrome Solution add 1 mL of a solution of sodium hydroxide (1 in 6), and proceed as directed in the Identification (4) under Mercurochrome.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Meropenem Hydrate**

メロペネム水和物

Meropenem Hydrate contains not less than 980 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis. The potency of Meropenem Hydrate is expressed as mass (potency) of meropenem (C₁₇H₂₅N₃O₅S)₃H₂O: 383.46.

**Description** Meropenem Hydrate occurs as a white to light yellow crystalline powder. It is sparingly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

**Identification (1)** Dissolve 10 mg of Meropenem Hydrate in 2 mL of water, add 3 mL of hydroxyammonium chloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Determine the absorption spectra of solutions of Meropenem Hydrate and Meropenem RS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>.

(3) Determine the infrared absorption spectra of Meropenem Hydrate and Meropenem RS as directed in the potassium bromide disk method under Infrared Spectropho-
under Liquid Chromatography

of the sample solution and standard solution as directed

\[
\text{pH} < 2.49 \quad \text{Dissolve 0.2 g of Meropenem Hydrate in 20 mL of water: the pH of the solution is between 4.0 and 6.0.}
\]

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Meropenem Hydrate in 10 mL of sodium hydrogen carbonate TS: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 0.3 mL of Cobalt (II) Chloride CS and 1.2 mL of Iron (III) Chloride CS add 18.5 mL of dilute hydrochloric acid (1 in 40).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Meropenem Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Meropenem Hydrate in 10 mL of triethylamine-phosphate buffer solution, pH 5.0, and use this solution as the sample solution. Prepare the sample solution before use. Pipet 1 mL of the sample solution, and add triethylamine-phosphate buffer solution, pH 5.0 to make exactly 100 mL. Pipet 3 mL of this solution, add triethylamine-phosphate buffer solution, pH 5.0 to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of ring-opened meropenem, having the relative retention time about 0.5 to meropenem, and the peak area of the dimer, having the relative retention time about 2.2 to meropenem, obtained from the sample solution are not larger than the peak area of meropenem from the standard solution, the area of the peak other than meropenem and the peaks mentioned above from the sample solution is not larger than 1/3 times the peak area of meropenem from the standard solution, and the total area of the peaks other than meropenem from the sample solution is not larger than 3 times the peak area of meropenem from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 40\(^\circ\)C.

Mobile phase: A mixture of triethylamine-phosphate buffer solution, pH 5.0 and acetonitrile (100:7).

Flow rate: Adjust the flow rate so that the retention time of meropenem is about 6 minutes.

Time span of measurement: About 7 times as long as the retention time of meropenem.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add triethylamine-phosphate buffer solution, pH 5.0 to make exactly 25 mL. Confirm that the peak area of meropenem obtained from 10 \(\mu\)L of this solution is equivalent to 16 to 24% of that from 10 \(\mu\)L of the standard solution.

System performance: Warm the sample solution at 60\(^\circ\)C for 30 minutes. When the procedure is run with 10 \(\mu\)L of the sample solution under the above operating conditions, the ring-opened meropenem, meropenem and the dimer are eluted in this order, and the resolution between the peaks of the ring-opened meropenem and meropenem is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of meropenem is not more than 1.5%.

Water <2.49> Not less than 11.4% and not more than 13.4% (0.35 g, volumetric titration, direct titration).

Residue on ignition <2.49> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Meropenem Hydrate and Meropenem RS, equivalent to about 50 mg (potency), add exactly 10 mL of the internal standard solution to dissolve, add triethylamine-phosphate buffer solution, pH 5.0 to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 \(\mu\)L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_1\) and \(Q_2\), of the peak area of meropenem to that of the internal standard.

\[
\text{Amount [mg (potency)] of meropenem (C_{17}H_{25}N_{3}O_{5}S)} = \frac{M_5 \times Q_1}{Q_2} \times 1000
\]

\(M_5\): Amount [mg (potency)] of Meropenem RS

Internal standard solution—A solution of benzyl alcohol in triethylamine-phosphate buffer solution, pH 5.0 (1 in 300).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 25\(^\circ\)C.

Mobile phase: A mixture of triethylamine-phosphate buffer solution, pH 5.0 and methanol (5:1).

Flow rate: Adjust the flow rate so that the retention time of meropenem is about 7 minutes.

System suitability—

System performance: When the procedure is run with 5 \(\mu\)L of the standard solution under the above operating conditions, meropenem and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 5 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of meropenem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Meropenem for Injection

注射用メロペネム

Meropenem for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of meropenem (C₁₇H₂₅N₃O₅S: 383.46).

Method of preparation Prepare as directed under Injections, with Meropenem Hydrate.

Description Meropenem for Injection occurs as a white to light yellow crystalline powder.

Identification Determine the infrared absorption spectrum of Meropenem for Injection as directed in the potassium bromide disk method under Infrared Spectrophotometry §2.25: it exhibits absorption at the wave numbers of about 3410 cm⁻¹, 1750 cm⁻¹, 1655 cm⁻¹, 1583 cm⁻¹ and 1391 cm⁻¹.

pH <2.54 Dissolve an amount of Meropenem for Injection, equivalent to 0.25 g (potency) of Meropenem Hydrate according to the labeled amount, in 5 mL of water: the pH of the solution is between 7.3 and 8.3.

Purity (1) Clarity and color of solution—Dissolve an amount of Meropenem for Injection, equivalent to 1.0 g (potency) of Meropenem Hydrate according to the labeled amount, in 20 mL of water: the solution is clear and is not more intensely colored than the following matching fluid.

Matching fluid: To a mixture of 0.3 mL of Cobalt (II) Chloride CS and 1.2 mL of Iron (III) Chloride CS add 18.5 mL of triethylamine-phosphate buffer solution, pH 5.0 (1 in 40). The solution is practically colorless.

Amount, in 20 mL of water: the solution is clear and is not more intensely colored than the following matching fluid.

(2) Related substances—Being specified separately.

Loss on drying <2.41> 9.5 – 12.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 0.12 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass on the contents of not less than 10 containers of Meropenem for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of Meropenem Hydrate, dissolve in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution, pH 5.0, to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Meropenem RS, equivalent to about 50 mg (potency), dissolve in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution, pH 5.0, to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01D> according to the following conditions, and calculate the ratios, Q₁ and Q₅, of the peak area of meropenem to that of the internal standard.

Amount [mg (potency)] of meropenem (C₁₇H₂₅N₃O₅S) = M₅ × Q₁/Q₅

M₅: Amount [mg (potency)] of Meropenem RS

Internal standard solution—A solution of benzyl alcohol in triethylamine-phosphate buffer solution, pH 5.0 (1 in 300).

Operating conditions—Proceed as directed in the operating conditions in the Assay under Meropenem Hydrate.

System suitability—System performance: Proceed as directed in the system suitability in the Assay under Meropenem Hydrate.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of meropenem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Mestranol

メストラノール

C₂₁H₂₆O₂·310.43
3-Methoxy-nor-17α-pregn-1,3,5(10)-tri-en-20-yn-17-ol [72-33-3]

Mestranol, when dried, contains not less than 97.0% and not more than 102.0% of C₂₁H₂₅O₂.

Description Mestranol occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in ethanol (99.5%) and in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 2 mg of Mestranol in 1 mL of a mixture of sulfuric acid and ethanol (99.5) (2:1): a red-purple color develops with a yellow-green fluorescence.

(2) Determine the absorption spectrum of a solution of Mestranol in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mestranol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Mestranol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or
the spectrum of previously dried Mestranol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** $<2.49^\circ \ [\alpha]_{D}^{20} +2+8^\circ$ (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

**Melting point** $<2.60^\circ \ 148 – 154^\circ C$

**Purity** (1) Heavy metals $<1.07^\circ$—Produce with 1.0 g of Mestranol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic $<1.11^\circ$—Produce the test solution with 1.0 g of Mestranol according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Mestranol in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.01^\circ$. Spot 10 µL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (99.5) (29:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly with a mixture of chloroform and ethanol (99.5) (29:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, and heat at 105°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Residue on ignition** $<2.44^\circ$ Not more than 0.1% (0.5 g, 105°C, 3 hours).

**Assay** Weigh accurately about 10 mg each of Mestranol and Mestranol RS, previously dried, dissolve in ethanol (99.5) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, $A_7$ and $A_8$, of the sample solution and the standard solution at 279 nm as directed under Ultraviolet-visible Spectrophotometry $<2.24^\circ$.

Amount (mg) of $C_{21}H_{26}O_{2}$ = $M_s \times A_7/A_8$

$M_s$: Amount (mg) of Mestranol RS

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Metenolone Acetate**

メテノロン酢酸エステル

C$_{22}$H$_{32}$O$_3$: 344.49
1-Methyl-3-oxo-5α-androst-1-en-17β-y1 acetate

Metenolone Acetate, when dried, contains not less than 97.0% and not more than 103.0% of C$_{22}$H$_{32}$O$_3$.

**Description** Metenolone Acetate occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetone, in 1,4-dioxane and in chloroform, soluble in methanol and in ethanol (95%), sparingly soluble in diethyl ether and in sesame oil, slightly soluble in hexane and in petroleum ether, and practically insoluble in water.

**Identification** (1) Dissolve 1 mg of Metenolone Acetate in 5 mL of a mixture of ethanol (95%) and sulfuric acid (1:1), and heat for 30 minutes in a water bath: a red-brown color develops.

(2) To 10 mg of Metenolone Acetate add 0.5 mL of dilute sodium hydroxide-ethanol TS, and heat for 1 minute on a water bath. After cooling, add 0.5 mL of diluted sulfuric acid (1 in 2), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

(3) Dissolve 50 mg of Metenolone Acetate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), and boil for 2 hours under a reflux condenser. After cooling, add this solution gradually to 50 mL of cold water, and stir for 15 minutes. Filter the precipitate so obtained by suction through a glass filter (G4), wash with 10 mL of water, and dry at 105°C for 1 hour: it melts $<2.60^\circ$ between 157°C and 161°C.

(4) Determine the infrared absorption spectrum of Metenolone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25^\circ$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** $<2.49^\circ \ [\alpha]_{D}^{20} +39 – +42^\circ$ (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

**Melting point** $<2.60^\circ \ 141 – 144^\circ C$

**Purity** (1) Clarity and color of solution—Dissolve 0.50 g of Metenolone Acetate in 10 mL of 1,4-dioxane: the solution is clear and colorless to pale yellow.

(2) Heavy metals $<1.07^\circ$—Produce with 2.0 g of Metenolone Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 35 mg of Metenolone Acetate in 20 mL of chloroform, and use this solution as the
sample solution. Pipet 1 mL of the sample solution, dilute with chloroform to exactly 250 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.60 > \). Spot 10 \( \mu L \) each of the standard solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and cyclohexane (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

Loss on drying \( <2.47 > \) Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition \( <2.44 > \) Not more than 0.1% (0.5 g).

Assay Weigh accurately about 10 mg of Metenolone Acetate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and dilute with methanol to exactly 50 mL. Determine the absorbance \( A \) of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry \( <2.24 > \).

\[ \text{Amount (mg) of } C_{27}H_{42}O_3 = A/391 \times 10,000 \]

Containers and storage Containers—Tight containers. Storage—Light-resistant.

**Metenolone Enanthate**

メテノロンエナント酸エステル

![](image)

\( C_{27}H_{42}O_3: 414.62 \)

1-Methyl-3-oxo-5α-androst-1-en-17β-yl heptanoate

[303-42-4]

Metenolone Enanthate, when dried, contains not less than 97.0% and not more than 103.0% of \( C_{27}H_{42}O_3 \).

Description Metenolone Enanthate occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in ethanol (95), in acetone, in 1,4-dioxane, and in chloroform, freely soluble in methanol, in ethyl acetate, in diethyl ether, in cyclohexane, in petroleum ether and in toluene, soluble in sesame oil, and practically insoluble in water.

Identification (1) Heat 1 mg of Metenolone Enanthate with 5 mL of a mixture of ethanol (95) and sulfuric acid (1:1) on a water bath for 30 minutes: a red-brown color develops.

(2) Dissolve 0.05 g of Metenolone Enanthate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), boil under a reflux condenser for 2 hours, cool, add slowly this solution to 50 mL of cold water, and stir for 15 minutes. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the washings become neutral, and dry at 105°C for 1 hour: it melts \( <2.60 > \) between 156°C and 162°C.

Optical rotation \( <2.49 > \) \([\alpha]_{D}^2: +39° – +43° \) (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

Melting point \( <2.60 > \) 67 – 72°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Metenolone Enanthate in 10 mL of 1,4-dioxane: the solution is clear and colorless.

(2) Heavy metals \( <1.07 > \)—Proceed with 2.0 g of Metenolone Enanthate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Metenolone Enanthate in exactly 10 mL of chloroform, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography \( <2.60 > \). Spot 10 \( \mu L \) of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and cyclohexane (1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

Loss on drying \( <2.47 > \) Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition \( <2.44 > \) Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.1 g of Metenolone Enanthate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute with methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute again with methanol to make exactly 100 mL. Determine the absorbance, \( A \), of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry \( <2.24 > \).

\[ \text{Amount (mg) of } C_{27}H_{42}O_3 = A/325 \times 100,000 \]

Containers and storage Containers—Tight containers. Storage—Light-resistant.

**Metenolone Enanthate Injection**

メテノロンエナント酸エステル注射液

Metenolone Enanthate Injection is an oily solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of metenolone enanthate \( (C_{27}H_{42}O_3: 414.62) \).

Method of preparation Prepare as directed under Injections, with Metenolone Enanthate.

Description Metenolone Enanthate Injection is a clear, pale yellow, oily liquid.

Identification (1) Measure a volume of Metenolone Enanthate Injection, equivalent to 0.1 g of Metenolone Enanthate according to the labeled amount, add 20 mL of
Metformin Hydrochloride

メトホルミン塩酸塩

C₇H₁₁N₅.HCl: 165.62
1,1-Dimethylbiguanide monohydrochloride
[1115-70-4]

Metformin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of C₇H₁₁N₅.HCl.

Description Metformin Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in acetic acid (100), and slightly soluble in ethanol (99.5).

Melting point: about 221°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Metformin Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metformin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Metformin Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Metformin Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 2.5 g of Metformin Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add water to make exactly 50 mL, and use this solution as the standard solution (2). Separately, to 0.10 g of 1-cyanoguanidine add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 4-methyl-2-pentanone, 2-methoxyethanol, water, and acetic acid (100) (30:20:5:3) to a distance of about 10 cm, air-dry the plate, then dry at 105°C for 10 minutes. Spray evenly sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS on the plate: the spot other than the principal spot with the sample solution is not more intense than the spot with the standard solution (1), the num-
Metformin Hydrochloride Tablets

メトホルミン塩酸塩錠

Metformin Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of metformin hydrochloride (C₄H₁₁N₅.HCl: 165.62).

Method of preparation Prepare as directed under Tablets, with Metformin Hydrochloride.

Identification Shake an amount of powdered Metformin Hydrochloride Tablets, equivalent to 250 mg of Metformin Hydrochloride according to the labeled amount, with 25 mL of 2-propanol, and filter. Evaporate the filtrate under reduced pressure in a water bath at 40°C, and determine the infrared absorption spectrum of the residue as directed in the potassium chloride disk method under Infrared Spectrophotometry. It exhibits absorption at the wave numbers of about 3370 cm⁻¹, 3160 cm⁻¹, 1627 cm⁻¹, 1569 cm⁻¹ and 1419 cm⁻¹.

Uniformity of dosage unit According to the following conditions, it meets the requirement of the Mass variation test.

Dissolution Being specified separately.

Assay Weigh accurately about 0.1 g of Metformin Hydrochloride Tablets, previously dried at 105°C for 3 hours, and dissolve in 40 mL of acetic anhydride, and titrate with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 4.141 mg of C₄H₁₁N₅.HCl

Containers and storage Contain—Tight containers.

Methamphetamine Hydrochloride

メタンフェタミン塩酸塩

C₉H₁₅N.HCl: 185.69
(2S)-N-Methyl-1-phenylpropan-2-amine monohydrochloride [51-57-0]

Methamphetamine Hydrochloride, when dried, contains not less than 98.5% of C₉H₁₅N.HCl.

Description Methamphetamine Hydrochloride occurs as colorless crystals or a white, crystalline powder. It is odorless.

It is freely soluble in water, in ethanol (95) and in chlo-
Melting point

0.2 g, water, 10 mL, 100 mm).

Weigh accurately about 0.4 g of Methamphetamine.

Assay

2 hours).

Optical rotation

mol/L sodium hydroxide VS: a yellow color develops.

mol/L sulfuric acid VS: a red color develops.

solution as the sample solution.

and cooled water, add 2 drops of methyl red TS, and use this

Methamphetamine Hydrochloride in 40 mL of freshly boiled

Acidity or alkalinity—Dissolve 2.0 g of

Purity (1)

<

mixture of acetic anhydride and acetic acid (100) (7:3).

98.5

Loss on drying

<

stand for 10 minutes: the solution remains unchanged.

Optical rotation

<2.49> [α]D: +16 – +19° (after drying, 0.2 g, water, 10 mL, 100 mm).

Melting point

<2.60> 171 – 175°C

Purity (1) Acidity or alkalinity—Dissolve 2.0 g of Methamphetamine Hydrochloride in 40 mL of freshly boiled and cooled water, add 2 drops of methyl red TS, and use this solution as the sample solution.

(i) To 20 mL of the sample solution add 0.20 mL of 0.01 mol/L sulfuric acid VS: a red color develops.

(ii) To 20 mL of the sample solution add 0.20 mL of 0.02 mol/L sodium hydroxide VS: a yellow color develops.

2.41

Loss on drying

<2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition

<2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Methamphetamine Hydrochloride, previously dried, and digest in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

= 18.57 mg of C₁₀H₁₅N.HCl

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

L-Methionine

L-メチオニン

C₃H₁₁NO₂S: 149.21

(2S)-2-Amino-4-(methylsulfonyl)butanoic acid

[63-69-3]

L-Methionine, when dried, contains not less than 98.5% of C₃H₁₁NO₂S.

Description L-Methionine occurs as white crystals or crystalline powder. It has a characteristic odor.

It is freely soluble in formic acid, soluble in water, and very slightly soluble in ethanol (95). It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Methionine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D: +21.0 – +25.0° (after drying, 0.5 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 0.5 g of L-Methionine in 20 mL of water: the pH of this solution is between 5.2 and 6.2.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Methionine in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of L-Methionine in 20 mL of water, and add 6 mL of dilute nitric acid and water to make 40 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 40 mL. In this test, to the test solution and the control solution add 10 mL each of silver nitrate TS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.25 g of L-Methionine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Methionine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Dissolve 1.0 g of L-Methionine in 40 mL of water and 2 mL of dilute acetic acid, dissolve by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic <1.11>—Transfer 1.0 g of L-Methionine to a 100-mL decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add two 2-mL portions of nitric acid, heat, add 2 mL portions of hydrogen peroxide (30) several times, and heat until the solution becomes colorless or pale yellow. After cooling, add 2 mL of saturated ammonium oxalate monohydrate solution, and heat again until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Methionine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. After air-drying, immediately develop the plate with a mixture of 1-butanol, water
and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**<sub>2.47</sub> Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition**<sub>2.48</sub> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.15 g of L-Methionine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate<sub>2.50</sub> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
\[= 14.92 \text{ mg of C}_2\text{H}_3\text{NO}_3\text{S}\]

**Containers and storage** Containers—Tight containers.

### Methotrexate

メトトレキサート

![Methotrexate structure](Image)

C<sub>20</sub>H<sub>22</sub>N<sub>8</sub>O<sub>5</sub>: 454.44

N-[4-[2,4-Diaminopterin-6-ylmethyl](methyl)amino]benzoyl-L-glutamic acid [59-05-2]

Methotrexate is a mixture of 4-amino-10-methylfolic acid and closely related compounds.

It contains not less than 94.0% and not more than 102.0% of C<sub>20</sub>H<sub>22</sub>N<sub>8</sub>O<sub>5</sub>, calculated on the anhydrous basis.

**Description** Methotrexate occurs as a yellow-brown, crystalline powder.

It is slightly soluble in pyridine, and practically insoluble in water, in acetonitrile, in ethanol (95) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS and in dilute sodium carbonate TS.

It is gradually affected by light.

**Identification** (1) Dissolve 1 mg of Methotrexate in 100 mL of 0.1 mol/L hydrochloric acid TS. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry<sub>2.24</sub>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methotrexate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methotrexate as directed in the potassium bromide disk method under Infrared Spectrophotometry<sub>2.25</sub>, and compare the spectrum with the Reference Spectrum or the spectrum of Methotrexate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Water**<sub>2.48</sub> Take 5 mL of pyridine for water determination and 20 mL of methanol for Karl Fischer method in a dried titration flask, and titrate with water determination TS until the end point. Weigh accurately about 0.2 g of Methotrexate, immediately place in the titration flask, and add a known excess volume of Karl Fischer TS. Mix well for 30 minutes, and perform the test: the water content is not more than 12.0%.

**Residue on ignition**<sub>2.48</sub> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 25 mg each of Methotrexate and Methotrexate RS, dissolve in the mobile phase to make exactly 250 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 µL each of these solutions as directed under Liquid Chromatography<sub>2.07</sub> according to the following conditions, and determine the peak areas, A<sub>T</sub> and A<sub>S</sub>, of methotrexate in each solution.

\[
\text{Amount (mg) of C}_{20}\text{H}_{22}\text{N}_8\text{O}_5 = M_S \times \frac{A_T}{A_S}
\]

\[M_S: \text{Amount (mg) of Methotrexate RS, calculated on the anhydrous basis}\]

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 302 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 and acetonitrile (89:11).

Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 8 minutes.

**System suitability**—

System performance: Dissolve 10 mg each of Methotrexate and folic acid in 100 mL of the mobile phase. When the procedure is run with 10 µL of this solution under the above operating conditions, folic acid and methotrexate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

### Methotrexate Capsules

メトトレキサートカプセル

Methotrexate Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of methotrexate (C<sub>20</sub>H<sub>22</sub>N<sub>8</sub>O<sub>5</sub>; 454.44).

**Method of preparation** Prepare as directed under Capsules, with Methotrexate.
Identification To an amount of the content of Methotrexate Capsules, equivalent to 2 mg of Methotrexate according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 10 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $<2.245$; it exhibits maxima between 240 nm and 244 nm and between 304 nm and 308 nm.

Uniformity of dosage units $<6.02>$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To the content of 1 capsule of Methotrexate Capsules add $3 V/5 mL$ of the mobile phase, agitate with the aid of ultrasonic waves for 15 minutes, then shake for 25 minutes, and add the mobile phase to make exactly $V$ mL so that each mL contains about $20 \mu g$ of methotrexate ($C_{20}H_{22}N_{8}O_{5}$). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water $<2.48>$ in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Perform the test with 20 $\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography $<2.07>$ according to the following conditions, and determine the peak areas, $A_{T}$ and $A_{S}$, of methotrexate of both solutions.

\[
\text{Dissolution rate (\%)} = \frac{M_{S} \times A_{T}/A_{S} \times V/V 	imes 1/C \times 18}{M_{S}} \times C
\]

$M_{S}$: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

C: Labeled amount (mg) of methotrexate ($C_{20}H_{22}N_{8}O_{5}$) in 1 capsule

Operating conditions—
Proceed as directed in the operating conditions in the Assay.

System suitability—
System performance: When the procedure is run with 50 $\mu L$ of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of methotrexate are not less than 3500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 $\mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

Assay Accurately weigh the mass of not less than 20 Methotrexate Capsules, take out all of the content, and accurately weigh the mass of the empty capsules. Powder the content, weigh accurately a portion of the powder, equivalent to about 10 mg of methotrexate ($C_{20}H_{22}N_{8}O_{5}$), add 60 mL of the mobile phase, shake for 25 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water $<2.48>$ in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 20 $\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography $<2.07>$, and calculate the ratios, $Q_{T}$ and $Q_{S}$, of the peak area of methotrexate to that of the internal standard.

\[
\text{Amount (mg) of methotrexate} = M_{S} \times Q_{T}/Q_{S}
\]

$M_{S}$: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

Internal standard solution—A solution of 4-nitrophenol in
methanol (1 in 10,000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 302 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 250 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 28.5 mL of 0.2 mol/L sodium hydroxide TS and water to make 1000 mL. To 890 mL of this solution add 110 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 6 minutes.

**System suitability**—

System performance: Dissolve 10 mg each of methotrexate and folic acid in 100 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, folic acid and methotrexate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methotrexate to that of the internal standard is not more than 1.0%.

**Containers and storage**  Containers—Tight containers.

### Methoxsalen

メトキサレン

C₁₂H₈O₄: 216.19

9-Methoxy-7H-furo[3,2-g]chromen-7-one

[298-81-7]

Methoxsalen occurs as white to pale yellow crystals or crystalline powder. It is odorless and tasteless. It is freely soluble in chloroform, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

**Description**  Methoxsalen occurs as white to pale yellow crystals or crystalline powder. It is odorless and tasteless. It is freely soluble in chloroform, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

**Identification** (1)  To 10 mg of Methoxsalen add 5 mL of dilute nitric acid, and heat: a yellow color develops. Make this solution alkaline with a solution of sodium hydroxide (2 in 5): the color changes to red-brown.

(2)  To 10 mg of Methoxsalen add 5 mL of sulfuric acid, and shake: a yellow color develops.

(3)  Determine the absorption spectrum of a solution of Methoxsalen in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methoxsalen RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point**  <2.60°  145 – 149°C

**Purity** (1)  Heavy metals <1.07>—Proceed with 1.0 g of Methoxsalen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2)  Arsenic <1.11>—Prepare the test solution with 1.0 g of Methoxsalen according to Method 3, and perform the test (not more than 2 ppm).

(3)  Related substances—Dissolve 50 mg of Methoxsalen in 10 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add chloroform to make exactly 50 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, hexane and ethyl acetate (40:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Water** <2.48>  Not more than 0.5% (1 g, volumetric titration, direct titration).

**Residue on ignition** <2.44>  Not more than 0.1% (1 g).

**Assay**  Weigh accurately about 50 mg each of Methoxsalen and Methoxsalen RS, and dissolve each in ethanol (95) to make exactly 100 mL. Pipet 2 mL each of these solutions, and dilute each with ethanol (95) to make exactly 25 mL. Pipet 10 mL each of these solutions, and dilute each again with ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A₉ and A₈, of the sample solution and the standard solution at 300 nm as directed under Ultraviolet-visible Spectrophotometry 2.24.

\[
\text{Amount (mg) of } \text{C}_12\text{H}_8\text{O}_4 = M_S \times A_9 / A_8
\]

\[
M_S: \text{Amount (mg) of Methoxsalen RS, calculated on the anhydrous basis}
\]

**Containers and storage**  Containers—Well-closed containers.

**Storage**—Light-resistant.
Methylbenactyzium Bromide
メチルベナクチジウム臭化物

\[
\text{C}_2\text{H}_2\text{BrNO}_3: 422.36
\]
\[
N,N\text{-Diethyl}-2\{[\text{hydroxyl}]\text{(diphenyl)acetoxyl}\}-N\text{-methyl}methylaminium bromide
\]

Methylbenactyzium Bromide, when dried, contains not less than 99.0\% of \(\text{C}_2\text{H}_3\text{BrNO}_3\).

**Description**
Methylbenactyzium Bromide occurs as white crystals or crystalline powder. It is odorless, and has an extremely bitter taste.

It is freely soluble in water and in acetic acid (100), soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Methylbenactyzium Bromide (1 in 100) is between 5.0 and 6.0.

**Identification** (1)
Shake 0.5 mL of a solution of Methylbenactyzium Bromide (1 in 100) with 5 mL of phosphate buffer solution, pH 7.0, 2 to 3 drops of bromothymol blue TS and 5 mL of chloroform: a yellow color develops in the chloroform layer.

(2) To about 1 g of Methylbenactyzium Bromide add 5 mL of water and 10 mL of sodium hydroxide TS, allow to stand for 5 minutes, add 5 mL of dilute hydrochloric acid, collect the precipitate, wash well with water, recrystallize from a mixture of water and ethanol (95) (10:3), and dry at 105°C for 1 hour: the crystals melt \(<2.60\) between 145°C and 150°C. Continue the heating up to about 200°C: a red color develops.

(3) Add 2 mL of dilute nitric acid to 5 mL of a solution of Methylbenactyzium Bromide (1 in 10): the solution responds to the Qualitative Tests \(<1.09\) (1) for bromide.

**Melting point** \(<2.60\> 168 – 172°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Methylbenactyzium Bromide in 10 mL of water: the solution is clear and colorless.

(2) Sulfate \(<1.14\>—Perform the test with 0.5 g of Methylbenactyzium Bromide. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals \(<1.07\>—Proceed with 2.0 g of Methylbenactyzium Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Loss on drying** \(<2.41\> Not more than 0.5\% (2 g, 105°C, 2 hours).

**Residue on ignition** \(<2.44\> Not more than 0.1\% (1 g).

**Assay**
Weigh accurately about 0.5 g of Methylbenactyzium Bromide, previously dried, and dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (4:1). Titrate \(<2.50\> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 42.24 mg of \(\text{C}_2\text{H}_3\text{BrNO}_3\)

**Containers and storage**
Containers—Tight containers.

Methylcellulose
メチルセルロース

**Cellulose, methyl ether**

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (** )

Methylcellulose is a methyl ether of cellulose. It contains not less than 26.0\% and not more than 33.0\% of methoxy group (\(-\text{OCH}_3\): 31.03), calculated on the dried basis.

The viscosity of Methylcellulose is shown in milipascal second (mPa-s).

**Description**
Methylcellulose occurs as a white to yellowish white, powder or granules. It is practically insoluble in ethanol (99.5).
Methylcellulose swells, when water is added, and forms a clear or slightly turbid, viscous liquid.

**Identification** (1)
Disperse evenly 1.0 g of Methylcellulose over the surface of 100 mL of water in a beaker, while gently tapping the top of the container, if necessary, and allow the beaker to stand: it aggregates on the surface of water.

(2) Add 1.0 g of Methylcellulose to 100 mL of hot water, and stir: it becomes a suspension. Cool the suspension to 5°C, and stir: the resulting liquid is a clear or a slightly cloudy, viscous fluid.

(3) To 0.1 mL of the viscous fluid obtained in (2) add 9 mL of dilute sulfuric acid (9 in 10), stir, heat in a water bath for exactly 3 minutes, and immediately cool in ice water. Add carefully 0.6 mL of ninhydrin TS, stir, and allow to stand at 25°C: the solution shows a light red color, and it does not change to purple color within 100 minutes.

(4) Pour and spread out 2 to 3 mL of the viscous fluid obtained in (2) onto a glass plate, and allow the water to evaporate: a transparent film results.

(5) Pipet 50 mL of water, add exactly 50 mL of the viscous fluid obtained in (2), and warm to rise the temperature at a rate of 2 to 5°C per minute while stirring: the temperature, when a white turbidity of the solution starts to increase, is not less than 50°C.

**Viscosity** \(<2.55\>

(1) Method I: Apply to Methylcellulose having a labeled viscosity of less than 600 mPa-s. Put exactly an amount of Methylcellulose, equivalent to 4.000 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 200.0 g, stopper the bottle, stir by mechanical means at 350- to 450-
revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath not exceeding 5°C for 20 to 40 minutes. Add cooled water, if necessary, to make 200.0 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

(ii) Method II: Apply to Methylcellulose having a labeled viscosity of not less than 600 mPa·s. Put exactly an amount of Methylcellulose, equivalent to 10.00 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 500.0 g, stopper the bottle, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method II (2) under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

Operating conditions—
Apparatus: Brookfield type viscometer LV model.
Rotor No., rotation frequency, and conversion factor: According to the following table, depending on the labeled viscosity.

<table>
<thead>
<tr>
<th>Labeled viscosity (mPa·s)</th>
<th>Rotor No.</th>
<th>Rotation frequency /min</th>
<th>Conversion factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not less than 600 and less than 1400</td>
<td>3</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>n = 1400</td>
<td>n = 3500</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>n = 3500</td>
<td>n = 9500</td>
<td>4</td>
<td>60</td>
</tr>
<tr>
<td>n = 9500</td>
<td>n = 99,500</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Procedure of apparatus: Read value after 2 minutes of rotation, and stop the rotation for 2 minutes. Repeat this procedure more two times, and average three observed values.

pH <2.54> Allow the sample solution obtained in the Viscosity to stand at 20 ± 2°C for 5 minutes: the pH of the solution thus obtained is between 5.0 and 8.0.

Purity Heavy metals—Put 1.0 g of Methylcellulose in a 100-mL kjeldahl flask, add a sufficient amount of a mixture of nitric acid and sulfuric acid (5:4) to wet the sample, and heat gently. Repeat this procedure until use totally 18 mL of the mixture of nitric acid and sulfuric acid. Then boil gently until the solution changes to black. After cooling, add 2 mL of nitric acid, and heat until the solution changes to black. Repeat this procedure until the solution no longer changes to black, and heat strongly until dense white fumes are evolved. After cooling, add 5 mL of water, boil gently until dense white fumes are evolved, then heat until the volume of the solution becomes to 2 to 3 mL. After cooling, if the solution reveals yellow color by addition of 5 mL of water, add 1 mL of hydrogen peroxide (30), and heat until the volume of the solution becomes to 2 to 3 mL. After cooling, dilute the solution with 2 to 3 mL of water, transfer to a Nessler tube, add water to make 25 mL, and use this solution as the test solution. Separately, put 2.0 mL of Standard Lead Solution in a 100-mL kjeldahl flask, add 18 mL of the mixture of nitric acid and sulfuric acid (5:4) and an amount of nitric acid equal to that used for preparation of the test solution, and heat until white fumes are evolved. After cooling, add 10 mL of water. In the case where hydrogen peroxide (30) is added for the preparation of the test solution, add the same amount of hydrogen peroxide (30), then proceed in the same manner for preparation of the test solution, and use so obtained solution as the control solution. Adjust the test solution and the control solution to pH 3.0 to 4.0 with ammonia solution (28), and add water to make 40 mL, respectively. To these solutions add 1.2 mL of thioacetamide-alkaline glycerin TS, 2 mL of acetate buffer solution, pH 3.5 and water to make 50 mL, separately. After allowing to stand for 5 minutes, observe vertically both tubes on a white background: the color obtained with the test solution is not more intense than that with the control solution (not more than 20 ppm).

Loss on drying <2.4f> Not more than 5.0% (1 g, 105°C, 1 hour).

Residue on ignition <2.4g> Not more than 1.5% (1 g).

Assay (i) Apparatus—Reaction bottle: A 5-mL pressure-tight glass vial, having 20 mm in outside diameter and 50 mm in height, the neck 20 mm in outside diameter and 13 mm in inside diameter, equipped with a septum of butyl-rubber processed the surface with fluoroplastics, which can be fixed tightly to vial with aluminum cap, or equivalent. Heater: A square-shaped aluminum block, having holes 20 mm in diameter and 32 mm in depth, adopted to the reaction bottle. Capable of stirring the content of the reaction bottle by means of magnetic stirrer or of reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Methylcellulose, transfer to the reaction bottle, add 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Stir or shake for 60 minutes while heating so that the temperature of the bottle content is 130 ± 2°C. In the case when the stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the bottle to cool, and again weigh accurately. If the mass loss is less than 0.50% or there is no evidence of a leak, use the upper layer of the mixture as the sample solution. Separately, put 0.06 to 0.10 g of adipic acid in a reaction bottle, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Add 45 μL of iodomethane for assay through the septum using micro-syringe, weigh accurately, stir thoroughly, and use the upper layer of the mixture as the standard solution. Perform the test with 1 to 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Qr and Qs, of the peak area of iodomethane to that of the internal standard.

\[ \text{Content (%) of methoxy group (CH}_3\text{O)} = \frac{M_s}{M_s/M_s + Q_r/Q_s} \times 21.86 \]

M_s: Amount (mg) of iodomethane in the standard solution
M: Amount (mg) of sample, calculated on the dried basis
Internal standard solution—A solution of n-octane in o-xylene (3 in 100).

Operating conditions—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column 3–4 mm in inside diameter and 1.8–3 m in length, packed with silicaceous earth for gas chromatography, 125 to 150 μm in diameter, coated with methyl silicone polymer at the ratio of 10–20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium for thermal conductivity detector, or Helium or Nitrogen for hydrogen, flame-ionization detector.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

System suitability—

System performance: When the procedure is run with 1–2 μL of the standard solution under the above operating conditions, iodomethane and the internal standard are eluted in this order, with complete separation of these peaks.

*Containers and storage* Containers—Well-closed containers.

**Methyldopa Hydrate**

メチルドパ水和物

C₁₀H₁₃NO₄·½H₂O: 238.24

(2S)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate

[M1372-08-1]

Methyldopa Hydrate contains not less than 98.0% of methyldopa (C₁₀H₁₃NO₄·½H₂O: 211.21), calculated on the anhydrous basis.

**Description** Methyldopa Hydrate occurs as a white to pale grayish white, crystalline powder.

It is slightly soluble in water, in methanol and in acetic acid (100), very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid.

**Identification** (1) To 10 mg of Methyldopa Hydrate add 3 drops of ninhydrin TS, and heat in a water bath for 3 minutes: a purple color develops.

(2) Determine the absorption spectrum of a solution of Methyldopa Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.49>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyldopa RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Methyldopa Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Methyldopa RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [α]D: −25−28° (calculated on the anhydrous basis, 1 g, aluminum (III) chloride TS, 20 mL, 100 mm).

**Purity** (1) Acidity—Shake 1.0 g of Methyldopa Hydrate with 100 mL of freshly boiled and cooled water, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of methyl red TS: a yellow color develops.

(2) Chloride <1.03>—Perform the test with 0.5 g of Methyldopa Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Methyldopa Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Methyldopa Hydrate in 5 mL of dilute hydrochloric acid, and perform the test (not more than 2 ppm).

(5) 3-O-Methylmethyldopa—Dissolve 0.10 g of Methyldopa Hydrate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 5 mg of 3-O-methylmethyldopa for thin-layer chromatography in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (13:5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-nitroaniline-sodium nitrite TS on the plate, and air-dry the plate, then spray evenly a solution of sodium carbonate decahydrate (1 in 4) on the plate: the spot from the sample solution corresponding to that from the standard solution is not more intense than the spot from the standard solution.

**Water** <2.48> 10.0–13.0% (0.2 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Methyldopa Hydrate, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 to 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 21.12 mg of C₁₀H₁₃NO₄.

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.
Methyldopa Tablets

Methyldopa Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methyldopa (C_{10}H_{13}NO_{4}: 211.21).

Method of preparation Prepare as directed under Tablets, with Methyldopa Hydrate.

Identification (1) To a quantity of powdered Methyldopa Tablets, equivalent to 0.1 g of Methyldopa Hydrate according to the labeled amount, add 10 mL of water, heat in a water bath for 5 minutes with occasional shaking. After cooling, centrifuge for 5 minutes at 2000 rotations per minute, apply 1 drop of the supernatant solution to a filter paper, and dry with warm air. Place 1 drop of ninhydrin TS over the spot, and heat for 5 minutes at 100°C: a purple color develops.

(2) To 0.5 mL of the supernatant liquid obtained in the Identification (1) add 2 mL of 0.05 mol/L sulfuric acid TS, 2 mL of iron (II) tartrate TS and 4 drops of ammonia TS, and shake well: a deep purple color develops.

(3) To 0.7 mL of the supernatant liquid obtained in the Identification (1) add 0.1 mol/L hydrochloric acid TS to make 20 mL. To 10 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 277 nm and 283 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Methyldopa Tablets add 50 mL of 0.05 mol/L sulfuric acid TS, shake for 15 minutes, then add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate equivalent to about 5 mg of methyldopa (C_{10}H_{13}NO_{4}), add exactly 5 mL of iron (II) tartrate TS, then add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (previously dry at 125°C for 2 hours), and dissolve in 0.05 mol/L sulfuric acid TS, shake thoroughly for 15 minutes, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (previously dry at 125°C for 2 hours), and determine the loss on drying <2.4>, dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 5 mL of iron (II) tartrate TS, and add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solutions and the standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Dissolution rate (\%)} = \frac{M_S}{M_T} \times \frac{A_T}{A_S} \times \frac{V}{V} \times \frac{1}{C} \times 45
\]

M_S: Amount (mg) of methyldopa for assay, calculated on the dried basis

C: Labeled amount (mg) of methyldopa (C_{10}H_{13}NO_{4}) in 1 tablet

Assay Weigh accurately and powder not less than 20 Methyldopa Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of methyldopa (C_{10}H_{13}NO_{4}), add 50 mL of 0.05 mol/L sulfuric acid TS, shake thoroughly for 15 minutes, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (previously dry at 125°C for 2 hours), and determine the loss on drying <2.4>, dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 5 mL of iron (II) tartrate TS, and add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of 0.05 mol/L sulfuric acid TS in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 520 nm, respectively.

\[
\text{Amount (mg) of methyldopa (C}_{10}\text{H}_{13}\text{NO}_{4}) = M_S \times \frac{A_T}{A_S} \times \frac{V}{V}
\]

M_S: Amount (mg) of Methyldopa RS, calculated on the dried basis

Containers and storage Containers—Well-closed containers.

Start the test with 1 tablet of Methyldopa Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 25 μg of methyldopa (C_{10}H_{13}NO_{4}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of methyldopa for assay (separately determine the loss on drying <2.4> at 125°C for 2 hours), and dissolve in water to make exactly 200 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Dissolution rate (\%)} = \frac{M_S}{M_T} \times \frac{A_T}{A_S} \times \frac{V}{V} \times \frac{1}{C} \times 45
\]

M_S: Amount (mg) of methyldopa for assay, calculated on the dried basis

C: Labeled amount (mg) of methyldopa (C_{10}H_{13}NO_{4}) in 1 tablet

Assay Weigh accurately and powder not less than 20 Methyldopa Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of methyldopa (C_{10}H_{13}NO_{4}), add 50 mL of 0.05 mol/L sulfuric acid TS, shake thoroughly for 15 minutes, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (previously dry at 125°C for 2 hours), and determine the loss on drying <2.4>, dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 5 mL of iron (II) tartrate TS, and add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of 0.05 mol/L sulfuric acid TS in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 520 nm, respectively.

\[
\text{Amount (mg) of methyldopa (C}_{10}\text{H}_{13}\text{NO}_{4}) = M_S \times \frac{A_T}{A_S} \times \frac{V}{V}
\]

M_S: Amount (mg) of Methyldopa RS, calculated on the dried basis

Containers and storage Containers—Well-closed containers.


**dl-Methylephedrine Hydrochloride**

*dl-メチルエフェドリン塩酸塩*

\[ \text{C}_{11}\text{H}_{17}\text{NO.HCl}: 215.72 \]

\[(1R,5S,2R)-2\text{-Dimethylamino-1-phenylpropan-1-ol monohydrochloride} [18760-80-0]\]

*dl-Methylephedrine Hydrochloride,* when dried, contains not less than 99.0% and not more than 101.0% of \( \text{C}_{11}\text{H}_{17}\text{NO.HCl} \).

**Description**  *dl-Methylephedrine Hydrochloride* occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in water, sparingly soluble in ethanol (99.5), slightly soluble in acetic acid (100), and practically insoluble in acetic anhydride.

A solution of *dl-Methylephedrine Hydrochloride* (1 in 20) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of *dl-Methylephedrine Hydrochloride* (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry \(<2.34>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of *dl-Methylephedrine Hydrochloride,* previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of *dl-Methylephedrine Hydrochloride* (1 in 10) responds to the Qualitative Tests \(<1.09>\) for chloride.

**pH \(<2.54>\)** The pH of a solution prepared by dissolving 1.0 g of *dl-Methylephedrine Hydrochloride* in 20 mL of water is between 4.5 and 6.0.

**Melting point \(<2.60>\)** 207–211\(^\circ\)C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of *dl-Methylephedrine Hydrochloride* in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals \(<1.07>\)—Proceed with 1.0 g of *dl-Methylephedrine Hydrochloride* according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of *dl-Methylephedrine Hydrochloride* in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L of each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of methylephedrine is not larger than the peak area of methylephedrine from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40\(^\circ\)C.

Mobile phase: Dissolve 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylephedrine is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of methylephedrine beginning after the solvent peak.

**System suitability**—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of methylephedrine obtained from 20 \( \mu \)L of this solution is equivalent to 7 to 13% of that from 20 \( \mu \)L of the standard solution.

System performance: Dissolve 50 mg of *dl-Methylephedrine Hydrochloride* and 0.4 mg of methyl parahydroxybenzoate in 50 mL of water. When the procedure is run with 20 \( \mu \)L of this solution under the above operating conditions, methylephedrine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylephedrine is not more than 2.0%.

**Loss on drying \(<2.4I>\)** Not more than 0.5% (1 g, 105\(^\circ\)C, 3 hours).

**Residue on ignition \(<2.44>\)** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of *dl-Methylephedrine Hydrochloride,* previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate \(<2.5p>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[ \text{Each mL of 0.1 mol/L perchloric acid VS} \]
\[ = 21.57 \text{mg of C}_{11}\text{H}_{17}\text{NO.HCl} \]

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.
10% *dl*-Methylephedrine Hydrochloride Powder

*dl*-Methylephedrine Hydrochloride Powder

*dl*-メチルエフェドリン塩酸塩散 10%

10% *dl*-Methylephedrine Hydrochloride Powder contains not less than 9.3% and not more than 10.7% of *dl*-methylephedrine hydrochloride (C₇H₁₇NO·HCl: 215.72).

**Method of preparation**

<table>
<thead>
<tr>
<th>dl*-Methylephedrine Hydrochloride</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch, Lactose Hydrate or</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

Prepare as directed under Granules or Powders, with the above ingredients.

**Identification** Determine the absorption spectrum of a solution of 10% *dl*-Methylephedrine Hydrochloride Powder (1 in 200) as directed under Ultraviolet-visible Spectrophotometry (c2.2d): it exhibits maxima between 250 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 264 nm.

**Assay** Weigh accurately about 0.5 g of 10% *dl*-Methylephedrine Hydrochloride Powder, add exactly 4 mL of the internal standard solution and 25 mL of water, shake vigorously for 20 minutes to dissolve, add water to make 50 mL, filter through a membrane filter with pore size of 0.45 μm, if necessary, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of *dl*-methylephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 4 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (c2.01) according to the following conditions, and calculate the ratios of the peak area, Q₁ and Q₂, of methylephedrine to that of the internal standard.

Amount (mg) of *dl*-methylephedrine hydrochloride

\[ M_s = \frac{M_0 \times Q_1}{Q_2} \]

where

- **M₀**: Amount (mg) of *dl*-methylephedrine hydrochloride for assay
- **Mₘ**: Amount (mg) of *dl*-methylephedrine hydrochloride

**Internal standard solution**—A solution of methyl parahydroxybenzoate in acetonitrile (1 in 10,000).

**Operating conditions**

- **Detector**: An ultraviolet absorption photometer (wavelength: 257 nm).
- **Column**: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature**: A constant temperature of about 40°C.
- **Mobile phase**: Dissolve 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.
- **Flow rate**: Adjust the flow rate so that the retention time of methylephedrine is about 10 minutes.
- **System suitability**—
  - System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, methylephedrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.
  - System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methylephedrine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers. Storage—Light-resistant.

**Methylergometrine Maleate**

メチルエルゴメトリンマレイン酸塩

\[
\text{C}_{20}\text{H}_{25}\text{N}_3\text{O}_2\cdot\text{C}_4\text{H}_4\text{O}_4: 455.50 \quad (8S)-N-[(1S)-1-(Hydroxymethyl)propyl]-6-methyl-9,10-didehydroergoline-8-carboxamide monomaleate [7054-07-1]
\]

Methylergometrine Maleate, when dried, contains not less than 95.0% and not more than 105.0% of C₂₀H₂₅N₃O₂·C₄H₄O₄.

**Description** Methylergometrine Maleate occurs as a white to pale yellow, crystalline powder. It is odorless. It is slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It gradually changes to yellow by light.

Melting point: about 190°C (with decomposition).

**Identification** (1) A solution of Methylergometrine Maleate (1 in 200) shows a blue fluorescence.

(2) The colored solution obtained in the Assay develops a deep blue in color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry (c2.2d), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylergometrine Maleate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) To 5 mL of a solution of Methylergometrine Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the test solution fades immediately.

**Optical rotation** (c2.4p) \([\alpha]_D^{20} +44° - +50°\) (after drying,
Methylergometrine Maleate Tablets / Official Monographs

**Purity**
Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 8 mg of Methylergometrine Maleate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (95) and ammonia solution (28) (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test immediately with these solutions as directed under Thin-layer Chromatography 

\[
\text{Rf} < 0.67
\]
Spot 10 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, and immediately develop the plate with a mixture of chloroform, methanol and water (75:25:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**
Not more than 2.0% (0.2 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Assay**
Weigh accurately about 10 mg each of Methylergometrine Maleate Tablets and Methylergometrine Maleate RS; previously dried to constant weight in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours (indeed for 4 hours). Weigh accurately about 10 mg each of Methylergometrine Maleate Tablets and Methylergometrine Maleate RS, previously dried in a desiccator for 4 hours (in vacuum, phosphorus (V) oxide), separately into brown glass-stoppered test tubes, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a brown glass-stoppered centrifuge tube, and add 3 g of sodium chloride and 2 mL of ammonia solution (28), add exactly 25 mL of chloroform, and after vigorous shaking for 10 minutes, centrifuge for 5 minutes. Discard the water layer, take the chloroform extracts, add chloroform to make exactly \( V \) mL of a solution containing about 5 \( \mu g \) of methylergometrine maleate \((C_{20}H_{25}N_3O_2\cdotC_4H_4O_4)\) per mL, and use this solution as the sample solution. Separately, weigh accurately about 1.25 mg of Methylergometrine Maleate RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a brown glass-stoppered centrifuge tube, and add 3 g of sodium chloride and 2 mL of ammonia solution (28). Add exactly 25 mL of chloroform, shake vigorously for 10 minutes, and centrifuge for 5 minutes. Discard the water layer, and use the chloroform layer as the standard solution. Pipet 20 mL each of the sample solution and the standard solution separately into brown glass-stoppered centrifuge tubes, add immediately exactly 10 mL of dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and shake for 5 minutes vigorously. Centrifuge these solutions for 5 minutes, take the water layers, and allow them to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), using dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS as the blank. Determine the absorbances, \( A_T \) and \( A_S \), of the subsequent solutions of the sample solution and standard solution at 545 nm, respectively.

\[
\text{Amount (mg) of } C_{20}H_{25}N_3O_2\cdotC_4H_4O_4 = M_S \times \frac{A_T}{A_S}
\]

\( M_S \): Amount (mg) of Methylergometrine Maleate RS

**Containers and storage**
Containers—Tight containers. Storage—Light-resistant.

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**Methylergometrine Maleate Tablets**
メチルエルゴメトリンマレイン酸塩錠

Methylergometrine Maleate Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methylergometrine maleate \((C_{20}H_{25}N_3O_2\cdotC_4H_4O_4) \approx 455.50\).

**Method of preparation**
Prepare as directed under Tablets, with Methylergometrine maleate.

**Identification**
(1) The sample solution obtained in the Assay shows a blue fluorescence.
(2) The colored solution obtained in the Assay shows a deep blue color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \): it exhibits maxima between 543 nm and 547 nm and between 620 nm and 630 nm.

**Uniformity of dosage unit**
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Methylergometrine Maleate Tablets to a brown glass-stoppered centrifuge tube, add 10 mL of water, shake for 10 minutes vigorously, and disintegrate the tablet. Add 3 g of sodium chloride and 2 mL of ammonia solution (28), add exactly 25 mL of chloroform, and after vigorous shaking for 10 minutes, centrifuge for 5 minutes. Discard the water layer, take the chloroform extracts, add chloroform to make exactly \( V \) mL of a solution containing about 5 \( \mu g \) of methylergometrine maleate \((C_{20}H_{25}N_3O_2\cdotC_4H_4O_4)\) per mL, and use this solution as the sample solution. Separately, weigh accurately about 1.25 mg of Methylergometrine Maleate RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a brown glass-stoppered centrifuge tube, and add 3 g of sodium chloride and 2 mL of ammonia solution (28). Add exactly 25 mL of chloroform, shake vigorously for 10 minutes, and centrifuge for 5 minutes. Discard the water layer, and use the chloroform layer as the standard solution. Pipet 20 mL each of the sample solution and the standard solution separately into brown glass-stoppered centrifuge tubes, add immediately exactly 10 mL of dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and shake for 5 minutes vigorously. Centrifuge these solutions for 5 minutes, take the water layers, and allow them to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), using dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS as the blank. Determine the absorbances, \( A_T \) and \( A_S \), of the subsequent solutions of the sample solution and standard solution at 545 nm, respectively.

\[
\text{Amount (mg) of methylergometrine maleate } (C_{20}H_{25}N_3O_2\cdotC_4H_4O_4) = M_S \times \frac{A_T}{A_S} \times \frac{V}{250}
\]

\( M_S \): Amount (mg) of Methylergometrine Maleate RS

**Dissolution**
When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Methylergometrine Maleate Tablets is not less than 70%.

Start the test with 1 tablet of Methylergometrine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \( \mu m \). Discard the first 10 mL of the filtrate, to exactly \( V \) mL of the subsequent filtrate add water to make exactly \( V \) mL so that each mL contains about 0.13 \( \mu g \) of methylergometrine maleate \((C_{20}H_{25}N_3O_2\cdotC_4H_4O_4)\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Methylergometrine Maleate RS, previously dried in a desiccator for 4 hours (in vacuum, phosphorus (V) oxide), and use this solution as the sample solution. Determine immediately the intensities of the
Methyl Parahydroxybenzoate

パラオキシ安息香酸メチル

C₈H₈O₃: 152.15
Methyl 4-hydroxybenzoate
[98-76-3]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (∗, †).

Methyl Parahydroxybenzoate, when dried, contains not less than 98.0% and not more than 102.0% of C₈H₈O₃.

∗Description Methyl Parahydroxybenzoate, occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and slightly soluble in water. ♦

Identification (1) The melting point <2.60° of Methyl Parahydroxybenzoate is between 125°C and 128°C.

∗(2) Determine the infrared absorption spectrum of Methyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. ♦

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Methyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add water to make 1000 mL.

(2) Acidity—Dissolve 0.20 g of Methyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromoresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

∗(3) Heavy metals <1.07>—Dissolve 1.0 g of Methyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm). ♦

(4) Related substances—Dissolve 0.10 g of Methyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot with the sample solution is not more intense than the spot obtained with the standard solution.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1.0 g of Methyl Parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS = 152.1 mg of C₈H₈O₃.
**Methylprednisolone**

メチルプレドニゾロン

C_{22}H_{30}O_{5}: 374.47

\[\text{11\beta,17,21-Trihydroxy-6\alpha-methylpregna-1,4-diene-3,20-dione} \]

[83-43-2]

**Description**  Methylprednisolone occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol and in 1,4-dioxane, slightly soluble in ethanol (95) and in chloroform, and practically insoluble in water and in diethyl ether.

Melting point: 232 – 240°C (with decomposition).

**Identification (1)** Add 2 mL of sulfuric acid to 2 mg of Methylprednisolone: a deep red color develops with no fluorescence. Then add 10 mL of water to this solution: the color fades, and a gray, flocculent precipitate is produced.

**Optical rotation** <2.49> \[ [\alpha]_{D}^{25} +79 - +86^\circ \] (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

**Purity** Related substances—Dissolve 50 mg of Methylprednisolone in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Methylprednisolone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Loss on drying** <2.47> Not more than 1.0% (0.5 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.2% (0.2 g).

**Assay** Weigh accurately about 10 mg of Methylprednisolone, previously dried, and dissolve in methanol to make exactly 100 mL. To exactly 5 mL of this solution add methanol to make exactly 50 mL, and determine the absorbance \( A \) at the wavelength of maximum absorption at about 243 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of \( C_{22}H_{30}O_{5} = \frac{A}{400} \times 10,000 \)

**Containers and storage** Containers—Well-closed containers.

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**Methylprednisolone Succinate**

メチルプレドニゾロンコハク酸エステル

C_{26}H_{34}O_{8}: 474.54

\[\text{11\beta,17,21-Trihydroxy-6\alpha-methylpregna-1,4-diene-3,20-dione 21-(hydrogen succinate)} \]

[2921-57-5]

**Description** Methylprednisolone Succinate occurs as a white, crystals or crystalline powder.

It is soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 235°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Methylprednisolone Succinate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylprednisolone Succinate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** <2.49> \[ [\alpha]_{D}^{25} +99 - +103^\circ \] (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Methylprednisolone Succinate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of
Methylrosanilinium Chloride

Crystal Violet

メチルロザニリン塩化物

C\textsubscript{2\text{5}}H\textsubscript{30}Cl\textsubscript{7}N\textsubscript{3}: 407.98

Methylrosanilinium Chloride is hexamethylpararosaniline chloride, and is usually admixed with penamethylpararosaniline chloride and tetramethylpararosaniline chloride.

It contains not less than 96.0% of methylrosanilinium chloride [as hexamethylpararosaniline chloride (C\textsubscript{2\text{5}}H\textsubscript{30}Cl\textsubscript{7}N\textsubscript{3})], calculated on the dried basis.

**Description**

Methylrosanilinium Chloride occurs as green fragments having a metallic luster or a dark green powder. It is odorless or has a slight odor.

It is soluble in ethanol (95), sparingly soluble in water, and practically insoluble in diethyl ether.

**Identification**

1. To 1 mL of sulfuric acid add 1 mg of Methylrosanilinium Chloride: it dissolves, and shows an orange to red-brown color. To this solution add water dropwise: the color of the solution changes from brown through green to blue.

2. Dissolve 0.02 g of Methylrosanilinium Chloride in 10 mL of water, add 5 drops of hydrochloric acid, and use this solution as the sample solution. To 5 mL of the sample solution add tannic acid TS dropwise: an intense blue precipitate is formed.
(3) To 5 mL of the sample solution obtained in (2) add 0.5 g of zinc powder, and shake: the solution is decolorized. Place 1 drop of this solution on filter paper, and apply 1 drop of ammonia TS adjacent to it: a blue color is produced at the zone of contact of the both solutions.

**Purity (1)** Ethanol-insoluble substances—Weigh accurately about 1 g of Methylrosanilinium Chloride, previously dried at 105°C for 4 hours, heat with 50 mL of ethanol (95) under a reflux condenser for 15 minutes in a water bath, and filter the mixture through a tared glass filter (G4). Wash the residue on the filter with warm ethanol (95) until the last washing does not show a purple color, and dry at 105°C for 2 hours: the mass of the residue is not more than 1.0%.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Methylrosanilinium Chloride according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Zinc—To 0.10 g of Methylrosanilinium Chloride add 0.1 mL of sulfuric acid, and incinerate by ignition. After cooling, boil with 5 mL of dilute hydrochloric acid, 0.5 mL of dilute nitric acid and 4 mL of water, add 5 mL of ammonia TS, boil again, and filter. To the filtrate add 2 to 3 drops of sodium sulfide TS: no turbidity is produced.

(4) Arsenic <1.11>—Prepare the test solution with 0.40 g of Methylrosanilinium Chloride, according to Method 3, and perform the test (not more than 5 ppm).

**Loss on drying <2.41>** Not more than 7.5% (1 g, 105°C, 4 hours).

**Residue on ignition <2.44>** Not more than 1.5% (0.5 g).

**Assay** Transfer about 0.4 g of Methylrosanilinium Chloride, accurately weighed, to a wide-mouthed, conical flask, add 25 mL of water and 10 mL of hydrochloric acid, dissolve, and add exactly 50 mL of 0.1 mol/L titanium (III) chloride VS while passing a stream of carbon dioxide through the flask. Heat to boil, and boil gently for 15 minutes, swirling the liquid frequently. Cool while passing a stream of carbon dioxide through the flask, titrate <2.50> the excess titanium (III) chloride VS with 0.5 mol/L ammonium iron (III) chloride TS until a faint, red color is produced (indicator: 5 mL of ammonium thiocyanate TS). Perform a blank determination.

Each mL of 0.1 mol/L titanium (III) chloride VS = 20.40 mg of C8H8ClN3

**Containers and storage** Containers—Tight containers.

---

**Methyl Salicylate**

サリチル酸メチル

\[
\text{C}_8\text{H}_8\text{O}_3: 152.15
\]

Methyl 2-hydroxybenzoate

\[ [119-36-8] \]

Methyl Salicylate contains not less than 98.0% of C8H8O3.

**Description** Methyl Salicylate is a colorless to pale yellow liquid. It has a strong, characteristic odor. It is miscible with ethanol (95) and with diethyl ether. It is very slightly soluble in water.

**Specific gravity** \( d_{20}^2: 1.182 - 1.192 \)

**Boiling point**: 219 – 224°C

**Identification** Shake 1 drop of Methyl Salicylate thoroughly with 5 mL of water for 1 minute, and add 1 drop of iron (III) chloride TS: a purple color develops.

**Purity (1)** Acidity—Shake 5.0 mL of Methyl Salicylate thoroughly with 25 mL of freshly boiled and cooled water and 1.0 mL of 0.1 mol/L sodium hydroxide VS for 1 minute, add 2 drops of phenol red TS, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS until the red color disappears: not more than 0.45 mL of 0.1 mol/L sodium hydroxide VS is consumed.

(2) Heavy metals—Shake 10.0 mL of Methyl Salicylate thoroughly with 10 mL of water, add 1 drop of hydrochloric acid, and saturate with hydrogen sulfide by passing it through the mixture: neither the oily layer nor the aqueous layer shows a dark color.

**Assay** Weigh accurately about 2 g of Methyl Salicylate, add an exactly measured 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and heat on a water bath for 2 hours under a reflux condenser. Cool, and titrate <2.50> the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L potassium hydroxide-ethanol VS = 76.08 mg of C8H8O3

**Containers and storage** Containers—Tight containers.
Compound Methyl Salicylate Spirit

複方サリチル酸メチル精

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl Salicylate</td>
<td>40 mL</td>
</tr>
<tr>
<td>Capsicum Tincture</td>
<td>100 mL</td>
</tr>
<tr>
<td>d- or dl-Camphor</td>
<td>50 g</td>
</tr>
<tr>
<td>Ethanol</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Medicated Spirits, with the above ingredients.

**Description** Compound Methyl Salicylate Spirit is a reddish yellow liquid, having a characteristic odor and a burning taste.

**Identification (1)** Shake 1 mL of Compound Methyl Salicylate Spirit with 5 mL of dilute ethanol, and add 1 drop of iron (III) chloride TS: a purple color is produced (methyl salicylate).

(2) Shake thoroughly 0.5 mL of Compound Methyl Salicylate Spirit with 10 mL of chloroform, and use this solution as the sample solution. Perform the test with these solutions as directed under Thin-layer Chromatography.

Prepare as directed under Medicated Spirits, with the above ingredients.

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyltestosterone</td>
<td>40 g</td>
</tr>
<tr>
<td>Capsicum Tincture</td>
<td>100 mL</td>
</tr>
<tr>
<td>d- or dl-Camphor</td>
<td>50 g</td>
</tr>
<tr>
<td>Ethanol</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Medicated Spirits, with the above ingredients.

**Description** Methyltestosterone occurs as white to pale yellow, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

**Identification (1)** Determine the absorption spectrum of a solution of Methyltestosterone in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry.<sup>2.24</sup>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyltestosterone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methyltestosterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry.<sup>2.25</sup>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Methyltestosterone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation**<sup>2.49</sup> [α]<sub>D</sub>: +79 – +85° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

**Melting point**<sup>2.66</sup> 163 – 168°C

**Purity** Related substances—Dissolve 40 mg of Methyltestosterone in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.<sup>2.03</sup>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**<sup>2.41</sup> Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 10 hours).

**Residue on ignition**<sup>2.44</sup> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 20 mg each of Methyltestosterone and Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve each in methanol to make exactly 200 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography.<sup>2.01</sup> according to the following conditions, and calculate the ratios, Q<sub>T</sub> and Q<sub>S</sub>, of the peak area of methyltestosterone to that of the internal standard.

\[
\text{Amount (mg) of } \text{C}_{20}\text{H}_{30}\text{O}_{2} = M_S \times Q_T/Q_S
\]

\[
M_S: \text{Amount (mg) of Methyltestosterone RS}
\]

**Internal standard solution**—A solution of propyl parahydroxybenzoato in methanol (1 in 10,000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about
35°C.

Mobile phase: A mixture of acetonitrile and water (11:9).

Flow rate: Adjust the flow rate so that the retention time of methyltestosterone is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and methyltestosterone are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of methyltestosterone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Methyltestosterone Tablets

メチルテストステロン錠

Methyltestosterone Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methyltestosterone (C_{20}H_{30}O_{2}: 302.45).

Method of preparation Prepare as directed under Tablets, with Methyltestosterone.

Identification To a portion of powdered Methyltestosterone Tablets, equivalent to 10 mg of Methyltestosterone according to the labeled amount, add 50 mL of acetone, shake for 30 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 10 mL of acetone, and use this solution as the sample solution. Separately, dissolve 10 mg of Methyltestosterone RS in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95): (9:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 10 minutes: the spot from the sample solution and the standard solution show the same Rf value.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Methyltestosterone Tablets add 5 mL of water to disintegrate, add 50 mL of methanol, and shake for 30 minutes. Add methanol to make exactly 100 mL, and centrifuge. Measure exactly V mL of the supernatant liquid, add methanol to make exactly V' mL of a solution containing about 10 μg of methyltestosterone (C_{20}H_{30}O_{2}) per mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, and dissolve in 5 mL of water and 50 mL of methanol, then add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at the wavelength of maximum absorption at about 241 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Amount (mg) of methyltestosterone (C}_{20}\text{H}_{30}\text{O}_{2}) = M_S \times C \times V' \times V \times 1/10
\]

\[
\begin{align*}
M_S & : \text{Amount (mg) of Methyltestosterone RS} \\
C & : \text{Labeled amount (mg) of methyltestosterone (C}_{20}\text{H}_{30}\text{O}_{2}) \text{ in 1 tablet}
\end{align*}
\]

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of a solution prepared by dissolving 1 g of polysorbate 80 in water to make 5 L as the dissolution medium, the dissolution rate in 30 minutes of a 10-mg tablet is not less than 75% and that in 60 minutes of a 25-mg tablet is not less than 70%.

Start the test with 1 tablet of Methyltestosterone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 11 μg of methyltestosterone (C_{20}H_{30}O_{2}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Methyltestosterone RS, previously dried in vacuum using phosphorus (V) oxide as a desiccant for 10 hours, and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 249 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of methyltestosterone (C_{20}H_{30}O_{2})

\[
\begin{align*}
M_S & : \text{Amount (mg) of Methyltestosterone RS} \\
C & : \text{Labeled amount (mg) of methyltestosterone (C}_{20}\text{H}_{30}\text{O}_{2}) \text{ in 1 tablet}
\end{align*}
\]

\[
\begin{align*}
\text{Assay} & \text{ Weigh accurately the mass of not less than 20 Methyltestosterone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of methyltestosterone (C}_{20}\text{H}_{30}\text{O}_{2}), add about 70 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, filter through a membrane filter (not exceeding 0.45 μm in pore size), and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of methyltestosterone to that of the internal standard.
\end{align*}
\]
Amount (mg) of methyltestosterone \((C_{20}H_{30}O_2)\)

\[ M_s = M_{0.5} \times Q_1/Q_0 \times 5/4 \]

\(M_s\): Amount (mg) of Methyltestosterone RS

**Internal standard solution—** A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile and water (11:9).

Flow rate: Adjust the flow rate so that the retention time of methyltestosterone is about 10 minutes.

**System suitability—**

System performance: When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, the internal standard and methyltestosterone are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methyltestosterone to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

---

**Meticrane**

メチクラン

\[ C_{10}H_{13}NO_4S_2 \]

6-Methylthiochromane-7-sulfonamide 1,1-dioxide

[1084-65-7]

Meticrane, when dried, contains not less than 98.0% of \(C_{10}H_{13}NO_4S_2\).

**Description** Meticrane occurs as white, crystals or crystalline powder. It is odorless and has a slightly bitter taste.

It is freely soluble in dimethylformamide, slightly soluble in acetonitrile and in methanol, very slightly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 234°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Meticrane in methanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)** Ammonium <1.02—Perform the test with 0.10 g of Meticrane. Prepare the control solution with 3.0 mL of Standard Ammonium Solution (not more than 0.03%).

**Heavy metals <1.07—** Proceed with 1.0 g of Meticrane according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Related substances—** Dissolve 50 mg of Meticrane in 50 mL of acetonitrile. To 5 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu\)L of each of the sample solution and standard solution as directed under Liquid Chromatography (2.27,1) according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than meticrane from the sample solution is not larger than the peak area of meticrane from the standard solution.

**Operating conditions 1—**

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (17:3).

Flow rate: Adjust the flow rate so that the retention time of meticrane is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of meticrane beginning after the solvent peak.

**System suitability 1—**

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 \(\mu\)L of this solution is equivalent to 7 to 13% of that from 10 \(\mu\)L of the standard solution.

System performance: Dissolve 10 mg each of Meticrane and caffeine in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL.

When the procedure is run with 10 \(\mu\)L of this solution under the above operating conditions 1, caffeine and meticrane are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions 1, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

**Operating conditions 2—**

Detector, column, and column temperature: Proceed as directed in the operating conditions 1.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of meticrane is about 2 minutes.
Metildigoxin

メチルジゴキシン

C₄₂H₆₆O₁₄. ½ C₃H₆O: 824.00
3β-[2,6-Dideoxy-4-O-methyl-β-D-ribo-hexopyranosyl-(1→4)]-2,6-dideoxy-β-D-ribo-hexopyranosyl(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl[oxyl]-12β,14-dihydroxy-5β-card-20(22)-enolide—acetone (1/1)
[30683-43-9, acetone-free]

Metildigoxin contains not less than 96.0% and not more than 103.0% of C₄₂H₆₆O₁₄. ½ C₃H₆O, calculated on the anhydrous basis.

Description Metildigoxin occurs as a white to light yellowish white, crystalline powder.

It is freely soluble in N,N-dimethylformamide, in pyridine and in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, very slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Dissolve 2 mg of Metildigoxin in 2 mL of acetic acid (100), shake well with 1 drop of iron (III) chloride TS, and add gently 2 mL of sulfuric acid to divide into two layers: a brown color develops at the interface, and a deep blue color gradually develops in the acetic acid layer.

(2) Dissolve 2 mg of Metildigoxin in 2 mL of 1,3-dinitrobenzene TS, add 2 mL of a solution of tetramethylammonium hydroxide in ethanol (95) (1 in 200), and shake: a purple color gradually develops, and changes to blue-purple.

(3) Determine the absorption spectrum of a solution of Metildigoxin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Metildigoxin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Determine the infrared absorption spectrum of Metildigoxin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Metildigoxin RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Metildigoxin and Metildigoxin RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Containers and storage Containers—Well-closed containers.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Meticrane, previously dried, dissolve in 50 mL of dimethylformamide, add 5 mL of water, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 27.54 mg of C₁₀H₁₃NO₄S₂

Containers and storage Containers—Well-closed containers.

Time span of measurement: About 10 times as long as the retention time of meticrane beginning after the solvent peak.

System suitability 2—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 µL of this solution is equivalent to 7 to 13% of that from 10 µL of the standard solution.

System performance: Dissolve 20 mg each of Meticrane and methyl parahydroxybenzoate in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 µL of this solution under the above operating conditions 2, meticrane and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

 Containers—Well-closed containers.
Metildigoxin RS (separately, determine the water 2.48° in the same manner as Metildigoxin), and dissolve each in methanol to make exactly 50 mL. Pipet 5 mL of each of the solutions, add methanol to each to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL of each of the sample solution and standard solution, add 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS to each, shake well, add methanol to make exactly 25 mL, and allow to stand at 20 ± 0.5°C for 20 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry 2.26° using a solution prepared by mixing 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS and adding methanol to make exactly 25 mL as the blank. Determine the maximum absorbances, \( A_t \) and \( A_s \), of the subsequent solutions obtained from the sample solution and the standard solution, respectively, by measuring every 5 minutes, at 495 nm.

\[
\text{Amount (mg) of } C_{14}H_{22}ClN_3O_2 = M_s \times \frac{A_t}{A_s}
\]

\( M_s \): Amount (mg) of Metildigoxin RS, calculated on the anhydrous basis.

Containers and storage

Containers—Tight containers.

**Metoclopramide**

メトクロプラミド

\[
\text{C}_{14}\text{H}_{22}\text{ClN}_3\text{O}_2: \quad 299.80
\]

4-Amino-5-chloro-N-2-(diethylamino)ethyl)-2-methoxybenzamide

[364-62-5]

Metoclopramide, when dried, contains not less than 99.0% of \( C_{14}H_{22}ClN_3O_2 \).

**Description**

Metoclopramide occurs as white crystals or a crystalline powder, and is odorless.

It is freely soluble in acetic acid (100), soluble in methanol and in chloroform, sparingly soluble in acetic anhydride, in ethanol (95) and in acetone, very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

**Identification**

(1) Dissolve 10 mg of Metoclopramide in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests 1.09° for Primary Aromatic Amines.

(2) Dissolve 10 mg of Metoclopramide in 5 mL of dilute hydrochloric acid and 20 mL of water, and to 5 mL of this solution add 1 mL of Dragendorff’s TS: a reddish orange precipitate is produced.

(3) Dissolve 0.1 g of Metoclopramide in 1 mL of 1 mol/L hydrochloric acid TS, and dilute with water to make 100 mL. To 1 mL of the solution add water to make 100 mL.
Metoclopramide Tablets

メトクロプラミド錠

Metoclopramide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of metoclopramide (C\textsubscript{14}H\textsubscript{22}ClN\textsubscript{3}O\textsubscript{2}: 299.80).

**Method of preparation** Prepare as directed under Tablets, with Metoclopramide.

**Identification** (1) To a quantity of powdered Metoclopramide Tablets, equivalent to 50 mg of Metoclopramide according to the labeled amount, add 15 mL of 0.5 mol/L hydrochloric acid TS, and heat in a water bath at 70°C for 15 minutes while frequent shaking. After cooling, centrifuge for 10 minutes, and to 5 mL of the supernatant liquid add 1 mL of 4-dimethylaminobenzaldehyde-hydrochloric acid TS: a yellow color develops.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \): it exhibits maxima between 270 nm and 274 nm, and between 306 nm and 310 nm.

**Uniformity of dosage units** \( <6.02> \) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Metoclopramide Tablets add 10 mL of 0.1 mol/L hydrochloric acid TS, disperse the particles with the aid of ultrasonic waves, then add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \).

\[
\text{Amount (mg) of metoclopramide (C}_{14}\text{H}_{22}\text{ClN}_{3}\text{O}_{2}) = M_S \times A_T / A_S \times V / 1000
\]

\( M_S \): Amount (mg) of metoclopramide for assay

**Dissolution** Being specified separately.

**Assay** Weigh accurately not less than 20 Metoclopramide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of metoclopramide (C\textsubscript{14}H\textsubscript{22}ClN\textsubscript{3}O\textsubscript{2}), add 300 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Centrifuge for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \).

\[
\text{Amount (mg) of metoclopramide (C}_{14}\text{H}_{22}\text{ClN}_{3}\text{O}_{2}) = M_S \times A_T / A_S
\]

\( M_S \): Amount (mg) of metoclopramide for assay

**Containers and storage** Containers—Well-closed containers.

**Containers and storage** Containers—Tight containers.
Metoprolol Tartrate

メトプロロール酒石酸塩

(\(\text{C}_{15}\text{H}_{25}\text{NO}_{3}\))\(_2\cdot\text{C}_4\text{H}_6\text{O}_6\): 684.81
(2R5)-1-\{4-(2-Methoxyethyl)phenoxyl\}-3-\{(1-methylethyl)amino\}propan-2-ol hemi-(2R,3R)-tartrate

Metoprolol Tartrate, when dried, contains not less than 99.0% and not more than 101.0% of \((\text{C}_{15}\text{H}_{25}\text{NO}_{3})\)_2\cdot\text{C}_4\text{H}_6\text{O}_6\).

**Description** Metoprolol Tartrate occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in methanol, in ethanol (95) and in acetic acid (100).

Optical rotation \([\alpha]_D^{\text{p}}: +7.0 - +10.0^\circ\) (after drying, 1 g, water, 50 mL, 100 mm).

**Identification** (1) Determine the absorption spectrum of a solution of Metoprolol Tartrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metoprolol Tartrate, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Metoprolol Tartrate from a solution in acetone (23 in 1000), filter and dry the crystals, and perform the test with the crystals.

(3) A solution of Metoprolol Tartrate (1 in 5) responds to the Qualitative Tests <1.09> (1) for tartrate.

**pH** <2.54> The pH of a solution obtained by dissolving 1.0 g of Metoprolol Tartrate in 10 mL of water is between 6.0 and 7.0.

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 g of Metoprolol Tartrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Metoprolol Tartrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \(\mu\text{L}\) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. After saturating the plate with the atmosphere by allowing to stand in a developing vessel, which contains the developing solvent and a glass vessel containing ammonium water (28), develop with the developing solvent, a mixture of ethyl acetate and methanol (4:1), to a distance of about 12 cm, and air-dry the plate. Allow to stand the plate in an iodine vapors until the spot with the standard solution appears obviously: the spot other than the principal spot and other than the spot on the original point with the sample solution is not more than three spots, and they are not more intense than the spot with the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Metoprolol Tartrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.35> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 34.24 mg of \((\text{C}_{15}\text{H}_{25}\text{NO}_{3})\)_2\cdot\text{C}_4\text{H}_6\text{O}_6\).

**Containers and storage** Containers—Well-closed containers.

Metoprolol Tartrate Tablets

メトプロロール酒石酸塩錠

Metoprolol Tartrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of metoprolol tartrate \([(\text{C}_{15}\text{H}_{25}\text{NO}_{3})\)_2\cdot\text{C}_4\text{H}_6\text{O}_6\): 684.81].

**Method of preparation** Prepare as directed under Tablets, with Metoprolol Tartrate.

**Identification** To an amount of powdered Metoprolol Tartrate Tablets, equivalent to 10 mg of Metoprolol Tartrate according to the labeled amount, add 100 mL of ethanol (95), shake for 15 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 274 nm and 278 nm and between 281 nm and 285 nm.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Metoprolol Tartrate Tablets add 1 mL of water for every 10 mg of Metoprolol Tartrate, shake for 20 minutes, then add 75 mL of ethanol (95), shake for 15 minutes, add ethanol (95) to make exactly 100 mL, and centrifuge. Pipet \(V\) mL of the supernatant liquid, add ethanol (95) to make exactly \(V\) so that each mL contains about 0.1 mg of metoprolol tartrate \([(\text{C}_{15}\text{H}_{25}\text{NO}_{3})\)_2\cdot\text{C}_4\text{H}_6\text{O}_6]\), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, dissolve in 5 mL of water, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), of the sample solution and standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using ethanol (95) as the blank.
**Dissolution**  
When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Metoprolol Tartrate Tablets is not less than 80%.

Start the test with 1 tablet of Metoprolol Tartrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 22 μg of metoprolol tartrate ((C_{15}H_{25}NO_{3})_{2}.C_{4}H_{6}O_{6}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in 60 mL of the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1), add exactly 10 mL of the internal standard solution, and centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.12 g of metoprolol tartrate ((C_{15}H_{25}NO_{3})_{2}.C_{4}H_{6}O_{6}) in 1 tablet and add water to make exactly 200 mL. Pipet 8 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution.

Perform the test with exactly 50 mL of the sample solution add 250 mL of acetonitrile.

Dissolution rate (% with respect to the labeled amount of metoprolol tartrate ((C_{15}H_{25}NO_{3})_{2}.C_{4}H_{6}O_{6})

\[ M_{S} \times A_{1}/A_{3} \times V'/V \times 1/C \times 36 \]

\[ M_{S}: \text{Amount (mg) of metoprolol tartrate for assay} \]

C: Labeled amount (mg) of metoprolol tartrate ((C_{15}H_{25}NO_{3})_{2}.C_{4}H_{6}O_{6}) in 1 tablet

**Operating conditions**—
Proceed as directed in the Assay.

**System suitability**—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, metoprolol and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of metoprolol to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Well-closed containers.

**Metronidazole**

メトロニダゾール

\[ C_{6}H_{12}N_{3}O_{3}: 171.15 \]

2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethanol [445-48-1]

Metronidazole, when dried, contains not less than 99.0% and not more than 101.0% of C_{6}H_{12}N_{3}O_{3}.

**Description**

Metronidazole occurs as white to pale yellowish-white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (99.5) and in acetone, and slightly soluble in water.

It dissolves in dilute hydrochloric acid.

It is colored to yellow-brown by light.

**Identification (1)**

Determine the absorption spectrum of a
solution of Metronidazole in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metronidazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 159 – 163°C.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Metronidazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) 2-Methyl-5-nitroimidazole—Dissolve 0.10 g of Metronidazole in acetic acid to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 2-methyl-5-nitroimidazole for thin-layer chromatography in acetic acid to make exactly 20 mL, then pipet 5 mL of this solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate immediately with a mixture of acetone, water and ethyl acetate (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution is the same.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Metronidazole, previously dried, and dissolve in 30 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 0.5 mL of p-naphtholbenzein TS) until the color of the solution changes from orange-yellow to green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 17.12 mg of C6H5NO3

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Metronidazole Tablets

メトロニダゾール錠

Metronidazole Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of metronidazole (C6H5NO3: 171.15).

Method of preparation Prepare as directed under Tablets, with Metronidazole.

Identification (1) To an amount of powdered Metronida-
Dissolution rate (%) with respect to the labeled amount of metronidazole (C<sub>35</sub>H<sub>37</sub>N<sub>5</sub>O<sub>3</sub>)

\[ \text{Dissolution rate} = \frac{M_5 \times A_T / A_S \times V \times V / 1 \times C \times 45}{M_3} \]

\( M_3 \): Amount (mg) of metronidazole for assay
\( C \): Labeled amount (mg) of metronidazole (C<sub>35</sub>H<sub>37</sub>N<sub>5</sub>O<sub>3</sub>) in 1 tablet

**Assay**
Weigh accurately the mass of not less than 20 Metronidazole Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.25 g of metronidazole (C<sub>35</sub>H<sub>37</sub>N<sub>5</sub>O<sub>3</sub>), add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 10 minutes, and add the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Filter this solution through a membrane filter with pore size of 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of metronidazole for assay, previously dried in vacuum on silica gel for 24 hours, dissolve in the mixture of water and methanol (4:1) to make exactly 100 mL. Filter this solution through a membrane filter with pore size of 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of metronidazole.

\[ \frac{M_S \times A_T}{A_S} \times 10 \]

\( M_S \): Amount (mg) of metronidazole (C<sub>35</sub>H<sub>37</sub>N<sub>5</sub>O<sub>3</sub>) for assay

**Operating conditions—**
- Detector: An ultraviolet absorption photometer (wavelength: 320 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of water and methanol (4:1).
- Flow rate: Adjust the flow rate so that the retention time of metronidazole is about 5 minutes.

**System suitability—**
- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metronidazole are not less than 3000 and not more than 1.5, respectively.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metronidazole is not more than 1.0%.

**Containers and storage**
Containers—Tight containers.

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**Metyrapone**

メチラボン

\[ \text{C}_{14}H_{18}N_2O: 226.27 \]

2-Methyl-1,2-di(pyridin-3-yl)propan-1-one [54-36-4]

Metyrapone, when dried, contains not less than 98.0% of \( \text{C}_{14}H_{18}N_2O \).

**Description**
Metyrapone occurs as a white to pale yellow, crystalline powder. It has a characteristic odor and a bitter taste.

It is very soluble in methanol, in ethanol (95), in acetic anhydride, in chloroform, in diethyl ether and in nitrobenzene, and sparingly soluble in water.

It dissolves in 0.5 mol/L sulfuric acid TS.

**Identification**
(1) Mix 5 mg of Metyrapone with 10 mg of 1-chloro-2,4-dinitrobenzene, melt by gently heating for 5 to 6 seconds, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

(2) Determine the absorption spectrum of a solution of Metyrapone in 0.5 mol/L sulfuric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point**
\(<2.60>\) 50 – 54°C

**Purity**
(1) Clarity and color of solution—Dissolve 0.5 g of Metyrapone in 5 mL of methanol: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Metyrapone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Metyrapone, according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Metyrapone in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 2 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (15:1) to a distance of about 10 cm, and air-dry the plate for about 15 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying**
\(<2.41>\) Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).
Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Metyrapone, previously dried, dissolve in 10 mL of nitrobenzene and 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 11.31 mg of C\textsubscript{13}H\textsubscript{18}N\textsubscript{2}O

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Mexiletine Hydrochloride

メキシレチン塩酸塩

C\textsubscript{11}H\textsubscript{17}NO.HCl: 215.72
(1RS)-2-(2,6-Dimethylphenoxy)-1-methylethylamine monohydrochloride

 Mexiletine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of C\textsubscript{11}H\textsubscript{17}NO.HCl.

Description Mexiletine Hydrochloride occurs as a white powder.

It is freely soluble in water and in ethanol (95), slightly soluble in acetonitrile, and practically insoluble in diethyl ether.

A solution of Mexiletine Hydrochloride (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Mexiletine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.26>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mexiletine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mexiletine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Mexiletine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Mexiletine Hydrochloride from ethanol (95), filter, dry the crystals, and repeat the test on the crystals.

(3) A solution of Mexiletine Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the pH of this solution is between 3.8 and 5.8.

Melting point <2.60> 200 - 204°C.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy Metals <1.07>—Proceed with 2.0 g of Mexiletine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Mexiletine Hydrochloride in 20 mL of the mobile phase, and use this solution as the standard solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each peak area of the peaks other than the peak of mexiletine from the sample solution is not larger than the peak area of mexiletine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of mexiletine obtained from 20 μL of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About 3 times as long as the retention time of mexiletine beginning after peaks of the solvent.

Loss on drying <2.44> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Mexiletine Hydrochloride and Mexiletine Hydrochloride RS, each previously dried, and dissolve each in the mobile phase to make exactly 20 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q\textsubscript{T} and Q\textsubscript{S}, of the peak area of mexiletine to that of the internal standard, respectively.

\[
\text{Amount (mg) of C}_{11}\text{H}_{17}\text{NO.HCl} = M_S \times Q_T/Q_S
\]

\(M_S\): Amount (mg) of Mexiletine Hydrochloride RS

Internal standard solution—A solution of phenetylamine hydrochloride in the mobile phase (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (about 7 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 2.5 g of sodium lauryl sulfate and 3 g of sodium dihydrogenphosphate dihydrate in 600 mL of
Miconazole

ミコナゾール

C₁₈H₁₄Cl₄N₂O: 416.13
1-[(2RS)-2-(2,4-Dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole
[22916-47-8]

Miconazole, when dried, contains not less than 98.5% of C₁₈H₁₄Cl₄N₂O.

Description Miconazole occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetic acid (100), soluble in diethyl ether, and practically insoluble in water.

A solution of Miconazole in methanol (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Miconazole in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Miconazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 84 – 87°C.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Miconazole according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Miconazole according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Miconazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 50 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, chloroform, methanol and ammonia solution (28:60:30:10:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.4>—Not more than 0.5% (1 g, in vacuum, silica gel, 60%, 3 hours).

Residue on ignition <2.4>—Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Miconazole, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <2.25> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of p-naphtholbenzein TS) until the color of the solution changes from light yellow-brown to light yellow-green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 41.61 mg of C₁₈H₁₄Cl₄N₂O

Miconazole Nitrate

ミコナゾール硝酸塩

C₁₈H₁₄Cl₄N₂O.HNO₃: 479.14
1-[(2RS)-2-(2,4-Dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole mononitrate
[22832-87-7]

Miconazole Nitrate, when dried, contains not less than 98.5% of C₁₈H₁₄Cl₄N₂O.HNO₃.

Description Miconazole Nitrate occurs as a white crystalline powder.

It is freely soluble in N,N-dimethylformamide, sparingly soluble in methanol, slightly soluble in ethanol (95), in acetic acid (100), and very slightly soluble in water and in diethyl ether.

Melting point: about 180°C (with decomposition).

Identification (1) To 2 mL of a solution of Miconazole Nitrate in methanol (1 in 100) add 2 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Miconazole Nitrate in methanol (1 in 2500) as directed under...
Ultraviolet-visible Spectrophotometry <2.4>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

3. Perform the test with a solution of Miconazole Nitrate in methanol (1 in 100) as directed under Flame Coloration Test <1.04> (2); a green color appears.

4. A solution of Miconazole Nitrate in methanol (1 in 100) responds to the Qualitative Tests <1.09> for nitrate.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Miconazole Nitrate in 100 mL of methanol; the solution is clear and colorless.

2. Chloride <1.03>—Dissolve 0.10 g of Miconazole Nitrate in 6 mL of dilute nitric acid and $\text{Ni}_2\text{N}$-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and $\text{Ni}_2\text{N}$-dimethylformamide to make 50 mL (not more than 0.09%).

3. Heavy metals <1.07>—Proceed with 1.0 g of Miconazole Nitrate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

4. Arsenic <1.11>—Prepare the test solution with 1.0 g of Miconazole Nitrate according to Method 3, and perform the test (not more than 2 ppm).

5. Related substances—Dissolve 0.10 g of Miconazole Nitrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of n-hexane, chloroform, methanol and ammonia solution (28) (60:30:10:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate in iodine vapor for 20 minutes; the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.4>—Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

**Residue on ignition** <2.44>—Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Miconazole Nitrate, previously dried, dissolve in 30 mL of acetic acid (100) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 47.91 mg of $\text{C}_18\text{H}_{14}\text{Cl}_4\text{N}_2\text{O}$.\text{HNO}_3

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Micronomicin Sulfate**

ミクロノマイシン硫酸塩

[(C$_{20}$H$_{41}$N$_5$O$_{17}$)$_2$.5H$_2$SO$_4$; 1417.53 2-Amino-2,3,4,6-tetraoxy-6-methylamino-$\alpha$-$\beta$-erythro-hexopyranosyl(1→4)[3-deoxy-4-C-methyl-3-methylamino-$\beta$-L-arabinopyranosyl(1→6)]-2-deoxy-$\beta$-streptamine hemipentasulfate [52093-21-7, Micronomicin]]

Micronomicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micromonospora sagamiensis*.

It contains not less than 590 μg (potency) and not more than 660 μg (potency) per mg, calculated on the anhydrous basis. The potency of Micronomicin Sulfate is expressed as mass (potency) of micromonomicin (C$_{20}$H$_{41}$N$_5$O$_{17}$; 463.57).

**Description** Micronomicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, sparingly soluble in ethylene glycol, and practically insoluble in methanol and in ethanol (99.5).

It is hygroscopic.

**Identification** (1) Dissolve 50 mg each of Micronomicin Sulfate and Micronomicin Sulfate RS in 10 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-buthanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of ethanol (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spots obtained from the sample solution and standard solution are red-purple to red-brown and their Rf values are the same.

2. To 5 mL of a solution of Micronomicin Sulfate (1 in 100) add 1 mL of barium chloride TS: a white precipitate is formed, and it does not dissolve by addition of dilute nitric acid.

**Optical rotation** <2.4> [α]$_D^20$ + 110 – + 130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).
pH <2.5> The pH of a solution obtained by dissolving 1.0 g of Micronomic Sulfate in 10 mL of water is between 3.5 and 5.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.5 g of Micronomic Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Micronomic Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.40 g of Micronomic Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.02>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and pyridine (25:1) (1 in 500), and heat and air-dry the plate. Spray evenly a solution of ninhydrin in ammonia solution (28) (10:8:7) to a distance of about 10 cm, and dry the plate. The spot obtained from the sample solution is not more intense than the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Water** <2.48> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Micronomic Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

(iv) Sample solutions—Weigh accurately an amount of Micronomic Sulfate, equivalent to about 20 mg (potency), and 0.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

**Purity (1)** Clarity and color of solution—Dissolve 1.5 g of Micronomic Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

**Water** <2.48> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Micronomic Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Water** <2.48> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Micronomic Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Water** <2.48> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Micronomic Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Water** <2.48> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Micronomic Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Water** <2.48> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Micronomic Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Water** <2.48> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Micronomic Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Midecamycin is a macrolide substance having antibacterial activity produced by the growth of *Streptomyces mycarofaciens*.

It contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Midecamycin is expressed as mass (potency) of midecamycin (C₄₁H₆₇NO₁₅).  

**Description** Midecamycin occurs as a white crystalline powder.

- It is very soluble in methanol, freely soluble in ethanol (95), and very slightly soluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Midecamycin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Midecamycin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelength.

(2) Determine the infrared absorption spectrum of Midecamycin as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Midecamycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.6b> 153 – 158°C.

**Purity** Heavy metals <1.07>—Proceed with 1.0 g of Midecamycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**Loss on drying** <2.4f> Not more than 2.0% (1.0 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

**Residue on ignition** <2.4f> Not more than 0.2% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <
Midecamycin Acetate

ミデカマイシン酢酸エステル

\[
\text{C}_{45}\text{H}_{71}\text{NO}_{17}: 898.04
\]

\[
(3R,4S,5S,6R,8R,9R,10E,12E,15R)-9-Acetoxy-5-[3-O-acetyl-2,6-dideoxy-3-C-methyl-4-O-propanoyl-\alpha-L-\beta-D-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methyl-3-propioylhexadeca-10,12-dien-15-olide
\]

Midecamycin Acetate is a derivative of midecamycin.

It contains not less than 950 \( \mu \text{g} \) (potency) and not more than 1010 \( \mu \text{g} \) (potency) per mg, calculated on the dried basis. The potency of Midecamycin Acetate is expressed as mass of midecamycin acetate (\( \text{C}_{45}\text{H}_{71}\text{NO}_{17} \)).

**Description**

Midecamycin Acetate occurs as white, crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water.

**Identification**

(1) Determine the absorption spectrum of a solution of Midecamycin Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.4>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Midecamycin Acetate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Midecamycin Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or spectrum of dried Midecamycin Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity**

Heavy metals <1.07>—Proceed with 1.0 g of Midecamycin Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <2.41> Not more than 2.0% (1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Assay**

Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Micrococcus luteus* ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Midecamycin Acetate RS, previously dried, equivalent to about 25 mg (potency), and dissolve in methanol to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 – 15°C and use within 7 days. Take exactly a suitable amount of the stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 \( \mu \text{g} \) (potency) and 5 \( \mu \text{g} \) (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Midecamycin, previously dried, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, and add water to make exactly 50 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 \( \mu \text{g} \) (potency) and 5 \( \mu \text{g} \) (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage**

Containers—Tight containers.
Migrenin

ミグレニン

Migrenin is composed of 90 parts of antipyrine, 9 parts of caffeine, and 1 part of citric acid in mass.

Migrenin, when dried, contains not less than 87.0% and not more than 93.0% of antipyrine (C₁₁H₁₂N₂O: 188.23) and not less than 8.6% and not more than 9.5% of caffeine (C₈H₁₀N₄O₂: 194.19).

**Description** Migrenin occurs as a white powder or crystalline powder. It is odorless and has a bitter taste.

It is very soluble in water, freely soluble in ethanol (95%) and in chloroform, and slightly soluble in diethyl ether.

The pH of a solution of Migrenin (1 in 10) is between 3.0 and 4.0.

It is affected by moisture and light.

**Identification (1)** To 5 mL of a solution of Migrenin (1 in 100) add 2 drops of sodium nitrite TS and 1 mL of dilute sulfuric acid: a deep green color develops.

(2) To 5 mL of a solution of Migrenin (1 in 50) add 1 drop of hydrochloric acid and 0.2 mL of formaldehyde solution, heat in a water bath for 30 minutes, add an excess of ammonia TS, and filter. Acidify the filtrate with hydrochloric acid, shake with 3 mL of chloroform, and separate the chloroform layer. Evaporate the chloroform solution on a water bath, add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid to the residue, and evaporate on a water bath to dryness: the residue shows a yellow-red color. Invert the residue over a vessel containing 3 drops of ammonia TS: a red-purple color develops, disappearing on the addition of 2 to 3 drops of sodium hydroxide TS.

(3) A solution of Migrenin (1 in 10) responds to the Qualitative Tests for citrate.

**Melting point** 104 - 110°C.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Migrenin in 40 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Migrenin according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** Not more than 0.1% (1 g).

**Assay** (1) Antipyrine—Weigh accurately about 0.25 g of Migrenin, previously dried in an iodine flask, dissolve in 25 mL of sodium acetate TS, add exactly 30 mL of 0.05 mol/L iodine VS, and allow to stand for 20 minutes with occasional shaking. Add 15 mL of chloroform to dissolve the precipitate so obtained, and titrate the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 9.411 mg of C₁₁H₁₂N₂O

(2) Caffeine—To about 1 g of Migrenin, previously dried and accurately weighed, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 90 mg of Caffeine RS, previously dried at 80°C for 4 hours, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the standard solution. Perform the test with 1 μL of each of the sample solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of caffeine to that of the internal standard.

Amount (mg) of caffeine (C₈H₁₀N₄O₂) = \( M_S \times Q_1/Q_2 \)

M₅: Amount (mg) of Caffeine RS

**Internal standard solution**—A solution of ethenzamide in chloroform (1 in 50).

**Operating conditions**—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 210 cm in length, packed with silicic acid for gas chromatography (180 to 250 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 15%.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of ethenzamide is about 4 minutes.

**System suitability**—

System performance: Dissolve 0.9 g of antipyrine and 0.09 g of caffeine in 10 mL of chloroform. When the procedure is run with 1 μL of this solution under the above operating conditions, caffeine and antipyrine are eluted in this order with the retention time of these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of caffeine to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

Storage—Light-resistant.
Minocycline Hydrochloride

Minocycline Hydrochloride is the hydrochloride of a derivative of tetracycline. It contains not less than 890 μg (potency) and not more than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Minocycline Hydrochloride is expressed as mass (potency) of minocycline (C₂₃H₂₇N₃O₇·HCl: 457.48).

Description
Minocycline Hydrochloride occurs as a yellow crystalline powder. It is freely soluble in N,N-dimethylformamide, soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Minocycline Hydrochloride in a solution of hydrochloric acid in methanol (19 in 20,000) (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.2₄>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Minocycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the infrared absorption spectrum of Minocycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.2₅>, and compare the spectrum with the Reference Spectrum or the spectrum of Minocycline Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Minocycline Hydrochloride (1 in 100) responds to the Qualitative Tests <1.0₉> (2) for chloride.

pH <2.5₄> Dissolve 1.0 g of Minocycline Hydrochloride in 100 mL of water: the pH of the solution is between 3.5 and 4.5.

Purity (1) A solution of Minocycline Hydrochloride (1 in 100) is clear, and when the test is performed within 1 hour after preparation of this solution, the absorbance of the solution at 560 nm, determined as directed under Ultraviolet-visible Spectrophotometry <2.2₄>, is not more than 0.06.

(2) Heavy metals <1.0₇>—Proceed with 0.5 g of Minocycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(3) Related substances—Dissolve 50 mg of Minocycline Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test immediately after the preparation of the sample solution with 20 μL of the sample solution as directed under Liquid Chromatography <2.0₁₇> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of each peak area by the area percentage method: the amount of epiminocycline is not more than 1.2%, the amount of each peak other than minocycline and epiminocycline is not more than 1.0%, and the total area of the peaks other than minocycline and epiminocycline is not more than 2.0%.

Operating conditions—
Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay. Flow rate: Adjust the flow rate so that the retention time of minocycline is about 12 minutes. The retention time of epiminocycline is about 10 minutes under this condition. Time span of measurement: About 2.5 times as long as the retention time of minocycline beginning after the solvent peak.

System suitability—
Test for required detection: To exactly 2 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

Water <2.4₅> Not less than 4.3% and not more than 8.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.4₄> Not more than 0.5% (1 g).

Assay Weigh accurately an amount of Minocycline Hydrochloride and Minocycline Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0₁₇> according to the following conditions, and determine the peak areas, A₁ and A₃, of minocycline of these solutions.

Amount [μg (potency)] of minocycline (C₂₃H₂₇N₃O₇·HCl) = Mₕ × A₁/A₃ × 1000

Mₕ: Amount [mg (potency)] of Minocycline Hydrochloride RS

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octysilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about
Minocycline Hydrochloride for Injection / Official Monographs  JP XVI

25°C.

Mobile phase: Adjust to pH 6.5 of a mixture of a solution of ammonium oxalate monohydrate (7 in 250), N,N-dimethylformamide and 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (11:5:4) with tetrabutylammonium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of minocycline is about 12 minutes.

System suitability—

System performance: Dissolve 50 mg of Minocycline Hydrochloride in 25 mL of water. Heat 5 mL of this solution on a water bath for 60 minutes, then add water to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, epiminocycline and minocycline are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of peak areas of minocycline is not more than 1.0%.

Containers and storage—Tight containers. Storage—Light-resistant.

Minocycline Hydrochloride for Injection

注射用ミノサイクリン塩酸塩

Minocycline Hydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of minocycline (C21H20N2O7: 457.48).

Method of preparation—Prepare as directed under Injections, with Minocycline Hydrochloride.

Description—Minocycline Hydrochloride for Injection occurs as a yellow to yellow-brown powder or flakes.

Identification—Dissolve 4 mg of Minocycline Hydrochloride for Injection in 250 mL of a solution of hydrochloric acid in methanol (19 in 20,000). Dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution under the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of each peak by the area percentage method: the amount of epiminocycline, having the relative retention time of about 0.83 with respect to minocycline, is not more than 6.0%.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of minocycline, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

Water <2.48> Weigh accurately the mass of the content of one container of Minocycline Hydrochloride for Injection, dissolve in exactly 2 mL of methanol for water determination, and perform the test with exactly 1 mL of this solution as directed in the Volumetric titration (back titration): not more than 3.0%.

Bacterial endotoxins <4.01> Less than 1.25 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay—Weigh accurately the mass of the contents of not less than 10 containers of Minocycline Hydrochloride for Injection. Weigh accurately an amount of Minocycline Hydrochloride, equivalent to about 0.1 g (potency) of Minocycline Hydrochloride according to the labeled amount, in 10 mL of water is 2.0 to 3.5.

Purity—Related substances—Conduct this procedure rapidly after the preparation of the sample solution. Take an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride according to the labeled amount, dissolve in the mobile phase to make 100 mL. To 25 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of each peak by the area percentage method: the amount of epiminocycline, having the relative retention time of about 0.83 with respect to minocycline, is not more than 6.0%.

Amount [mg (potency)] of minocycline (C21H20N2O7) = M5 × A5/A5 × 4

M5: Amount [mg (potency)] of Minocycline Hydrochloride
Minocycline Hydrochloride Tablets

ミノサイクリン塩酸塩錠

Minocycline Hydrochloride Tablets contain not less than 90.0% and not more than 110.0% of the labeled potency of Minocycline (C_{23}H_{27}N_{3}O_{7}·HCl: 457.48).

Method of preparation Prepare as directed under Tablets, with Minocycline Hydrochloride.

Identification To a quantity of powdered Minocycline Hydrochloride Tablets, equivalent to 50 mg (potency) of Minocycline Hydrochloride according to the labeled amount, add 625 mL of a solution of hydrochloric acid in methanol (19 in 20,000), shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 221 nm and 225 nm, between 261 nm and 265 nm, and between 354 nm and 358 nm.

Purity Related substances—Conduct this procedure rapidly after preparation of the sample solution. Powder not less than 5 Minocycline Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to 50 mg (potency) of Minocycline Hydrochloride according to the labeled amount, add 60 mL of the mobile phase, shake vigorously, and add the mobile phase to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the area percentage method: the amount of Minocycline Hydrochloride is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of minocycline, beginning after the solvent peak.

System suitability—

Test for required detectability: To 2 mL of the sample solution add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of minocycline from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Minocycline Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Minocycline Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 9 μg (potency) of Minocycline Hydrochloride according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_{T} and A_{S}, at 348 nm.

Dissolution rate (%) with respect to the labeled amount of minocycline (C_{23}H_{27}N_{3}O_{7}·HCl): 
\[ M_{5} = \frac{A_{T}}{A_{S}} \times \frac{V}{V} \times 1/C \times 36 \]

M_{5}: Amount [mg (potency)] of Minocycline Hydrochloride RS
C: Labeled amount [mg (potency)] of minocycline (C_{23}H_{27}N_{3}O_{7}·HCl) in 1 tablet

Assay To a number of Minocycline Hydrochloride Tablets, equivalent to about 1 g (potency) of Minocycline Hydrochloride, add 120 mL of the mobile phase, treat with ultrasonic waves for 15 minutes, and add the mobile phase to make exactly 200 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, and add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 25 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Minocycline Hydrochloride.

Water <2.48> Not more than 12.0% (0.5 g of powdered Minocycline Hydrochloride Tablets, volumetric titration, back titration).

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Minocycline Hydrochloride Tablets add 60 mL of the mobile phase, treat with ultrasonic waves for 15 minutes, and add the mobile phase to make exactly V mL so that each mL contains about 0.5 mg (potency) of Minocycline Hydrochloride. Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of minocycline (C_{23}H_{27}N_{3}O_{7}·HCl) 
\[ M_{5} = \frac{A_{T}}{A_{S}} \times \frac{V}{50} \]

M_{5}: Amount [mg (potency)] of Minocycline Hydrochloride RS
Mitomycin C

Mitomycin C is a substance having antitumor activity produced by the growth of Streptomyces caespitosus.

It contains not less than 970 µg (potency) and not more than 1030 µg (potency) per mg, calculated on the dried basis. The potency of Mitomycin C is expressed as mass (potency) of mitomycin C (C₁₅H₁₈N₄O₅).

**Description** Mitomycin C occurs as blue-purple, crystals or crystalline powder.

It is freely soluble in N,N-dimethylacetamide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5).

**Identification (1)** Determine the absorption spectrum of a solution of Mitomycin C (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mitomycin C RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mitomycin C as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Mitomycin C RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** Related substances—Conduct this procedure rapidly after the sample and the standard solutions are prepared. Dissolve 50 mg of Mitomycin C in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each area of the peak other than mitomycin C obtained from the sample solution is not larger than the peak area of mitomycin C from the standard solution, and the total area of the peaks other than mitomycin C from the sample solution is not larger than 3 times the peak area of mitomycin C from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecysilanolized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To 800 mL of this solution add 200 mL of methanol.

Mobile phase B: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To this solution add 1000 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>10 – 30</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>30 – 45</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: About 1.0 mL per minute.

Time span of measurement: About 2 times as long as the retention time of mitomycin C beginning after the solvent peak.

**System suitability—**

Test for required detection: Pipet 10 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of mitomycin C obtained from 10 µL of this solution is equivalent to 7 to 13% of that from 10 µL of the standard solution.

System performance: Dissolve 25 mg of Mitomycin C and 40 mg of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of methanol. When the procedure is run with 10 µL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 3 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 3.0%.

**Loss on drying <2.41>** Not more than 1.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

**Assay** Weigh accurately an amount of Mitomycin C and Mitomycin C RS, equivalent to about 25 mg (potency), dissolve each in N,N-dimethylacetamide to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the fol-
Mitomycin C for Injection

Mitomycin C for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of mitomycin C (C₁₅H₁₈N₄O₅: 334.33).

Method of preparation Prepare as directed under Injectons, with Mitomycin C.

Description Mitomycin C for Injection occurs as a blue-purple powder.

Identification Dissolve an amount of Mitomycin C for Injection, equivalent to 2 mg (potency) of Mitomycin C according to the labeled amount, in 200 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 216 nm and 220 nm, and between 362 nm and 366 nm.

pH <2.54> The pH of a solution, prepared by dissolving 0.25 g of Mitomycin C for Injection in 20 mL of water, is 5.5 to 8.5.

Loss on drying <2.4l> Not more than 1.0% (0.4 g, in vacuo not exceeding 0.67 kPa, phosphorus (V) oxide, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 10 EU/mg (potency).

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 container of Mitomycin C for Injection add exactly V mL of N,N-dimethylacetamide so that each mL contains about 0.5 mg (potency) of Mitomycin C, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg (potency) of Mitomycin C RS, add N,N-dimethylacetamide to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mitomycin C.

Amount [mg (potency)] of mitomycin C (C₁₅H₁₈N₄O₅)
= Mₛ × Aₛ/Aᵣ × V/50

Mₛ: Amount [mg (potency)] of Mitomycin C RS

Containers and storage Containers—Tight containers.

Mizoribine

ミゾリビン

C₉H₁₃N₃O₆: 259.22
5-Hydroxy-1-β-D-ribofuranosyl-1H-imidazole-4-carboxamide [50924-49-7]

Mizoribine contains not less than 98.0% and not more than 102.0% of C₉H₁₃N₃O₆, calculated on the anhydrous basis.
Description  Mizoribine occurs as a white to yellowish white crystalline powder.

It is freely soluble in water, and practically insoluble in methanol and in ethanol (99.5).

Identification  (1) Determine the absorption spectrum of a solution of Mizoribine (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mizoribine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mizoribine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Mizoribine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation  <2.49>  \([\alpha]_D^{20} = -25 - -27^\circ (0.5 \text{ g calculated on the anhydrous basis, water, 25 mL, 100 mm})\).

Purity  (1) Heavy metals  <1.07>—Proceed with 1.0 g of Mizoribine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Mizoribine in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of mizoribine, \( A_1 \) and \( A_2 \), of both solutions.

\[
M:\text{Amount (mg) of Mizoribine RS, calculated on the anhydrous basis}
\]

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 279 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \text{m} \) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Diluted phosphoric acid (1 in 1500).

Flow rate: Adjust the flow rate so that the retention time of mizoribine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 \( \mu \text{L} \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mizoribine are not less than 10,000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 5 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mizoribine is not more than 1.0%.

Containers and storage  Containers—Tight containers.

Mizoribine Tablets

ミゾリビン錠

Mizoribine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of mizoribine (\( \text{C}_9\text{H}_{13}\text{N}_3\text{O}_6: 259.22 \)).

Method of preparation  Prepare as directed under Tablets, with Mizoribine.

Identification  To a quantity of powdered Mizoribine Tablets, equivalent to 0.1 g of Mizoribine according to the labeled amount, add 5 mL of water, shake, filter, and use the filtrate as the sample solution. Separately, dissolve 20 mg of Mizoribine RS in 1 mL of water, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Thin-Layer Chromatography <2.03>. Spot 1 \( \mu \text{L} \) each of the sample solu-
tion and standard solution on a plate of silica gel for thin-layer chromatography. Then develop the plate with a mixture of methanol, ammonia solution (28) and 1-propanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the principal spot from the sample solution and the spot from the standard solution show a red-brown color and the same Rf value.

Purity Related substances—To a quantity of powdered Mizoribine Tablets, equivalent to 0.10 g of Mizoribine anhydrous, add water to make exactly 100 mL. Pipet 2 mL of the solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A2, at 279 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[ \text{Amount of mizoribine (C9H13N3O6)} = M_5 \times A_1/A_2 \times V/V \times 1/50 \]

M5: Amount (mg) of Mizoribine RS, calculated on the anhydrous basis

Dissolution <5.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Mizoribine Tablets is not less than 80%.

Start the test with 1 tablet of Mizoribine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 14 μg of mizoribine (C9H13N3O6) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Mizoribine RS (separately determine the water content of Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A2, at 279 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[ \text{Dissolution rate (\%)} = \frac{A_1}{A_2} \times \frac{V}{V} \times \frac{1}{C} \times 45 \]

C: Labeled amount (mg) of mizoribine (C9H13N3O6) in 1 tablet

Assay Weigh accurately not less than 20 Mizoribine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of mizoribine (C9H13N3O6), add 50 mL of water and shake, then add water to make exactly 100 mL. Filter the solution, discard not less than 10 mL of the first filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Mizoribine RS (separately determine the water content of Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 2 mL of the solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A2, at 279 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[ \text{Amount (mg) of mizoribine (C9H13N3O6)} = M_5 \times A_1/A_2 \times V/V \times 1/50 \]

M5: Amount (mg) of Mizoribine RS, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Official Monographs / Mizoribine Tablets 1129
Morphine and Atropine Injection

Morphine and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.91 w/v% and not more than 1.09 w/v% of morphine hydrochloride hydrate \((\text{C}_{17}\text{H}_{19}\text{NO}_3\cdot\text{HCl}\cdot3\text{H}_2\text{O}: 375.84)\), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate \([\text{C}_{17}\text{H}_{33}\text{NO}_3\cdot2\cdot\text{H}_2\text{SO}_4\cdot\text{H}_2\text{O}: 694.83]\).

**Method of preparation**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine Hydrochloride Hydrate</td>
<td>10 g</td>
</tr>
<tr>
<td>Atropine Sulfate Hydrate</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Water for Injection</td>
<td>a significant quantity</td>
</tr>
</tbody>
</table>

Prepare as directed under Injections, with the above ingredients.

**Description** Morphine and Atropine Injection is a clear, colorless liquid. It is gradually colored by light. pH: 2.5 – 5.0

**Identification** To 2 mL of Morphine and Atropine Injection add 2 mL of ammonia TS, and extract with 10 mL of diethyl ether. Filter the extract with a filter paper, evaporate the filtrate on a water bath to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Separately, dissolve 0.1 g of morphine hydrochloride in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (1). Separately, dissolve 3 mg of atropine sulfate in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 µL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28:200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: the two spots obtained from the sample solution show the same color tone and the same Rf value with either spot of orange color obtained from the standard solution (1) or the standard solution (2) (morphine and atropine).

**Extractable volume** It meets the requirement.

**Assay** (1) Morphine hydrochloride hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, add exactly 10 mL of the internal standard solution to dissolve, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 µL of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios,  and  of the peak area of morphine to that of the internal standard.

\[
\text{Amount (mg) of morphine hydrochloride} = \text{M}_5 \times \frac{Q_T}{Q_S} \times 1.168
\]

\[
\text{M}_5: \text{Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis}
\]

**Internal standard solution**—A solution of etilefrine hydrochloride (1 in 500).

**Operating conditions**


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH with sodium hydroxide TS to 3.0. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

**System suitability**

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

(2) Atropine sulfate hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Atropine Sulfate RS (separately determine the loss on drying under the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios  and  of the peak areas of atropine to that of the internal standard.

\[
\text{Amount (mg) of atropine sulfate hydrate} = \text{M}_5 \times \frac{Q_T}{Q_S} \times 1/25 \times 1.027
\]

\[
\text{M}_5: \text{Amount (mg) of Atropine Sulfate RS, calculated on the dried basis}
\]

**Internal standard solution**—A solution of etilefrine hydrochloride (1 in 12,500).

**Operating conditions**

Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (1).
Detector: An ultraviolet absorption photometer (wavelength: 225 nm).
Flow rate: Adjust the flow rate so that the retention time of morphine is about 7 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, morphine, the internal standard and atropine are eluted in this order, and the resolution between morphine and the internal standard is not less than 3.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Morphine Hydrochloride Hydrate

モルヒネ塩酸塩水和物

C_{17}H_{19}NO_3.HCl.3H_2O: 375.84
(5R,6S)-4,5-Epoxy-17-methyl-7,8-didehydromorphinan-3,6-diol monohydrochloride trihydrate
[6055-06-7]

Morphine Hydrochloride Hydrate contains not less than 98.0% and not more than 102.0% of morphine hydrochloride (C_{17}H_{19}NO_3.HCl: 321.80), calculated on the anhydrous basis.

Description Morphine Hydrochloride Hydrate occurs as white, crystals or crystalline powder.
It is freely soluble in formic acid, soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).
It gradually becomes yellow-brown by light.

Identification (1) Determine the absorption spectrum of a solution of Morphine Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry 2.2.4, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Morphine Hydrochloride in dilute sodium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Morphine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.2.5, and compare the spectrum with the Reference Spectrum 1: both spectra show similar absorptivities at the same wave numbers.

Assay

(1) A solution of Morphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests 1.099 (2) for chloride.

Optical rotation <2.49> [α]_D: -111 – -116° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Morphine Hydrochloride Hydrate in 10 mL of water is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 0.40 g of Morphine Hydrochloride Hydrate in 10 mL of water; the solution is clear. When perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry 2.2.4, the absorbance at 420 nm is not more than 0.12.

(2) Sulfate <1.14>—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 2 to 3 drops of barium chloride TS: no turbidity is produced.

(3) Meconic acid—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 5 mL of dilute hydrochloric acid and 2 drops of iron (III) chloride TS: no red color develops.

(4) Related substances—Dissolve 0.1 g of Morphine Hydrochloride Hydrate in 10 mL of diluted ethanol (95) (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (95) (1 in 2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Water <2.48> 13 – 15% (0.1 g, direct titration).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Morphine Hydrochloride Injection

モルヒネ塩酸塩注射液

Morphine Hydrochloride Injection is an aqueous solution for injection.
It contains not less than 93.0% and not more than 107.0% of the labeled amount of morphine hydrochlo-
ride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O: 375.84).

**Method of preparation** Prepare as directed under Injections, with Morphine Hydrochloride Hydrate.

**Description** Morphine Hydrochloride Injection is a clear, colorless or pale yellow-brown liquid. It gradually becomes yellow-brown by light.

**pH** 2.5 – 5.0

**Identification** Take a volume of Morphine Hydrochloride Injection, equivalent to 0.04 g of Morphine Hydrochloride Hydrate according to the labeled amount, add water to make 20 mL, and use this solution as the sample solution. To 5 mL of the sample solution add water to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry (C₂₄H₂₄O₅N₂Cl): it exhibits a maximum between 283 nm and 287 nm. And to 5 mL of the sample solution add dilute sodium hydroxide TS to make 100 mL, and determine the absorption spectrum: it exhibits a maximum between 296 nm and 300 nm.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Take exactly a volume of Morphine Hydrochloride Injection, equivalent to about 80 mg of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O), add water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (C₂₄H₂₄O₅N₂Cl: it exhibits a maximum between 283 nm and 287 nm. And weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of water, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry (C₂₄H₂₄O₅N₂Cl: it exhibits a maximum between 283 nm and 287 nm. And weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of dilute sodium hydroxide TS, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate: it exhibits a maximum between 296 nm and 300 nm.

**Bacterial endotoxins** <4.07> Less than 1.5 EU/mg.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

**Morphine Hydrochloride Tablets**

Morphine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O: 375.84).

**Method of preparation** Prepare as directed under Tablets, with Morphine Hydrochloride Hydrate.

**Identification** Weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of water, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry (C₂₄H₂₄O₅N₂Cl: it exhibits a maximum between 283 nm and 287 nm. And weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of dilute sodium hydroxide TS, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate: it exhibits a maximum between 296 nm and 300 nm.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Morphine Hydrochloride Tablets add exactly 1 mL of the internal standard solution per 2 mg of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O), disperse the tablet into a small particles using ultrasonic waves, then treat with ultrasonic waves for 15 minutes with occasional stirring, and add water to make V mL so that each mL contains about 0.4 mg of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O). Filter the solution, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O) = \(M_s \times \frac{Q_t}{Q_s} \times \frac{V}{50} \times 1.168\)

Where:
- \(M_s\): Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis
- \(Q_t\): Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis
- \(Q_s\): Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis
- \(V\): Volume (mL) of the solution used for analysis
- \(50\): Volume (mL) of the reference solution
- \(1.168\): Conversion factor

**System suitability**—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Take exactly a volume of Morphine Hydrochloride Injection, equivalent to about 80 mg of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O), add water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (C₂₄H₂₄O₅N₂Cl: it exhibits a maximum between 283 nm and 287 nm. And weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of dilute sodium hydroxide TS, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate: it exhibits a maximum between 296 nm and 300 nm.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Morphine Hydrochloride Tablets add exactly 1 mL of the internal standard solution per 2 mg of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O), disperse the tablet into a small particles using ultrasonic waves, then treat with ultrasonic waves for 15 minutes with occasional stirring, and add water to make V mL so that each mL contains about 0.4 mg of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O). Filter the solution, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O) = \(M_s \times \frac{Q_t}{Q_s} \times \frac{V}{50} \times 1.168\)

Where:
- \(M_s\): Amount (mg) of morphine hydrochloride for assay,
calculated on the anhydrous basis

**Internal standard solution**—A solution of etilefrine hydrochloride (1 in 500).

**Dissolution** ⑤ ⑥ When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Morphine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Morphine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay (separately, determine the water <2.48> in the same manner as Morphine Hydrochloride Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of morphine in each solution.

Dissolution rate (%) with respect to the labeled amount of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O) = \( M_S \times A_1/\text{A_S} \times 1/C \times 36 \times 1.168 \)

\( M_S \): Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

\( C \): Labeled amount (mg) of morphine hydrochloride hydrate (C₁₇H₁₉NO₃.HCl.3H₂O) in 1 tablet

**Operating conditions**—
Proceed as directed in the operating conditions in the Assay.

**System suitability**—
System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of morphine are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of morphine is about 10 minutes.

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

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**Mosapride Citrate Hydrate**

Mosapride Citrate Hydrate occurs as a white to yellowish white crystalline powder.

\[ \text{C}_{21}\text{H}_{25}\text{ClFN}_{3}\text{O}_{3}.\text{C}_{6}\text{H}_{8}\text{O}_{7}.2\text{H}_{2}\text{O}: 650.05} \]

4-Amino-5-chloro-2-ethoxy-N-\{[(2RS)-4-(fluorobenzyl)morpholin-2-yl][methyl]benzamide monocitrate dihydrate

\[ [636582-62-2] \]

Mosapride Citrate Hydrate contains not less than 98.5% and not more than 101.0% of mosapride citrate (C₁₇H₁₉NO₃.HCl.3H₂O:: 614.02), calculated on the anhydrous basis.

**Description** Mosapride Citrate Hydrate occurs as a white to yellowish white crystalline powder.
It is freely soluble in N,N-dimethylformamide and in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.
A solution of Mosapride Citrate Hydrate in N,N-dimethylformamide (1 in 20) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Mosapride Citrate Hydrate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mosapride Citrate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Mosapride Citrate Hydrate in N,N-dimethylformamide (1 in 10) responds to the Qualitative Tests <1.09> (1) for citrate.

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Mosapride Citrate Hydrate in a platinum crucible according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Mosapride Citrate Hydrate in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.47 with respect to mosapride from the sample solution is not larger than 3 times the peak area of mosapride from the standard solution, and the area of each peak other than the peak of mosapride and other than the peak mentioned above from the sample solution is not larger than the peak area of mosapride from the standard solution. Furthermore, the total area of the peaks other than the peak of mosapride from the sample solution is not larger than 5 times the peak area of mosapride from the standard solution.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 274 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase A: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 4.0 with dilute hydrochloric acid, and add water to make 1000 mL.
Mobile phase B: Acetonitrile.
Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 35</td>
<td>80 → 45</td>
<td>20 → 55</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute.
Time span of measurement: Beginning after the solvent peak to 35 minutes after injection.

**System suitability—**
Test for required detectability: Pipet 4 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of mosapride obtained from 5 μL of this solution is equivalent to 15 to 25% of that of mosapride from 5 μL of the standard solution.
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 40,000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 5.0%.

(3) Residual solvent—Being specified separately.

**Water <2.48>** 5.0 - 6.5% (0.5 g, volumetric titration, back titration).

**Residue on ignition <2.44>** Not more than 0.1% (1 g, platinum crucible).

**Assay** Weigh accurately 0.5 g of Mosapride Citrate Hydrate, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 61.40 mg of C₂₁H₂₅ClFN₃O₇.C₆H₈O₇.

**Containers and storage** Containers—Well-closed containers.

**Mosapride Citrate Powder**

Mosapride Citrate Powder contains not less than 93.0% and not more than 107.0% of the labeled amount of mosapride citrate (C₂₁H₂₅ClFN₃O₇.C₆H₈O₇: 614.02).

**Method of preparation** Prepare as directed under Granules or Powders, with Mosapride Citrate Hydrate.

**Identification (1)** Powder Mosapride Citrate Powder. To a portion of the powder, equivalent to 10 mg of mosapride citrate (C₂₁H₂₅ClFN₃O₇.C₆H₈O₇) according to the labeled amount, add 10 mL of dilute acetic acid, shake for 10 minutes, and filter. To 5 mL of the filtrate add 0.3 mL of Dragendorff’s TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima be-
between 271 nm and 275 nm and between 306 nm and 310 nm.

**Purity** Related substances—Powder Mosapride Citrate Powder. To a portion of the powder, equivalent to 10 mg of mosapride citrate (C$_2$H$_2$ClF$_5$N$_3$O$_7$C$_6$H$_8$O$_7$), according to the labeled amount, moisten with 1 mL of water, then add 9 mL of methanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, according to the following conditions, and determine each peak area by the automatic integration method: the area of the two peaks, having the relative retention time of about 0.60 and about 0.85 with respect to mosapride obtained from the sample solution, is not larger than the peak area of mosapride from the standard solution, the area of other than the peak of mosapride and the peaks mentioned above is not larger than 2/5 times the peak area of mosapride from the standard solution, and the total area of the peak other than mosapride is not larger than 2 times the peak area of mosapride from the standard solution.

**Operating conditions**—
Detector, column, column temperature, mobile phases A and B, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Mosapride Citrate Hydrate.
Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 40</td>
<td>85 - 45</td>
<td>15 - 55</td>
</tr>
</tbody>
</table>

Time span of measurement: For 40 minutes after sample injection, beginning after the solvent peak.

**System suitability**—
Test for required detectability: To exactly 1 mL of the standard solution add methanol to make exactly 25 mL. Confirm that the peak area of mosapride obtained with 10 μL of this solution is equivalent to 3.0 to 5.0% of that with 10 μL of the standard solution.
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 40,000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 3.0%.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: the powder in single-unit container meets the requirement of the Content uniformity test.
To the total content of 1 container of Mosapride Citrate Powder add 5 mL of water, and shake. Then, add 20 mL of methanol, shake for 20 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V mL so that each mL contains about 20 μg of mosapride citrate (C$_2$H$_2$ClF$_5$N$_3$O$_7$C$_6$H$_8$O$_7$), and use this solution as the sample solution. Then, proceed as directed in the Assay.
Amount (mg) of mosapride citrate (C$_2$H$_2$ClF$_5$N$_3$O$_7$C$_6$H$_8$O$_7$) obtained with 10 mL of the 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Mosapride Citrate Powder is not less than 70%.
Start the test with an amount of Mosapride Citrate Powder, equivalent to about 2.5 mg of mosapride citrate (C$_2$H$_2$ClF$_5$N$_3$O$_7$C$_6$H$_8$O$_7$) according to the labeled amount, withdraw not more than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 30 mg of mosapride citrate for assay (separately determine the area of the two peaks, having the relative retention time of about 0.60 and about 0.85 with respect to mosapride obtained from the sample solution, and the total area of the peak other than mosapride is not larger than 2 times the peak area of mosapride from the standard solution.)

**System suitability**—
Detector: An ultraviolet absorption photometer (wavelength: 274 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeylsilanized silica gel for liquid chromatography (<5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust to pH 3.3 with dilute hydrochloric acid, and add water to make 1000 mL. To 240 mL of this solution add 90 mL of methanol and 70 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of mosapride is about 9 minutes.

**System suitability**—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 4000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operat-
ing conditions, the relative standard deviation of the peak area of mosapride is not more than 2.0%.

**Assay** Powder Mosapride Citrate Powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{7}.C_{6}H_{8}O_{7}), moisten with 2 mL of water, add 70 mL of methanol, shake for 20 minutes, then add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 53 mg of mosapride citrate for assay (separately determine the water and hydrate). Prepare as directed under Tablets, and dissolve in methanol to make exactly 100 mL, then add methanol to make exactly 50 mL, and centrifuge. This solution is used as the standard solution. Determine the absorbances, A_{T} and A_{S}, of the sample solution and the standard solution at 273 nm as directed under Ultraviolet-visible Spectrophotometry.<2.24≥.

**Amount (mg) of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{7}.C_{6}H_{8}O_{7})**

\[
M_{S} = M_{S} \times A_{T}/A_{S} \times 1/5
\]

**Containers and storage** Containers—Tight containers.

**Mosapride Citrate Tablets**

モサプリドクエン酸塩錠

Mosapride Citrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{7}.C_{6}H_{8}O_{7}: 614.02).

**Method of preparation** Prepare as directed under Tablets, with Mosapride Citrate Hydrate.

**Identification (1)** To an amount of powdered Mosapride Citrate Tablets, equivalent to 10 mg of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{7}.C_{6}H_{8}O_{7}) according to the labeled amount, add 10 mL of dilute acetic acid, shake for 10 minutes, and filter. To 5 mL of the filtrate add 0.3 mL of Dragendorff’s TS: an orange precipitate is formed.

**Operating conditions—**

- **Detector, column, column temperature, mobile phase A, mobile phase B, and flow rate:** Proceed as directed in the operating conditions in the Purity (2) under Mosapride Citrate Hydrate.
- **Flowing of the mobile phase:** Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 → 40</td>
<td>85 → 45</td>
<td>15 → 55</td>
</tr>
</tbody>
</table>

**System suitability—**

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 25 mL. Confirm that the peak area of mosapride obtained from 10 μL of this solution is equivalent to 3.0 to 5.0% of that of mosapride from 10 μL of the standard solution.

**System performance:** When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 40,000 and not more than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 3.0%.

**Uniformity of dosage units <6.02≥** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Mosapride Citrate Tablets add 5 mL of water, and shake well to disintegrate. Add 20 mL of methanol, shake for 20 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V mL so that each mL contains about 20 μg of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{7}.C_{6}H_{8}O_{7}), and use this solution as the sample solution. Proceed as directed in the Assay.

**Amount (mg) of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{7}.C_{6}H_{8}O_{7})**

\[
M_{S} = M_{S} \times A_{T}/A_{S} \times V/V' \times 1/50
\]

**Dissolution <6.10≥** When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of
Mosapride Citrate Tablets is not less than 80%.

Start the test with 1 tablet of Mosapride Citrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 1 mL of the subsequent filtrate, and add the dissolution medium to make exactly 15 mL so that each mL contains about 2.8 μg of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{3}.C_{6}H_{8}O_{7}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of mosapride citrate for assay (separately, determine the water <2.48% in the same manner as Mosapride Citrate Hydrate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 200 mL, and use this solution as the sample solution. Perform the test with exactly 50 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of mosapride of both solutions.

Dissolution rate (%) with respect to the labeled amount of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{3}.C_{6}H_{8}O_{7})
\[ M_S = \frac{A_T}{A_S} \times \frac{V}{V} \times \frac{1}{C} \times 9 \]

M_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis
C: Labeled amount (mg) of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{3}.C_{6}H_{8}O_{7}) in 1 tablet

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 274 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 3.3 with dilute hydrochloric acid, and add water to make 1000 mL. To 240 mL of this solution add 90 mL of methanol and 70 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of mosapride is about 9 minutes.

System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 4000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Mosapride Citrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{3}.C_{6}H_{8}O_{7}), and moisten with 2 mL of water. Add 70 mL of methanol, shake for 20 minutes, add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 53 mg of mosapride citrate for assay (separately, determine the water <2.48% in the same manner as Mosapride Citrate Hydrate), and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 273 nm.

Amount (mg) of mosapride citrate (C_{21}H_{25}ClFN_{3}O_{3}.C_{6}H_{8}O_{7})
\[ M_S = \frac{A_T}{A_S} \times \frac{1}{5} \]

M_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Freeze-dried Live Attenuated Mumps Vaccine

Freeze-dried Live Attenuated Mumps Vaccine is a dried preparation containing live attenuated mumps virus.

It conforms to the requirements of Freeze-dried Live Attenuated Mumps Vaccine in the Minimum Requirements of Biologic Products.

Description Freeze-dried Live Attenuated Mumps Vaccine becomes a clear, colorless, yellowish or reddish liquid on addition of solvent.

Mupirocin Calcium Hydrate

\[
\text{C}_{52}\text{H}_{86}\text{CaO}_{18}.2\text{H}_{2}\text{O}: 1075.34
\]

Monocalcium bis[(2E)-4-[(2S,3R,4R,5S)-5-[(2S,3S,4S,5S)-2,3-epoxy-5-hydroxy-4-methylhexyl]-3,4-dihydroxy-3,4,5,6-tetrahydro-2H-pyran-2-yl]-3-methylbut-2-enoyloxynonoate] dihydrate

[115074-43-6]

Mupirocin Calcium Hydrate is the calcium salt of a substance having antibacterial activity produced by the growth of Pseudomonas fluorescens.

It contains not less than 895 μg (potency) and not more than 970 μg (potency) per mg, calculated on the anhydrous basis. The potency of Mupirocin Calcium Hydrate is expressed as mass (potency) of mupirocin (C_{26}H_{44}O_{9}: 500.62).

Description Mupirocin Calcium Hydrate occurs as a white powder and has a bitter taste.

It is freely soluble in methanol and slightly soluble in water and in ethanol (95).
Identification (1) To 1 mL of a solution of Mupirocin Calcium Hydrate in methanol (1 in 200) add 4 mL of hydroxylamine perchlorate-ethanol TS and 1 mL of N,N'-dicyclohexylocarbodiimide-ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (II) perchlorate hexahydrate-ethanol TS to the solution, and shake; a dark purple color develops.

(2) Determine the absorption spectrum of a solution of Mupirocin Calcium Hydrate (1 in 50,000) as directed under Ultraviolet-Visible Spectrophotometry (2.24): it exhibits a maximum between 219 nm and 224 nm.

(3) Determine the infrared absorption spectrum of Mupirocin Calcium Hydrate as directed in the paste method under Infrared Spectrophotometry (2.25): it exhibits absorption at the wave numbers of about 1708 cm⁻¹, 1648 cm⁻¹, 1558 cm⁻¹, 1321 cm⁻¹, 1151 cm⁻¹ and 894 cm⁻¹.

(4) A solution of Mupirocin Calcium Hydrate (3 in 1000) responds to the Qualitative Tests (1.09) (3) for calcium salt.

Optical rotation (2.48) \( [\alpha]_D^{20} = -16 \sim -20^\circ \) (1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) Related substances—Dissolve 50 mg of Mupirocin Calcium Hydrate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make 10 mL, and use this solution as the sample solution (1). Pipet 2 mL of the sample solution (1), add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 100 mL, and use this solution as the sample solution (2). Preserve these solutions at a temperature between 10°C and 25°C. Perform the test with exactly 20 \( \mu \)L of the sample solution (1) and the sample solution (2) as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the areas of each peak of the sample solution (1) and the sample solution (2) by the automatic integration method. Calculate the amount of the related substances by the following formula: the amount of principal related substance (appeared at about 0.7 of the relative retention time to mupirocin) is not more than 4.0%, and the total amount of related substances (the total area of the peaks other than of the solvent and mupirocin) is not more than 6.0%.

Amount (%) of principal related substance

\[
\text{Amount (\%) of principal related substance} = \frac{A}{A + A_n} \times 100 \times \frac{P \times 100}{100 - \frac{A \times 100}{A + A_n}}
\]

Total amount (%) of related substances

\[
\text{Total amount (\%) of related substances} = \frac{A}{A + A_n} \times 100 \times \frac{P \times 100}{100 - \frac{A \times 100}{A + A_n}}
\]

A: Total peak areas other than of the solvent and mupirocin from the sample solution (1)

A₁: Peak area of the relative retention time of about 0.7 to mupirocin from the sample solution (1)

Aₙ: A value of 50 times of peak area of mupirocin from the sample solution (2)

P: Potency per mg obtained from the assay

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of mupirocin beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 1 mL of the sample solution (2), and add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 20 mL. Confirm that the peak area of mupirocin obtained from 20 \( \mu \)L of this solution is equivalent to 4 to 6% of that obtained from 20 \( \mu \)L of the sample solution (2).

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the sample solution (2) under the above operating conditions, the relative standard deviation of the peak areas of mupirocin is not more than 2.0%.

(2) Inorganic salt from manufacturing process—Being specified separately.

Water (2.48) Not less than 3.0% and not more than 4.5% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Mupirocin Calcium Hydrate and Mupirocin Lithium RS, equivalent to about 20 mg (potency), dissolve in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 200 mL, and use these solutions as the sample solution and the standard solution. Preserve these solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 \( \mu \)L of the sample solution (1) and the sample solution (2) as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, \( A_1 \) and \( A_S \), of mupirocin of each solution.

Amount \( [\mu g \text{ (potency)}] \) of mupirocin \( (C_{20}H_{33}O_8) \)

\[
M_S = \frac{M_S \times A_1/A_S \times 1000}{M_C}
\]

\( M_S \): Amount [mg (potency)] of Mupirocin Lithium RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.71 g of ammonium acetate in 750 mL of water, adjust the pH to 5.7 with acetic acid (100), and add water to make 1000 mL. To 300 mL of this solution add 100 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of mupirocin is about 12.5 minutes.

System suitability—

System performance: Dissolve about 20 mg of Mupirocin Lithium RS and about 5 mg of ethyl parahydroxybenzoate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and a solution of tetrahydrofuran (3 in 4) (1:1) to make 200 mL. When the procedure is run with 20 \( \mu \)L of this solution under the above operating conditions, mupirocin and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 12.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mupirocin is not more than 1.0%.

**Containers and storage**  Containers—Tight containers.

**Mupirocin Calcium Ointment**

ムピロシンカルシウム軟膏

Mupirocin Calcium Ointment is an oily ointment preparation.

Mupirocin Calcium Ointment contains not less than 95.0% and not more than 105.0% of the labeled potency of mupirocin (C_{26}H_{44}O_{9}: 500.62).

**Method of preparation**  Prepare as directed under Ointments, with Mupirocin Calcium Hydrate.

**Identification**  To an amount of Mupirocin Calcium Ointment, equivalent to 10 mg (potency) of Mupirocin Calcium Hydrate according to the labeled amount, add 5 mL of water, and warm on a water bath at 60°C for 10 minutes while occasional shaking. After cooling, filter, and to 1 mL of the filtrate add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.2>: it exhibits a maximum between 220 nm and 224 nm.

**Purity**  Related substances—To an amount of Mupirocin Calcium Ointment, equivalent to 50 mg (potency) of Mupirocin Calcium Hydrate according to the labeled amount, add 5 mL of diluted tetrahydrofuran (3 in 4), and shake vigorously. Then, add 5 mL of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, shake vigorously, filter through a glass wool filter, and use the filtrate as the sample solution. Pipet 2 mL of the sample solution, add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and diluted tetrahydrofuran (3 in 4) (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.6> according to the following conditions, and determine the area of the peak other than mupirocin obtained from the sample solution and the peak area of mupirocin from the standard solution by the automatic integration method. Calculate the amount of each related substance using the following equation: the amount of the related substance having the relative retention time of mupirocin (C_{26}H_{44}O_{9}: 500.62).

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mupirocin is not more than 2.0%.

**Assay**  Weigh accurately an amount of Mupirocin Calcium Ointment, equivalent to about 2 mg (potency) of Mupirocin Calcium Hydrate, add exactly 10 mL of diluted tetrahydrofuran (3 in 4), and shake vigorously. To this solution add exactly 10 mL of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, shake vigorously, filter through a glass wool filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Mupirocin Lithium RS, equivalent to about 20 mg (potency), dissolve in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and diluted tetrahydrofuran (3 in 4) (1:1) to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mupirocin Calcium Hydrate.

\[
\text{Amount [mg (potency)] of mupirocin (C}_{26}\text{H}_{44}\text{O}_{9}) = \frac{M_5 \times A_T/A_S \times 1/10}{M_5} = A_T/A_S \times 1/10
\]

\[
M_5: \text{Amount [mg (potency)] of Mupirocin Lithium RS}
\]

**Containers and storage**  Containers—Tight containers.

**Nabumetone**

ナブメトン

\[
\text{C}_{15}\text{H}_{16}\text{O}_{2}: 228.29
\]

4-(6-Methoxynaphthalen-2-yl)butan-2-one [42924-33-8]

Nabumetone contains not less than 98.0% and not more than 101.0% of C_{15}H_{16}O_{2}, calculated on the anhydrous basis.

**Description**  Nabumetone occurs as white to yellowish white crystals or a crystalline powder.

It is soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.
Identification (1) Determine the absorption spectrum of a solution of Nabumetone in methanol (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nabumetone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nabumetone as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Nabumetone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point: \( 2.60 \) \( ^\circ \text{C} \) to \( 79 – 84^\circ \text{C} \).

Purity (1) Heavy metals: Proceed with 1.0 g of Nabumetone according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Nabumetone in 20 mL of acetonitrile, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add acetonitrile to make exactly 50 mL. Pipet 1 mL of this solution, add acetonitrile to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the peak area of the related substance \( G \) obtained from the sample solution is not larger than 3/5 times the peak area of nabumetone from the standard solution, and each peak area other than nabumetone and the related substance \( G \) is not larger than 1/5 times the peak area of nabumetone from the standard solution. Furthermore, the total area of the peaks other than nabumetone is not larger than 1.6 times the peak area of nabumetone from the standard solution. For these calculations, use each peak area of the related substances \( A, B, C, D, E, F \) and \( G \), which are having the relative retention time of about 0.73, 0.85, 0.93, 1.2, 1.9, 2.6 and 2.7 with respect to nabumetone, after multiplying by their relative response factors, 0.12, 0.94, 0.25, 0.42, 1.02, 0.91 and 0.1, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Mobile phase A: A mixture of water and acetic acid (100:999:1).

Mobile phase B: A mixture of acetonitrile and tetrahydrofuran (7:3).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 12</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>12 – 28</td>
<td>60 ( \rightarrow ) 20</td>
<td>40 ( \rightarrow ) 80</td>
</tr>
</tbody>
</table>

Flow rate: 1.3 mL per minute.

Time span of measurement: About 3 times as long as the retention time of nabumetone, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nabumetone obtained from 10 \( \mu \)L of this solution is equivalent to 14 to 26% of that from 10 \( \mu \)L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nabumetone is not more than 5.0%.

Water: Not more than 0.2% (1 g, volumetric titration, direct titration).

Residue on ignition: Not more than 0.1% (1 g).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Mobile phase: To 600 mL of a mixture of water and acetic acid (100:999:1) add 400 mL of a mixture of acetonitrile and tetrahydrofuran (7:3). Flow rate: Adjust the flow rate so that the retention time of nabumetone is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nabumetone are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nabumetone is not more than 1.0%.

Containers and storage—

Containers—Tight containers.
Nabumetone Tablets

ナブメトン錠

Nabumetone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nabumetone (C_{15}H_{16}O_{2}: 228.29).

Method of preparation Prepare as directed under Tablets, with Nabumetone.

Identification To a quantity of powdered Nabumetone Tablets, equivalent to 80 mg of Nabumetone according to the labeled amount, add 50 mL of methanol, shake for 10 minutes and centrifuge the solution. To 1 mL of the supernatant liquid, add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.4Ω>: it exhibits maxima between 259 nm and 263 nm, between 268 nm and 272 nm, between 316 nm and 320 nm, and between 330 nm and 334 nm.

Uniformity of dosage units <6.0Ω> It meets the requirements of the Mass variation test.

Dissolution <6.10Ω> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of a solution of polysorbate 80 (dissolving 3 g of polysorbate 80 in water to make 100 mL) as the dissolution medium, the dissolution rate in 60 minutes of Nabumetone Tablets is not less than 70%.

Start the test with 1 tablet of Nabumetone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add a solution, prepared by adding to 20 mL of ethanol (99.5) the dissolution medium to make 50 mL, to make exactly V mL so that each mL contains about 89 μg of nabumetone (C_{15}H_{16}O_{2}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Nabumetone RS (separately determine the water <2.4Ω> in the same manner as Nabumetone), and dissolve by adding 50 mL of methanol and exactly 20 mL of the internal standard solution, then add methanol to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0Ω> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nabumetone to that of the internal standard.

\[
\text{Amount (mg) of nabumetone (C}_{15}\text{H}_{16}\text{O}_{2}) = \frac{M_S \times Q_T}{Q_S} \times 5
\]

M_S: Amount (mg) of Nabumetone RS, calculated on the anhydrous basis

Internal standard solution—Dissolve 0.12 g of 2-ethylhexyl parahydroxybenzoate in methanol to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (550:450:1).

Flow rate: Adjust the flow rate so that the retention time of nabumetone is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, nabumetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 13.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nabumetone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

methanol, shake for 30 minutes, and then add methanol to make exactly 100 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Nabumetone RS (separately determine the water <2.4Ω> in the same manner as Nabumetone), dissolve by adding 50 mL of methanol and exactly 20 mL of the internal standard solution, then add methanol to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0Ω> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nabumetone to that of the internal standard.

\[
\text{Amount (mg) of nabumetone (C}_{15}\text{H}_{16}\text{O}_{2}) = \frac{M_S \times Q_T}{Q_S} \times 5
\]

M_S: Amount (mg) of Nabumetone RS, calculated on the anhydrous basis

Internal standard solution—Dissolve 0.12 g of 2-ethylhexyl parahydroxybenzoate in methanol to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (550:450:1).

Flow rate: Adjust the flow rate so that the retention time of nabumetone is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, nabumetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 13.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nabumetone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.
Nadolol

Naドロール

\[
\text{C}_{17}\text{H}_{27}\text{NO}_4: 309.40 \\
R^1 = \text{OH}, R^2 = \text{H} \\
(2R5,3S,)-5-[(2SR)-3-\{(1,1-\text{Dimethylthelyl)}\text{amino}-2-\text{hydroxypropoxy}\}-1,2,3,4-\text{tetrahydronaphthalene}-2,3-\text{diol} \\
R^1 = \text{H}, R^2 = \text{OH} \\
(2R5,3S,)-5-[(2RS)-3-\{(1,1-\text{Dimethylthelyl)}\text{amino}-2-\text{hydroxypropoxy}\}-1,2,3,4-\text{tetrahydronaphthalene}-2,3-\text{diol} \\
[\{22000-33-9]\]

Nadolol, when dried, contains not less than 98.0% of C_{17}H_{27}NO_4.

**Description**

Nadolol occurs as a white to yellow-brownish white crystalline powder. It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and slightly soluble in water and in chloroform. A solution of Nadolol in methanol (1 in 100) shows no optical rotation. Melting point: about 137°C.

**Identification**

(1) Determine the absorption spectrum of a solution of Nadolol in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nadolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, so that its transmittance at an absorption band at a wave number of about 1585 cm\(^{-1}\) is 25 to 30%, and determine the infrared absorption spectrum between 1600 cm\(^{-1}\) and 1100 cm\(^{-1}\). Determine the absorbances, A_{1265} and A_{1250}, from the transmittances, T_{1265} and T_{1250}, at wave numbers of about 1265 cm\(^{-1}\) (racemic substance A) and 1250 cm\(^{-1}\) (racemic substance B), respectively: the ratio A_{1265}/A_{1250} is between 0.72 and 1.08.

**Assay**

Weigh accurately about 0.28 g of Nadolol, previously dried, dissolve in 30 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green-blue (indicator: 3 drops of crystal violet TS). Perform a blank determination to make correction. Amount of the related substances calculated by the following equation is not more than 2.0%.

**Loss on drying** <2.41> Not more than 1.0% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Isomer ratio**

Prepare a paste with 0.01 g of Nadolol as directed in the paste method under Infrared Spectrophotometry <2.25> so that its transmittance at an absorption band at a wave number of about 1585 cm\(^{-1}\) is 25 to 30%, and determine the infrared absorption spectrum between 1600 cm\(^{-1}\) and 1100 cm\(^{-1}\). Determine the absorbances, A_{1265} and A_{1250}, from the transmittances, T_{1265} and T_{1250}, at wave numbers of about 1265 cm\(^{-1}\) (racemic substance A) and 1250 cm\(^{-1}\) (racemic substance B), respectively: the ratio A_{1265}/A_{1250} is between 0.72 and 1.08.

**Containers and storage**

Containers—Tight containers. Storage—Light-resistant.
Nafamostat Mesilate

ナファモスタットメシル酸塩

\[
\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}: 539.58
\]

6-Amidinonaphthalen-2-yl 4-guanidinobenzoate bis(methanesulfonate)

[NJP XVI Official Monographs / Nafamostat Mesilate 1143]

Nafamostat Mesilate, when dried, contains not less than 99.0% and not more than 101.0% of \(\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}\).

**Description**
Nafamostat Mesilate occurs as a white crystalline powder.

- It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (99.5).
- It dissolves in 0.01 mol/L hydrochloric acid TS.
- Melting point: about 262°C (with decomposition).

**Identification**
1. Determine the absorption spectrum of a solution of Nafamostat Mesilate in 0.01 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Nafamostat Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

3. A 0.1-g portion of Nafamostat Mesilate responds to the Qualitative Tests <1.09> (1) for mesilate.

**pH**

The pH of a solution prepared by dissolving 1.0 g of Nafamostat Mesilate in 50 mL of water is between 4.7 and 5.7.

**Purity**

1. Clarity and color of solution—A solution prepared by dissolving 1.0 g of Nafamostat Mesilate in 50 mL of water is clear and colorless.

2. Heavy metals
   - Proceed with 2.0 g of Nafamostat Mesilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

3. Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.10 g of Nafamostat Mesilate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mobile phase to make exactly 100 mL. Then pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu\)L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the area of each peak other than nafamostat obtained from the sample solution is not larger than 1/5 times the peak area of nafamostat from the standard solution. Furthermore, the total area of the peaks other than nafamostat is not larger than the peak area of nafamostat from the standard solution.

   - Detector: An ultraviolet absorption photometer (wavelength: 260 nm).
   - Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
   - Column temperature: A constant temperature of about 40°C.
   - Mobile phase: Dissolve 6.07 g of sodium 1-heptane sulfonate in 1000 mL of diluted acetic acid (100) (3 in 500). To 700 mL of this solution add 300 mL of acetonitrile.
   - Flow rate: Adjust the flow rate so that the retention time of nafamostat is about 7 minutes.
   - Time span of measurement: About 4 times as long as the retention time of nafamostat, beginning after the solvent peak.

4. **System suitability**—Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Pipet 15 mL of this solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of nafamostat obtained from 10 \(\mu\)L of this solution is equivalent to 1.1 to 1.9% of that from 10 \(\mu\)L of the standard solution.

5. **System performance:** Dissolve 0.1 g of nafamostat mesilate in the mobile phase to make 100 mL. To 10 mL of this solution add the mobile phase to make 100 mL. To 5 mL of this solution add 5 mL of a solution of 6-amidino-2-naphthol methanesulfonate in the mobile phase (1 in 20,000). When the procedure is run with 10 \(\mu\)L of this solution under the above operating conditions, 6-amidino-2-naphthol and nafamostat are eluted in this order with the resolution between these peaks being not less than 6.

6. **System repeatability:** When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nafamostat is not more than 2.0%.

**Loss on drying**
Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition**
Not more than 0.1% (1 g).

**Assay**
Weigh accurately about 0.25 g of Nafamostat Mesilate, previously dried, dissolve in 4 mL of formic acid, add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 26.98 mg of \(\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}\)

**Containers and storage**
Containers—Tight containers.
Nalidixic Acid

Nalidixic Acid occurs as white to light yellow crystals or crystalline powder.

Identification (1) Determine the absorption spectrum of a solution of Nalidixic Acid in 0.01 mol/L sodium hydroxide TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry \( \geq 2.24 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nalidixic Acid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \( \geq 2.25 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point \( \geq 2.60 \): 225 - 231°C.

Purity (1) Chloride \( \leq 1.03 \): To 2.0 g of Nalidixic Acid add 50 mL of water, warm at 70°C for 5 minutes, cool quickly, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.012%).

(2) Heavy metals \( \leq 2.01 \): Proceed with 1.0 g of Nalidixic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Nalidixic Acid in 20 mL of 0.01 mol/L sodium hydroxide TS. To 5 mL of this solution, add water to make 10 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \( \geq 2.01 \) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than nalidixic acid with the sample solution is not larger than the peak area of nalidixic acid with the standard solution, and the total area of the peaks other than the peak of nalidixic acid is not larger than 2.5 times the peak area of nalidixic acid with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.24 g of sodium dihydrogen phosphate dihydrate in 950 mL of water, adjust the pH to 2.8 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nalidixic acid is about 19 minutes.

Time span of measurement: About 3 times as long as the retention time of nalidixic acid beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of nalidixic acid obtained with 10 μL of this solution is equivalent to 40 to 60% of that with 10 μL of the standard solution.

System performance: Dissolve 25 mg of methyl parahydroxybenzoate in 100 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add water to make 10 mL. To 5 mL of this solution add 5 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoate and nalidixic acid are eluted in this order with the resolution between these peaks being not less than 13.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nalidixic acid is not more than 2.0%.

Loss on drying \( \geq 2.41 \): Not more than 0.20% (1 g, 105°C, 3 hours).

Residue on ignition \( \geq 2.44 \): Not more than 0.2% (1 g).

Assay Weigh accurately about 0.3 g of Nalidixic Acid, previously dried, dissolve in 50 mL of N,N-dimethylformamide, and titrate \( \geq 2.50 \) with 0.1 mol/L tetramethyl ammonium hydroxide VS (potentiometric titration). Separately, to 50 mL of N,N-dimethylformamide add 13 mL of a mixture of water and methanol (89:11), perform a blank determination with the solution, and make any necessary correction.

Each mL of 0.1 mol/L tetramethyl ammonium hydroxide VS = 23.22 mg of C₁₂H₁₂N₂O₃.

Containers and storage Containers—Tight containers.
Naloxone Hydrochloride

C_{19}H_{21}NO_4.HCl: 363.84
(5R,14S)-17-Allyl-4,5-epoxy-3,14-dihydmorphinan-6-one monohydrochloride
[357-08-4]

Naloxone Hydrochloride contains not less than 98.5% of C_{19}H_{21}NO_4.HCl, calculated on the dried basis.

**Description** Naloxone Hydrochloride occurs as white to yellowish white, crystals or crystalline powder.

It is freely soluble in water, soluble in methanol, slightly soluble in ethanol (99.5) and in acetic acid (100), and very slightly soluble in acetic anhydride.

It is hygroscopic.

It is gradually colored by light.

**Identification** (1) Determine the absorption spectrum of a solution of Naloxone Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Naloxone Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Naloxone Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

**Optical rotation** <2.48> [α]_D^25 = -170° to -181° (0.25 g calculated on the dried basis, water, 10 mL, 100 mm).

**pH** <2.54> Dissolve 0.10 g of Naloxone Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 4.5 and 5.5.

**Purity** Related substances—Conduct this procedure as rapidly as possible without exposure to light, using light-resistant containers. Dissolve 0.08 g of Naloxone Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.67>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia-saturated 1-butanol TS and methanol (20:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly iron (III) chloride-potassium hexacyanoferrate (III) TS on the plate: the number of the spot other than the principal spot from the sample solution is not more than 1 and it is not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 2.0% [0.1 g, 105°C, 5 hours. Use a desiccator (phosphorus (V) oxide) for cooling].

**Residue on ignition** <2.44> Not more than 0.2% (0.1 g).

**Assay** Weigh accurately about 0.3 g of Naloxone Hydrochloride, dissolve in 80 mL of acetic acid (100) by warming. After cooling, add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 36.38 mg of C_{19}H_{21}NO_4.HCl

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

Naphazoline Hydrochloride

C_{14}H_{14}N_2.HCl: 246.74
2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1H-imidazole monohydrochloride
[550-99-2]

Naphazoline Hydrochloride, when dried, contains not less than 98.5% of C_{14}H_{14}N_2.HCl.

**Description** Naphazoline Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, soluble in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: 255 – 260°C (with decomposition).

**Identification** (1) To 10 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 5 mL of bromine TS, and boil: a deep purple color develops.

(2) To 30 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 2 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Evaporate the combined diethyl ether extracts to dryness with the aid of a current of air. Dry the residue at 80°C for 1 hour: the residue melts <2.60> between 117°C and 120°C.

(3) Dissolve 0.02 g of the residue obtained in (2) in 2 to 3 drops of dilute hydrochloric acid and 5 mL of water, and add 2 mL of Reinecke salt TS: a red-purple, crystalline precipitate is formed.

(4) A solution of Naphazoline Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

**pH** <2.54> Dissolve 0.10 g of Naphazoline Hydrochloride
in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Naphazoline Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Naphazoline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Naphazoline Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.67 mg of C\textsubscript{14}H\textsubscript{14}N\textsubscript{2}.HCl

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

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**Naphazoline Nitrate**

**C\textsubscript{14}H\textsubscript{14}N\textsubscript{2}.HNO\textsubscript{3}: 273.29**

\text{2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1H-imidazole mononitrate [5144-52-5]} 

Naphazoline Nitrate, when dried, contains not less than 98.5% of C\textsubscript{14}H\textsubscript{14}N\textsubscript{2}.HNO\textsubscript{3}.

**Description** Naphazoline Nitrate occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in acetic acid (100), soluble in ethanol (95), sparingly soluble in water, slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

**Identification (1)** To 10 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of sodium hydroxide TS, and boil: a deep purple color develops.

(2) To 20 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness with the aid of a current of air, and dry the residue at 80°C for 1 hour: the residue so obtained melts <2.60> between 117°C and 120°C.

(3) A solution of Naphazoline Nitrate (1 in 20) responds to the Qualitative Tests <1.09> for nitrate.

**pH** <2.54> Dissolve 0.1 g of Naphazoline Nitrate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

**Melting point** <2.60> 167 – 170°C.

**Purity (1)** Clarity and color of solution—Dissolve 0.5 g of Naphazoline Nitrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Naphazoline Nitrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Naphazoline Nitrate, previously dried, dissolve in 10 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 27.33 mg of C\textsubscript{14}H\textsubscript{14}N\textsubscript{2}.HNO\textsubscript{3}

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

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**Naphazoline and Chlorpheniramine Solution**

**Nファゾリン・クロルフェニラミン液**

Naphazoline and Chlorpheniramine Solution contains not less than 0.045 w/v% and not more than 0.055 w/v% of naphazoline nitrate (C\textsubscript{14}H\textsubscript{14}N\textsubscript{2}.HNO\textsubscript{3}: 273.29), and not less than 0.09 w/v% and not more than 0.11 w/v% of chlorpheniramine maleate (C\textsubscript{16}H\textsubscript{19}ClN\textsubscript{2}.C\textsubscript{4}H\textsubscript{4}O\textsubscript{4}: 390.86).

**Method of preparation**

| Naphazoline Nitrate | 0.5 g |
| Chlorpheniramine Maleate | 1 g |
| Chlorobutanol | 2 g |
| Glycerin | 50 mL |
| Purified Water or Purified Water in Containers | a sufficient quantity |

To make 1000 mL

Dissolve, and mix the above ingredients.

**Description** Naphazoline and Chlorpheniramine Solution is a clear, colorless liquid.

**Identification (1)** To 20 mL of Naphazoline and Chlorpheniramine Solution add 2 mL of a solution of potassium hydroxide (7 in 10) and 5 mL of pyridine, and heat at 100°C for 5 minutes: a red color is produced (chlorobutanol).

(2) Place 10 mL of Naphazoline and Chlorpheniramine Solution in a glass-stoppered test tube, add 10 mL of ethanol (95), 2 mL of sodium hydroxide TS and 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color is produced (glycerin).
(3) To 20 mL of Naphazoline and Chlorpheniramine Solution add 5 mL of sodium hydroxide TS, extract with 10 mL of diethyl ether, and separate the diethyl ether layer. Take 5 mL of this solution, distil off the solvent, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 0.01 g each of naphazoline nitrate and Chlorpheniramine Maleate RS in 10 mL and 5 mL of methanol, respectively, and use these solutions as standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots from the sample solution exhibit the same RT values as the spots from standard solutions (1) and (2). Spray evenly Dragendorff’s TS on the plate: the spots from standard solutions (1) and (2) and the corresponding spot from the sample solutions reveal an orange color.

**Assay** Pipet 4 mL of Naphazoline and Chlorpheniramine Solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of naphazoline nitrate for assay, dried at 105°C for 2 hours, and about 0.1 g of Chlorpheniramine Maleate RS, dried at 105°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solutions as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q_{Sa} and Q_{Sb}, of the peak height of naphazoline and chlorpheniramine to that of the internal standard of the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak height of naphazoline and chlorpheniramine to that of the internal standard of the standard solution.

\[
M_{Sa} = M_{Sb} \times Q_{Ts}/Q_{Sb} \times 1/25
\]

\[
M_{Sb} = M_{Sb} \times Q_{Ts}/Q_{Sb} \times 1/25
\]

Amount (mg) of naphazoline nitrate (C_{14}H_{14}N_{2}HNO_{3})

Amount (mg) of chlorpheniramine maleate (C_{16}H_{19}ClN_{2}C_{4}H_{4}O_{4})

Internal standard solution—A solution of ethenamide in methanol (1 in 1000).

**Operating conditions—**

- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column, about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: Room temperature.
- Mobile phase: A mixture of acetonitrile and a solution of sodium laurylsulfate (1 in 500) in diluted phosphoric acid (1 in 1000) (1:1).

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 10 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column giving well-resolved peaks of the internal standard, naphazoline and chlorpheniramine in this order.

**Containers and storage** Containers—Light-resistant.

### Naproxen

Naproxen, when dried, contains not less than 98.5% of C_{13}H_{13}O_{2}.

**Description** Naproxen occurs as white crystals or crystaline powder. It is odorless.

It is freely soluble in acetone, soluble in methanol, in ethanol (99.5) and in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

**Identification** (1) Dissolve 0.01 g of Naproxen in 5 mL of methanol, add 5 mL of water, then add 2 mL of potassium iodide TS and 5 mL of a solution of potassium iodate (1 in 100), and shake: a yellow to yellow-brown color develops. To this solution add 5 mL of chloroform, and shake: a light red-purple color develops in the chloroform layer.

(2) To 1 mL of a solution of Naproxen in ethanol (99.5) (1 in 300) add 4 mL of hydroxylamine perchlorate-dehydrated ethanol TS and 1 mL of N,N'-dicyclohexylcarbodiimide-dehydrated ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate-dehydrated ethanol TS, and shake: a red-purple color develops.

(3) Determine the absorption spectrum of a solution of Naproxen in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.27>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Naproxen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** [α]_{D}^{25} = +68.5° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

**Melting point** 154 – 158°C.

**Purity** (1) Clarity of solution—Dissolve 2.0 g of Naproxen in 20 mL of acetone: the solution is clear. Perform the
Nateglinide / Official Monographs

**Nateglinide**

<table>
<thead>
<tr>
<th>Nateglinide</th>
<th>ナテグリニド</th>
</tr>
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<tbody>
<tr>
<td>C_{19}H_{27}NO_{3}: 3 1 7 . 4 2</td>
<td>N-[trans-4-(1-Methylethyl)cyclohexanecarbonyl]-D-phenylalanine [105316-04-4]</td>
</tr>
</tbody>
</table>

Nateglinide, when dried, contains not less than 98.0% and not more than 102.0% of C_{19}H_{27}NO_{3}.

**Description**

Nateglinide occurs as a white crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), sparingly soluble in acetonitrile, and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

**Identification**

1. Determine the absorption spectrum of a solution of Nateglinide in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nateglinide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Nateglinide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Nateglinide RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the reference standard according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

**Optical rotation** <2.49> [α]D \(^{20}\) = −36.5 – −40.0° (after drying 0.2 g, dilute sodium hydroxide TS, 20 mL, 100 mm).

**Purity**

1. Heavy metals <1.07>—Proceed with 2.0 g of Nateglinide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

2. Related substances—Dissolve 0.25 g of Nateglinide in 20 mL of acetonitrile. To 4 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than nateglinide from the sample solution is not larger than the peak area of nateglinide from the standard solution.

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test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; the absorbance at 400 nm is not more than 0.070.

2. Heavy metals <1.07>—Proceed with 2.0 g of Naproxen according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

3. Arsenic <1.11>—Prepare the test solution with 2.0 g of Naproxen according to Method 3, and perform the test (not more than 1 ppm).

4. Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Naproxen in 10 mL of a mixture of chloroform and ethanol (99.5) (1:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of chloroform and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of chloroform and ethanol (99.5) (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, dichloromethane, tetrahydrofuran and acetic acid (100) (50:30:17:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.5 g of Naproxen, previously dried, add 100 mL of diluted methanol (4 in 5), dissolve by gentle warming if necessary, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 23.03 mg of C_{14}H_{14}O_{3}

**Containers and storage**

Containers—Well-closed containers.

Storage—Light-resistant.
Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 4 times as long as the retention time of nateglinide, beginning after the solvent peak.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nateglinide are not less than 6000 and not more than 1.2, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nateglinide is not more than 2.0%.
(3) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 0.2% (1 g, 105 °C, 2 hours).
Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay
Weigh accurately about 0.1 g of Nateglinide, previously dried, and dissolve in acetonitrile to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Nateglinide RS, previously dried, and dissolve in acetonitrile to make exactly 20 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, QT and QS, of the peak area of nateglinide to that of the internal standard.

\[ M_S = \frac{S \times Q_T}{Q_S} \times 2 \]

M₅: Amount (mg) of Nateglinide RS

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 500).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Adjust 0.05 mol/L sodium dihydrogen phosphate TS to pH 2.5 with phosphoric acid. To 550 mL of this solution add 450 mL of acetonitrile for liquid chromatography.
Flow rate: Adjust the flow rate so that the retention time of nateglinide is about 10 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and nateglinide are eluted in this order with the resolution between these peaks being not less than 19.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nateglinide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nateglinide Tablets

ナテグリニド錠

Nateglinide Tablets contain not less than 96.0% and not more than 104.0% of the labeled amount of nateglinide (C₁₉H₂₇NO₃: 317.42).

Method of preparation
Prepare as directed under Tablets, with Nateglinide.

Identification
To an amount of powdered Nateglinide Tablets, equivalent to 20 mg of Nateglinide according to the labeled amount, add 20 mL of methanol, shake, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24v>: it exhibits maxima between 246 nm and 250 nm, between 251 nm and 255 nm, between 257 nm and 261 nm and between 262 nm and 266 nm.

Uniformity of dosage units <6.02>
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Nateglinide Tablets add 10 mL of 0.05 mol/L sodium dihydrogen phosphate TS adjusted to pH 2.5 with phosphoric acid, shake to disintegrate the tablet, and disperse to fine particles with the aid of ultrasonic waves. Add exactly 3V/50 mL of the internal standard solution, add 3V/5 mL of acetonitrile, shake for 10 minutes, and add acetonitrile to make V mL so that each mL contains about 0.6 mg of nateglinide (C₁₉H₂₇NO₃). Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm, and discard the first 5 mL of the filtrate. To 8 mL of the subsequent filtrate add the mobile phase to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Nateglinide RS, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 10 mL. Pipet 6 mL of this solution, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. To 8 mL of this solution add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.07>, and calculate the ratios, QT and QS, of the peak area of nateglinide to that of the internal standard.

\[ M_S = \frac{S \times Q_T}{Q_S} \times 2.5 \]

M₅: Amount (mg) of Nateglinide RS

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 250).

Operating conditions—
Proceed as directed in the operating conditions in the
so that each mL contains about 33 mg of nateglinide of both solutions. (C19H27NO3) according to the labeled amount, and use this solution as the standard solution. Pipet 5 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 mL of each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Qt and Qs, of the peak area of nateglinide to that of the internal standard.

\[
M_5: \text{Amount (mg) of Nateglinide RS}
\]

\[
\text{Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (3 in 125).}
\]

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust to pH 2.5 of 0.05 mol/L sodium dihydrogen phosphate TS with phosphoric acid. To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nateglinide is about 10 minutes.

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and nateglinide are eluted in this order with the resolution between these peaks being not less than 19.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nateglinide is not more than 1.0%.

**Containers and storage**

Containers—Tight containers.
Neostigmine Methylsulfate

Neostigmine Methylsulfate, when dried, contains not less than 98.0% and not more than 102.0% of C_{13}H_{22}N_{2}O_{6}S.

**Description** Neostigmine Methylsulfate occurs as a white, crystalline powder.

It is very soluble in water, and freely soluble in acetonitrile and in ethanol (95).

**Identification**

1. Determine the absorption spectrum of a solution of Neostigmine Methylsulfate (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Neostigmine Methylsulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Neostigmine Methylsulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectroscopy, and compare the spectrum with the Reference Spectrum or the spectrum of dried Neostigmine Methylsulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity (1)**

Clarity and color of solution—Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 3.0 and 5.0.

**Melting point** <2.60> 145 – 149°C.

**Sulfate**—Dissolve 0.20 g of Neostigmine Methylsulfate in 10 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced immediately.

**Dimethylaminophenol**—Dissolve 0.10 g of Neostigmine Methylsulfate in 10 mL of water, add 1 mL of sodium hydroxide TS, and while cooling with ice, add 1 mL of diazobenzensulfonic acid TS: no color develops.

**Loss on drying** <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 25 mg each of Neostigmine Methylsulfate and Neostigmine Methylsulfate RS, previously dried, dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.80> according to the following conditions, and determine the peak areas, A_T and A_S, of neostigmine in each solution.

\[
M_s: \text{Amount (mg) of } C_{13}H_{22}N_{2}O_{6}S = M_s \times A_T/A_S
\]

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 259 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.12 g of sodium dihydrogenphosphate dihydrate in 1000 mL of water, adjust to pH 3.0 with phosphoric acid, and add 0.871 g of sodium 1-pentanesulfonate to dissolve. To 890 mL of this solution add 110 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of neostigmine is about 9 minutes.

**System suitability**—

System performance: Dissolve 25 mg of Neostigmine Methylsulfate and 4 mg of dimethylaminophenol in 50 mL of the mobile phase. When the procedure is run with 10 µL of this solution under the above operating conditions, dimethylaminophenol and neostigmine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of neostigmine methylsulfate is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Neostigmine Methylsulfate Injection

Neostigmine Methylsulfate Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of neostigmine methylsulfate (C_{13}H_{22}N_{2}O_{6}S: 334.39).

**Method of preparation** Prepare as directed under Injections, with Neostigmine Methylsulfate.

**Description** Neostigmine Methylsulfate Injection is a clear, colorless liquid.

It is slowly affected by light.

pH: 5.0 – 6.5

**Identification** Take a volume of Neostigmine Methylsulfate Injection equivalent to 5 mg of neostigmine methylsulfate according to the labeled amount, add water to make 10 mL if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectro-
Nicardipine Hydrochloride

ニカルジピン塩酸塩

C_{26}H_{29}N_3O_6.HCl: 515.99
2-[Benzy[l(methyl)amino]ethyl methyl (4RS)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate monohydrochloride [34527-84-3]

Nicardipine hydrochloride, when dried, contains not less than 98.5% of C_{26}H_{29}N_3O_6.HCl.

Description Nicardipine Hydrochloride occurs as a pale greenish yellow crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water, in acetonitrile and in acetic anhydride.

A solution of Nicardipine Hydrochloride in methanol (1 in 20) shows no optical rotation.

It is gradually affected by light.

Identification (1) Determine the absorption spectrum of a solution of Nicardipine Hydrochloride in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicardipine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.02 g of Nicardipine Hydrochloride in 10 mL of water and 3 mL of nitric acid: the solution responds to the Qualitative Tests <1.06> for chloride.

Melting point <2.06> 167 – 171°C.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Nicardipine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Nicardipine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, then take exactly 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of nicardipine from the sample solution is not larger than the peak area of nicardipine from the standard solution, and the total area of each peak other than the peak of nicardipine is not larger than 2 times the peak area of nicardipine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of a solution of perchloric acid (43 in 50,000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of nicardipine is about 6 minutes.

Time span of measurement: About 4 times as long as the retention time of nicardipine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicardipine obtained from 10 μL of this solution is equivalent to 8 to 12% of that from 10 μL of the standard solution.

System performance: Dissolve 2 mg each of Nicardipine Hydrochloride and nifedipine in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, nicardipine and nifedipine are eluted in this order with the resolution between these peaks being not less than 3.

System reproductability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 3%. 
Loss on drying <2.4% Not more than 1.0% (1 g, 105°C, 
2 hours).

Residue on ignition <2.4% Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to day-
light, using light-resistant vessels. Weigh accurately about 
0.9 g of Nicardipine Hydrochloride, previously dried, dis-
solve in 100 mL of a mixture of acetic anhydride and acetic 
acid (100) (7:3), and titrate with 0.1 mol/L perchloric 
acid VS (potentiometric titration). Perform a blank determin-
ation, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS 
= 51.60 mg of C₂₆H₂₉N₃O₆.HCl

Containers and storage Containers—Well-closed contain-
ers.
Storage—Light-resistant.

Nicardipine Hydrochloride Injection

Japanese

ニカルジピン塩酸塩注射液

Nicardipine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of nicardipine hydro-
chloride (C₂₆H₂₉N₃O₆.HCl: 515.99).

Method of preparation Prepare as directed under Injec-
tions, with Nicardipine Hydrochloride.

Description Nicardipine Hydrochloride Injection occurs as a clear pale yellow liquid.

It is gradually changed by light.

Identification To a volume of Nicardipine Hydrochloride Injection, equivalent to 1 mg of Nicardipine Hydrochloride according to the labeled amount, add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.4> it exhibits maxima between 235 nm and 239 nm, and between 351 nm and 355 nm.

pH <2.54> 3.0 - 4.5

Purity Related substances—Conduct the procedure without 
exposure to day-light using light-resistant vessels. To a 
volume of Nicardipine Hydrochloride Injection, equivalent 
to 5 mg of Nicardipine Hydrochloride according to the 
labeled amount, add the mobile phase to make 10 mL, and use this solution as the sample solution. To exactly 2 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of nicardipine to that of the internal standard.

Amount (mg) of nicardipine hydrochloride
(C₂₆H₂₉N₃O₆.HCl) 

\[ M₅ = \frac{M₅ \times Q₁}{Q₂} \times \frac{1}{25} \]

M₅: Amount (mg) of nicardipine hydrochloride for assay

Internal standard solution—A solution of di-n-butyl phtha-
late in methanol (1 in 625).

Operating conditions—
Detector: An ultraviolet absorption photometer (wave-
length: 254 nm).
Column: A stainless steel column 4.6 mm in inside diame-
ter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.

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Nicergoline

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL. To 320 mL of this solution add 680 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nicardipine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, nicardipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Colored containers may be used.

Storage—Light-resistant.

Nicergoline

ニセルゴリン

C_{24}H_{26}BrN_{3}O_{3}: 484.39

(8R,10S)-10-Methoxy-1,6-dimethylergolin-8-yl][methyl 5-bromopyridine-3-carboxylate [27848-84-6]

Nicergoline, when dried, contains not less than 98.5% and not more than 101.0% of C_{24}H_{26}BrN_{3}O_{3}.

Description Nicergoline occurs as white to light yellow, crystals or crystalline powder.

It is soluble in acetonitrile, in ethanol (99.5) and in acetic anhydride, and practically insoluble in water.

It is gradually colored to light brown by light.

Melting point: about 136°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Nicergoline in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2,24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicergoline as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2,49> [α]D{sup 20} +5.2 – +6.2° (after drying, 0.5 g, ethanol (95), 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Nicergoline according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 25 mg of Nicergoline in 25 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 100 mL. Pipet 10 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2,01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.5 with respect to nicergoline, is not larger than 4 times the peak area of nicergoline from the standard solution, and the area of the peak other than nicergoline and other than the peak mentioned above is not larger than 2.5 times the peak area of nicergoline from the standard solution. The peak which area is larger than the peak area of nicergoline from the standard solution is not more than two peaks, and the total area of the peaks other than the peak of nicergoline is not larger than 7.5 times the peak area of nicergoline from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add acetonitrile to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that with 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 4.0%.

Loss on drying <2,47> Not more than 0.5% (2 g, in vacuum, 60°C, 2 hours).

Residue on ignition <2,44> Not more than 0.1% (1 g).
JP XVI

Assay  Weigh accurately about 0.4 g of Nicergoline, previously dried, add 10 mL of acetic anhydride, and warm to dissolve. After cooling, add 40 mL of nitrobenzene, and titrate \( <2.50 \) with 0.1 mol/L perchloric acid VS until the color of the solution changes to blue-green from red through a blue-purple (indicator: 10 drops of neutral red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.22 mg of C\(_{24}\)H\(_{26}\)BrN\(_3\)O\(_3\).

Containers and storage  Containers—Well-closed containers.

Storage—Light-resistant.

Nicergoline Powder

ニセルゴリン散

Nicergoline Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline (C\(_{24}\)H\(_{26}\)BrN\(_3\)O\(_3\): 484.39).

Method of preparation  Prepare as directed under Granules or Powders, with Nicergoline.

Identification  Vigorously shake for 10 minutes a quantity of Nicergoline Powder, equivalent to 10 mg of Nicergoline according to the labeled amount, with 20 mL of diluted ethanol (4 in 5), and centrifuge for 10 minutes. To 2 mL of the supernatant liquid add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \( <2.24 \): it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

Purity  Related substances—Perform the test with 20 \( \mu \)L of the sample solution obtained in the Assay as directed under Liquid Chromatography \( <2.01 \) according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of substances other than nicergoline by the area percentage method: the total amount of them is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of nicergoline after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 \( \mu \)L of this solution is equivalent to 3 to 7% of that with 20 \( \mu \)L of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.5%.

Uniformity of dosage unit \( <6.02 \)  The Nicergoline Powder in single-unit container meets the requirement of the Mass variation test.

Dissolution \( <6.10 \)  When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Nicergoline Powder is not less than 80%.

Start the test with an accurately weighed amount of Nicergoline Powder, equivalent to about 5 mg of nicergoline (C\(_{24}\)H\(_{26}\)BrN\(_3\)O\(_3\)) according to the labeled amount, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a laminated polyester fiber. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 225 nm, \( A_{T1} \), and at 250 nm, \( A_{T2} \), and at 250 nm, \( A_{S1} \), and at 250 nm, \( A_{S2} \), of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \( <2.24 \), using the dissolution medium as the blank.

Dissolution rate (\%) with respect to the labeled amount of nicergoline (C\(_{24}\)H\(_{26}\)BrN\(_3\)O\(_3\))

\[ \frac{M_s}{M_t} \times \left( \frac{A_{T1} - A_{T2}}{(A_{S1} - A_{S2})} \times 1/C \times 9 \right) \]

\( M_s \): Amount (mg) of nicergoline for assay

\( M_t \): Amount (g) of sample

C: Labeled amount (mg) of nicergoline (C\(_{24}\)H\(_{26}\)BrN\(_3\)O\(_3\)) in 1 g

Assay  Weigh accurately a quantity of Nicergoline Powder, equivalent to about 20 mg of nicergoline (C\(_{24}\)H\(_{26}\)BrN\(_3\)O\(_3\)), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 \) according to the following conditions, and determine the peak areas, \( A_{T1} \) and \( A_{S1} \), of nicergoline.

\[ \frac{M_s}{M_t} \times \frac{A_{S1}}{A_{T1}} \]

\( M_s \): Amount (mg) of nicergoline for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about
Nicergoline Tablets

ニセルゴリン錠

Nicergoline Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline (C_{24}H_{26}BrN_{3}O_{3}: 484.39).

**Method of preparation** Prepare as directed under Tablets, with Nicergoline.

**Identification** Take a quantity of powdered Nicergoline Tablets, equivalent to 10 mg of Nicergoline according to the labeled amount, add 20 mL of ethanol (99.5%), shake vigorously for 10 minutes, and filter through a 0.45-μm pore-size membrane filter. To 2 mL of the filtrate add ethanol (99.5%) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(2.24\): it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

**Purity** Related substances—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography \(2.01\) according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of substances other than nicergoline by the area percentage method: the total amount of them is not more than 2.0%.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

**System suitability**

Test for required detectability: To 1 mL of the standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that with 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability test in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.5%.

**Uniformity of dosage units** \(2.02\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Nicergoline Tablets add exactly 25 mL of diluted ethanol (4 in 5), disperse to fine particles with the aid of ultrasonic wave, and shake for 5 minutes. Centrifuge this solution for 10 minutes, pipet exactly 4 mL of the supernatant liquid, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in exactly 25 mL of diluted ethanol (4 in 5). Pipet 4 mL of this solution, add diluted ethanol (4 in 5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances at 288 nm, \(A_{12}\), and \(A_{32}\), and at 340 nm, \(A_{13}\) and \(A_{33}\), of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry \(2.24\).

Amount (mg) of nicergoline (C_{24}H_{26}BrN_{3}O_{3})

\[
M_{S} = M_{S} \times \frac{A_{T} - A_{12}}{A_{31} - A_{32}} \times \frac{1}{2}
\]

**Dissolution** Being specified separately.

**Assay** Weigh accurately the mass of not less than 20 Nicergoline Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of nicergoline (C_{24}H_{26}BrN_{3}O_{3}), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(2.01\) according to the following conditions, and determine the peak areas, \(A_{1}\) and \(A_{S}\), of nicergoline.

Amount (mg) of nicergoline (C_{24}H_{26}BrN_{3}O_{3})

\[
M_{S} = M_{S} \times \frac{A_{T}}{A_{S}}
\]

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.0%.

Containers and storage Containers—Tight containers.

### Niceritrol

ニセリトロール

C₉H₉N₃O₅: 556.52
Pentaerythritol tetranicotinate

[N568-05-3]

Niceritrol, when dried, contains not less than 99.0% of C₉H₉N₃O₅.

Description Niceritrol occurs as a white to pale yellowish white powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, soluble in N,N-dimethylformamide, very slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Niceritrol in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry TS (1), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Niceritrol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry TS, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 162 - 165°C.

Purity (1) Chloride <1.07>—To 2.0 g of Niceritrol add 50 mL of water, and warm at 70°C for 20 minutes, while shaking occasionally. After cooling, filter, and to 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Niceritrol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.17>—Prepare the test solution with 1.0 g of Niceritrol according to Method 3, and perform the test. Use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(4) Pyridine—Dissolve 0.5 g of Niceritrol in N,N-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pyridine, and add N,N-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add N,N-dimethylformamide to make exactly 100 mL, then pipet 0.5 mL of this solution, add N,N-dimethylformamide to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography TS (2.02) according to the following conditions. Determine each peak area of pyridine in both solutions: the peak area of pyridine from the sample solution is not larger than the peak area of pyridine from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with polyethylene glycol 20M for gas chromatography coated at the ratio of 10% on acid-treated and silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of pyridine is about 2 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the number of theoretical steps of the peak of pyridine is not less than 1500 steps.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pyridine is not more than 3.0%.

(5) Free acids—Transfer about 1 g of Niceritrol, weighed accurately, to a separator, dissolve in 20 mL of chloroform, and extract with 20 mL and then 10 mL of water while shaking well. Combine the whole extracts, and titrate <2.50> with 0.01 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, make any necessary correction, and calculate the amount of free acid by the following equation: it is not more than 0.1%.

Each mL of 0.01 mol/L sodium hydroxide VS = 1.231 mg of C₁₂H₂₅NO₂

(6) Related substances—Dissolve 0.10 g of Niceritrol in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet exactly 2 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography TS (2.02). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (4:1) to a distance of about
10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Niceritrol, previously dried, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS, boil gently for 20 minutes under a reflux condenser with a carbon dioxide absorber (soda lime). After cooling, titrate VS, boil gently for 20 minutes under a reflux condenser with dried, add exactly 25 mL of the filtrate add 0.60 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: a red color develops.

(3) Chloride <1.03>—Dissolve 0.6 g of Nicomol in 15 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 15 mL of dilute nitric acid and water to make 50 mL (not more than 0.024%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Nicomol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Nicomol according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.20 g of Nicomol in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 2 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethanol (95), acetonitrile and ethyl acetate (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1.5 g of Nicomol, previously dried, add exactly 40 mL of 0.5 mol/L sodium hydroxide VS, boil gently under a reflux condenser connected to a carbon dioxide absorption tube (soda lime) for 4 hours. After cooling, titrate <2.50> immediately the excess sodium hydroxide with 0.25 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS = 80.08 mg of C_{29}H_{24}N_{4}O_{8}

Containers and storage Containers—Well-closed containers.
Nicomol Tablets

ニコモール錠

Nicomol Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nicomol \((C_{34}H_{32}N_{4}O_{9}: 640.64)\). Prepare as directed under Tablets, with Nicomol. Evaporate the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1) and (2) under Nicomol.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Nicomol Tablets is not less than 75%.

Start the test with 1 tablet of Nicomol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add the dissolution medium to make exactly \(V\) mL so that each mL contains about 18 \(\mu\)g of nicomol \((C_{34}H_{32}N_{4}O_{9})\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in the dissolution medium to make exactly 100 mL, then pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_1\) and \(A_3\), of the sample solution and standard solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
M_S = \frac{S \times A_1 \times V \times \frac{C}{18}}{A_3 \times V' \times \frac{C}{25}}
\]

\(M_S\): Amount (mg) of nicomol for assay

\(C\): Labeled amount (mg) of nicomol \((C_{34}H_{32}N_{4}O_{9})\) in 1 tablet

Nicorandil

ニコランジル

C\(_6\)H\(_7\)N\(_3\)O\(_4\): 211.17
\(N\)-[2-(Nitrooxy)ethyl]pyridine-3-carboxamide [65141-46-0]

Nicorandil contains not less than 98.5% and not more than 101.0% of \(C_6H_7N_3O_4\), calculated on the anhydrous basis.

Description Nicorandil occurs as white crystals.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), soluble in acetic anhydride, and sparingly soluble in water.

Melting point: about 92°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Nicorandil \((1 \text{ in } 50,000)\) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicorandil as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Sulfate <1.14>—Dissolve 2.0 g of Nicorandil in 20 mL of dilute ethanol, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of dilute ethanol and 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.010%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Nicorandil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Nicorandil in 10 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 \(\mu\)L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of \(N\)-[3-hydroxyethyl]isonicotinamide nitric ester, having the relative retention time of about 0.86 with respect to nicorandil, is not more than 0.5% of the peak area of nicorandil, the area of all other peaks is less than 0.1%, and mine the absorbances, \(A_1\) and \(A_3\), of the sample solution and standard solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Amount (mg) of nicomol (C}_{34}\text{H}_{32}\text{N}_{4}\text{O}_{9}) = M_S \times A_1 / A_3 \times 25/2
\]

\(M_S\): Amount (mg) of nicomol for assay

Containers and storage Containers—Tight containers.
Nicotinamide / Official Monographs

Nicotinamide

ニコチン酸アミド

C₆H₄N₂O: 122.12
Pyridine-3-carboxamide
[98-92-0]

Nicotinamide, when dried, contains not less than 98.5% and not more than 102.0% of C₆H₄N₂O.

Description
Nicotinamide occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

Identification
(1) Mix 5 mg of Nicotinamide with 0.01 g of 1-chloro-2,4-dinitrobenzene, heat gently for 5 to 6 seconds, and fuse the mixture. Cool, and add 4 mL of potassium hydroxide-ethanol TS: a red color is produced.

(2) To 0.02 g of Nicotinamide add 5 mL of sodium hydroxide TS, and boil carefully: the gas evolved turns moistened red litmus paper blue.

(3) Dissolve 0.02 g of Nicotinamide in water to make 1000 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nicotinamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

pH <2.54> Dissolve 1.0 g of Nicotinamide in 20 mL of water: the pH of this solution is between 6.0 and 7.5.

Melting point <2.60> 128 – 131°C

Purity
(1) Clarity and color of solution—Dissolve 1.0 g of Nicotinamide in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Take 0.5 g of Nicotinamide, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Take 1.0 g of Nicotinamide, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Nicotinamide according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(5) Readily carbonizable substances <1.15>—Take 0.20 g of Nicotinamide, and perform the test. The solution has no more color than Matching Fluid A.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay
Weigh accurately about 25 mg each of Nicotinamide and titrate in 30 mL of a mixture of acetic anhydride and acetic acid.

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, tetrahydrofuran, triethylamine and trifluoroacetic acid (982:10:5:3).

Flow rate: Adjust the flow rate so that the retention time of nicorandil is about 18 minutes.

Time span of measurement: About 3 times as long as the retention time of nicorandil beginning after the solvent peak.

System suitability—
Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 500 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicorandil obtained with 10 μL of this solution is equivalent to 2 to 8% of that with 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Nicotinamide, when dried, contains not less than 98.5% and not more than 102.0% of C₆H₄N₂O.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicorandil is not more than 1.5%.

Water <2.48> Not more than 0.1% (2 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay
Weigh accurately about 0.3 g of Nicorandil, dissolve in 30 mL of a mixture of acetic anhydride and acetic acid (100:7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 21.12 mg of C₈H₉N₃O₄

Containers and storage
Containers—Tight containers.
Storage—At a temperature between 2°C and 8°C.
and Nicotinamide RS, both previously dried, dissolve separately in 3 mL of water, and add the mobile phase to make exactly 100 mL. Pipet 8 mL of each of these solutions, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, 20 and 25, of the peak area of nicotinamide to that of the internal standard.

\[ M = \frac{Q_2}{Q_5} \]

\[ M_s = \text{Amount (mg) of dried Nicotinamide RS} \]

**Internal standard solution**—A solution of nicotinic acid (1 in 25,000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in water to make 1000 mL. To 700 mL of this solution add 300 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of nicotinamide is about 7 minutes.

**System suitability**—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, nicotinic acid and nicotinamide are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nicotinamide to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

**Nicotinic Acid**

ニコチン酸

![Chemical Structure](image)

C₆H₅NO₂: 123.11
Pyridine-3-carboxylic acid
[59-67-6]

Nicotinic Acid, when dried, contains not less than 99.5% of C₆H₅NO₂.

Description

Nicotinic Acid occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste. It is sparingly soluble in water, slightly soluble in ethanol (95), and very slightly soluble in diethyl ether.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

**Identification** (1) Triturate 5 mg of Nicotinic Acid with 0.01 g of 1-chloro-2,4-dinitrobenzene, and fuse the mixture by gentle heating for 5 to 6 seconds. Cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color is produced.

(2) Dissolve 0.02 g of Nicotinic Acid in water to make 1000 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nicotinic Acid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**pH**

Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the pH of this solution is between 3.0 and 4.0.

**Melting point**

234 – 238°C

**Purity** (1) Clarity and color of solution—Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the solution is clear and colorless.

(2) Chloride (1.03)—Perform the test with 0.5 g of Nicotinic Acid. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate (1.14)—Dissolve 1.0 g of Nicotinic Acid in 3 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS and 3 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.019%).

(4) Nitro compounds—Dissolve 1.0 g of Nicotinic Acid in 8 mL of sodium hydroxide TS, and add water to make 20 mL: the solution has no more color than Matching Fluid A.

(5) Heavy metals (1.07)—Proceed with 1.0 g of Nicotinic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying**

Not more than 0.5% (1 g, 105°C, 1 hour).

**Residue on ignition**

Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.3 g of Nicotinic Acid, previously dried, dissolve in 50 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS = 12.31 mg of C₆H₅NO₂

**Containers and storage**—Containers—Well-closed containers.
Nicotinic Acid Injection

ニコチン酸注射液

Nicotinic Acid Injection is an aqueous solution for injection. It contains not less than 95.0% and not more than 110.0% of the labeled amount of nicotinic acid (C₆H₅NO₂: 123.11).

Method of preparation Prepare as directed under Injections, with Nicotinic Acid. It may contain Sodium Carbonate or Sodium Hydroxide as a solubilizer.

Description Nicotinic Acid Injection is a clear, colorless liquid. pH: 5.0 – 7.0

Identification (1) To a volume of Nicotinic Acid Injection, equivalent to 0.1 g of Nicotinic Acid according to the labeled amount, add 0.3 mL of dilute hydrochloric acid, and evaporate on a water bath to 2 mL. After cooling, collect the crystals formed, wash with small portions of ice-cold water until the last washing shows no turbidity on the addition of silver nitrate TS, and dry at 105°C for 1 hour: the crystals melt <2.60> between 234°C and 238°C. With the crystals, proceed as directed in the Identification (1) under Nicotinic Acid.

(2) Dissolve 0.02 g of the dried crystals obtained in (1) in water to make 1000 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 261 nm and 263 nm, and a minimum between 235 nm and 239 nm. Separately, determine the absorbances of this solution, A₁ and A₂, at each wavelength of maximum and minimum absorption, respectively: the ratio A₂/A₁ is between 0.35 and 0.39.

Bacterial endotoxins <4.06> Less than 3.0 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Nicotinic Acid Injection, equivalent to about 0.1 g of nicotinic acid (C₆H₅NO₂), and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Nicotinic Acid RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₃, of the peak area of nicotinic acid to that of the internal standard.

Amount (mg) of nicotinic acid (C₆H₅NO₂) = Mₛ × Q₃/Q₁

Mₛ: Amount (mg) of Nicotinic Acid RS

Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.1 g of sodium 1-octane sulfonate in a mixture of 0.05 mol/L sodium dihydrogenphosphate TS, pH 3.0 and methanol (4:1) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of caffeine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, nicotinic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of nicotinic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Nifedipine

ニフェジピン

C₇H₁₅N₂O₆: 346.33

Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
[21829-25-4]

Nifedipine contains not less than 98.0% and not more than 102.0% of C₇H₁₅N₂O₆, calculated on the dried basis.

Description Nifedipine occurs as a yellow, crystalline powder. It is odorless and tasteless. It is freely soluble in acetone and in dichloromethane, sparingly soluble in methanol, in ethanol (95) and in acetic acid (100), slightly soluble in diethyl ether, and practically insoluble in water. It is affected by light.

Identification (1) Dissolve 0.05 g of Nifedipine in 5 mL of ethanol (95), and add 5 mL of hydrochloric acid and 2 g
of zinc powder. Allow to stand for 5 minutes, and filter. Perform the test with the filtrate as directed under Qualitative Tests 1.09> for primary aromatic amines: a red-purple color develops.

2. Determine the absorption spectrum of a solution of Nifedipine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

3. Determine the infrared absorption spectrum of Nifedipine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point 2.60> 172 – 175°C.**

**Purity (1)** Clarity and color of solution—Dissolve 0.5 g of Nifedipine in 5 mL of acetone: the solution is clear and yellow.

(2) Chloride 1.03>—To 2.5 g of Nifedipine add 12 mL of dilute acetic acid and 13 mL of water, and heat to boil. After cooling, filter, and discard the first 10 mL of the filtrate. To 5 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate 1.14>—To 4 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.054%).

(4) Heavy metals 1.07>—Proceed with 2.0 g of Nifedipine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic 1.11>—Prepare the test solution with 1.0 g of Nifedipine according to Method 3, and perform the test (not more than 2 ppm).

(6) Basic substances—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 5.0 g of Nifedipine in 80 mL of a mixture of acetone and acetic acid (100) (5:3), and titrate 2.50> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction. Not more than 1.9 mL of 0.02 mol/L perchloric acid VS is consumed.

(7) Dimethyl-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 0.15 g of Nifedipine in dichloromethane to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of dimethyl-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylate for thin-layer chromatography in exactly 10 mL of dichloromethane. Measure exactly 1 mL of this solution, add dichloromethane to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.00>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution, corresponding to that from the standard solution, is not more intense than the spot from the standard solution.

**Loss on drying 2.41> Not more than 0.5% (0.5 g, 105°C, 2 hours).**

**Residue on ignition 2.44> Not more than 0.1% (1 g).**

**Assay** The procedure should be performed under protection from direct sunlight in light-resistant vessels. Weigh accurately about 0.12 g of Nifedipine, and dissolve in methanol to make exactly 200 mL. Measure exactly 5 mL of this solution, and add methanol to make exactly 100 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 350 nm as directed under Ultraviolet-visible Spectrophotometry 2.24>.

Amount (mg) of C19H19N3O6 = A/142.3 x 40,000

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Nilvadipine**

ニルバジピン

![](image)

C19H19N3O6: 385.37
3-Methyl 5-(1-methylethyl) (4RS)-2-cyano-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [75530-68-6]

Nilvadipine contains not less than 98.0% and not more than 102.0% of C19H19N3O6.

**Description** Nilvadipine occurs as a yellow crystalline powder.

It is freely soluble in acetonitrile, soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Nilvadipine in acetonitrile (1 in 20) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Nilvadipine in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nilvadipine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nilvadipine as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Nilvadipine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.
Nilvadipine Tablets

ニルバジピン錠

Nilvadipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nilvadipine (C_{19}H_{19}N_{3}O_{6}: 385.37).

Method of preparation Prepare as directed under Tablets, with Nilvadipine.

Identification To a quantity of powdered Nilvadipine Tablets, equivalent to 1 mg of nilvadipine according to the labeled amount, add 100 mL of ethanol (99.5), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 239 nm and 243 nm and a maximum having a broad-ranging absorption between 371 nm and 381 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the

Content uniformity

To 1 tablet of Nilvadipine Tablets add V mL of a mixture of acetonitrile and water (7:3) so that each mL of the solution contains about 0.2 mg of nilvadipine (C_{19}H_{19}N_{3}O_{6}), add acetonitrile to make exactly 10 mL. Confirm that the peak area of nilvadipine is about 12 minutes.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

Related substances—Dissolve 20 mg of Nilvadipine in 20 mL of acetonitrile, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of each related substance is not more than 0.3%, and the total of them is not more than 0.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

Time span of measurement: About 2.5 times as long as the retention time of nilvadipine beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nilvadipine obtained from 5 μL of this solution is equivalent to 7 to 13% of that from 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nilvadipine Tablets

Melting point <2.60> 167 to 171°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Nilvadipine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Nilvadipine in 20 mL of acetonitrile, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each related substance is not more than 0.3%, and the total of them is not more than 0.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.


Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

Time span of measurement: About 2.5 times as long as the retention time of nilvadipine beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nilvadipine obtained from 5 μL of this solution is equivalent to 7 to 13% of that from 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nilvadipine Tablets

ニルバジピン錠

Nilvadipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nilvadipine (C_{19}H_{19}N_{3}O_{6}: 385.37).

Method of preparation Prepare as directed under Tablets, with Nilvadipine.

Identification To a quantity of powdered Nilvadipine Tablets, equivalent to 1 mg of nilvadipine according to the labeled amount, add 100 mL of ethanol (99.5), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 239 nm and 243 nm and a maximum having a broad-ranging absorption between 371 nm and 381 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the

Content uniformity

To 1 tablet of Nilvadipine Tablets add V mL of a mixture of acetonitrile and water (7:3) so that each mL of the solution contains about 0.2 mg of nilvadipine (C_{19}H_{19}N_{3}O_{6}), add acetonitrile to make exactly 10 mL. Confirm that the peak area of nilvadipine is about 12 minutes.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nilvadipine obtained from 5 μL of this solution is equivalent to 7 to 13% of that from 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.
Dissolution

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Nilvadipine Tablets is not less than 85%.

Start the test with 1 tablet of Nilvadipine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 1 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately an amount of Nilvadipine RS, equivalent to 10 times the labeled amount of sample solution, and make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Amount (mg) of nilvadipine (C₁₉H₁₉N₃O₆) = M₅ × Q₁/Q₈ × V/100

M₅: Amount (mg) of Nilvadipine RS

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 500).

Dissolution

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Nilvadipine Tablets is not less than 85%.

Start the test with 1 tablet of Nilvadipine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 1 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately an amount of Nilvadipine RS, equivalent to 10 times the labeled amount of sample solution, and make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Amount (mg) of nilvadipine (C₁₉H₁₉N₃O₆) = M₅ × Q₁/Q₈ × V/100

M₅: Amount (mg) of Nilvadipine RS

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of phosphate buffer solution, pH 7.4, methanol and acetonitrile (7:7:6).

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nilvadipine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nilvadipine is not more than 1.5%.

Assay

Weigh accurately not less than 20 Nilvadipine Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 5 mg of nilvadipine (C₁₉H₁₉N₃O₆), add 10 mL of a mixture of acetonitrile and water (7:3) and exactly 25 mL of the internal standard solution, shake for 15 minutes, and add the mixture of acetonitrile and water (7:3) to make 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine RS, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of acetonitrile and water (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography.

Amount (mg) of nilvadipine (C₁₉H₁₉N₃O₆) = M₅ × Q₁/Q₈ × 1/4

M₅: Amount (mg) of Nilvadipine RS

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydroxide TS, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), and add 900 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Well-closed containers.
Nitrazepam

Nitrazepam, when dried, contains not less than 99.0% of C₁₅H₁₁N₃O₃.

Description Nitrazepam occurs as white to yellow crystals or crystalline powder. It is odorless.

It is freely soluble in acetic acid (100), soluble in acetone and in chloroform, slightly soluble in methanol, in ethanol (95) and in ethanol (99.5), very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 227°C (with decomposition).

Identification (1) To 3 mL of a solution of Nitrazepam in methanol (1 in 500) add 0.1 mL of sodium hydroxide TS: a yellow color is produced.

(2) To 0.02 g of Nitrazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests (1.09) for primary aromatic amines.

(3) Neutralize 0.5 mL of the filtrate obtained in (2) with sodium hydroxide TS, add 2 mL of ninhydrin TS, and heat on a water bath: a purple color is produced.

(4) Determine the absorption spectrum of a solution of Nitrazepam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Nitrazepam in 20 mL of acetone: the solution is clear and pale yellow to light yellow in color.

(2) Heavy metals (1.07)—Proceed with 1.0 g of Nitrazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic (1.11)—Prepare the test solution with 1.0 g of Nitrazepam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Nitrazepam in a 10 mL of mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and chloroform (1:1) to make exactly 20 mL, pipet 2 mL of this solution, add a mixture of methanol and chloroform (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of nitromethane and ethyl acetate (17:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying (2.41) Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition (2.44) Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Nitrazepam, previously dried, and dissolve in 40 mL of acetic acid (100). Titrate (2.50) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

C₁₅H₁₁N₃O₃ = 28.13 mg of C₁₅H₁₁N₃O₃

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Nitrendipine

Nitrendipine, when dried, contains not less than 98.5% and not more than 101.0% of C₁₈H₂₀N₂O₆.

Description Nitrendipine occurs as a yellow crystalline powder.

It is soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is gradually colored to brownish yellow by light.

A solution of Nitrendipine in acetonitrile (1 in 50) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Nitrendipine in methanol (1 in 80,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nitrendipine as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point (2.60) 157 – 161°C.

Purity (1) Heavy metals (1.07)—Proceed with 2.0 g of
Nitrendipine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure rapidly using light-resistant vessels. Dissolve 40 mg of Nitrendipine in 5 mL of acetonitrile, add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test immediately with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of related substances by the following equation: the amount of a related substance, having the relative retention time of about 0.8 with respect to nitrendipine, is not more than 1.0%, a related substance, having the relative retention time of about 1.3, is not more than 0.25%, and other related substances are not more than 0.2%, respectively. The total amount of the substances other than nitrendipine is not more than 2.0%.

Amount (%) of related substance = \( \frac{A_T}{A_S} \)

\( A_T \): Each peak area other than nitrendipine obtained from the sample solution

\( A_S \): Peak area of nitrendipine obtained from the standard solution

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (14:6:5).
Flow rate: Adjust the flow rate so that the retention time of nitrendipine is about 12 minutes.
Time span of measurement: About 2.5 times as long as the retention time of nitrendipine beginning after the solvent peak.

System suitability—
Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of nitrendipine obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.
System performance: Dissolve 10 mg of Nitrendipine and 3 mg of propyl parahydroxybenzoate in 5 mL of acetonitrile, and add the mobile phase to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, propyl parahydroxybenzoate and nitrendipine are eluted in this order with the resolution between these peaks being not less than 6.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrendipine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition <2.44>** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Nitrendipine, previously dried, dissolve in 60 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 100), add 50 mL of water, and titrate <2.50> with 0.1 mol/L serium (IV) tetraammonium sulfate VS until the red-orange color of the solution vanishes (indicator: 3 drops of 1,10-phenanthroline TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L serium (IV) tetraammonium sulfate VS = 18.02 mg of C_{18}H_{20}N_{2}O_{6}

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

### Nitrendipine Tablets

Nitrendipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nitrendipine (C_{18}H_{20}N_{2}O_{6}: 360.36).

**Method of preparation** Prepare as directed under Tablets, with Nitrendipine.

**Identification** Shake a quantity of powdered Nitrendipine Tablets, equivalent to 5 mg of Nitrendipine according to the labeled amount, with 70 mL of methanol, then add methanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 234 nm and 238 nm, and between 350 nm and 354 nm.

**Uniformity of dosage units <2.02>** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Nitrendipine Tablets add 15 mL of diluted acetonitrile (4 in 5), stir until the tablet is completely disintegrat-ed, and further stir for 10 minutes. Add diluted acetonitrile (4 in 5) to make exactly 20 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 1 mg of nitrendipine (C_{18}H_{20}N_{2}O_{6}), add exactly 5 mL of the internal standard solution, then add diluted acetonitrile (4 in 5) to make 25 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of nitrendipine (C_{18}H_{20}N_{2}O_{6}) = M_3 \times \frac{Q_T}{Q_S} \times \frac{1}{1 \times 5} \times \frac{1}{5}

M_3: Amount (mg) of nitrendipine for assay

**Internal standard solution**—A solution of propyl parahydroxybenzoate in diluted acetonitrile (4 in 5) (1 in 10,000).

**Dissolution <6.1D>** When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of the dissolution medium containing 3 g of polysorbate 80 in 5 L of water for 5-mg tablet and the dissolution medium containing 3 g of polysorbate 80 in 2000 mL of...
water for 10-mg tablet, the dissolution rate in 45 minutes of Nitrendipine Tablets is not less than 70%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Nitrendipine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the dissolution medium to make exactly V mL so that each mL contains about 5.6 μg of nitrendipine (C18H20N2O6) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of nitrendipine for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution, and add the dissolution medium to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A1 and A2, of nitrendipine.

Dissolution rate (%) with respect to the labeled amount of nitrendipine (C18H20N2O6)

\[
M_S = \frac{M_S}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 18
\]

M:\ Amount (mg) of nitrendipine for assay
C: Labeled amount (mg) of nitrendipine (C18H20N2O6) in 1 tablet

Operating conditions—

- Detector: An ultraviolet absorption photometer (wavelength: 356 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (14:6:5).
- Flow rate: Adjust the flow rate so that the retention time of nitrendipine is about 9 minutes.

System suitability—

- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and nitrendipine are eluted in this order with the resolution between these peaks being not less than 6.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrendipine is not more than 1.0%.

Containers and storage Containers—Light-resistant.

Storage—Light-resistant.

Nitrogen

窒素

N2: 28.01

Nitrogen is the nitrogen produced by the air liquefaction separation method. It contains not less than 99.5 vol% of N2.

Description Nitrogen is a colorless gas at room temperature and under atmospheric pressure, and is odorless.

1 mL of Nitrogen dissolves in 65 mL of water and in 9 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.

1000 mL of Nitrogen at 0°C and at a pressure of 101.3 kPa weighs 1.251 g.

Identification Introduce 1 mL each of Nitrogen and nitrogen into a gas-measuring tube or syringe for gas chromatography from a cylinder with a pressure-reducing valve, through a directly connected polyvinyl chloride or stainless
Nitroglycerin Tablets

Nitroglycerin Tablets contain not less than 80.0% and not more than 120.0% of the labeled amount of nitroglycerin (C₃H₅N₃O₉) according to the labeled amount, shake thoroughly with 12 mL of diethyl ether, filter, and use the filtrate as the sample solution. Evaporate 5 mL of the sample solution, dissolve the residue in 1 to 2 drops of sulfuric acid, and add 1 drop of diphenylamine TS: a deep blue color develops.

Evaporate 5 mL of the sample solution obtained in (1), add 5 drops of sodium hydroxide TS, heat over a low flame, and concentrate to about 0.1 mL. Cool, heat the residue with 0.02 g of potassium hydrogen sulfate: the odor of acrolein is perceptible.

Purity Free nitrate ion—Transfer an accurately measured quantity of powdered Nitroglycerin Tablets, equivalent to 20 mg of nitroglycerin (C₃H₅N₃O₉) according to the labeled amount, to a separator, add 40 mL of isopropanol and 40 mL of water, shake for 10 minutes, and allow the layers to separate. Collect the aqueous layer, add 40 mL of isopropyl ether, shake for 10 minutes, collect the aqueous layer, filter, and use the filtrate as the sample solution. Separately, transfer 10 mL of Standard Nitric Acid Solution to a separator, add 10 mL of water and 40 mL of the isopropyl ether layer of the first extraction of the sample solution, shake for 10 minutes, continue the procedure in the same manner as the sample solution, and use the solution so obtained as the standard solution. Transfer 20 mL each of the sample solution and the standard solution to Nessler tubes, respectively, shake well with 30 mL of water and 0.06 g of Griess-Romijn’s nitric acid reagent, allow to stand for 30 minutes, and observe the tubes horizontally: the sample solution has no more color than the standard solution.

Uniformity of dosage units — Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube and add exactly V mL of acetic acid (100) to provide a solution containing about 30 μg of nitroglycerin (C₃H₅N₃O₉) per mL. Shake vigorously for 1 hour, and after disintegrating the tablet, centrifuge, and use the supernatant liquid as the sample solution. When the tablet does not disintegrate during this procedure, transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, wet the tablet with 0.05 mL of acetic acid (100), and grind down it with a glass rod. While rinsing the glass rod, add acetic acid (100) to make exactly V mL of a solution containing about 30 μg of nitroglycerin (C₃H₅N₃O₉) per mL. Shake for 1 hour, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 5 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, add 2 mL each of salicylic acid TS shake, allow to stand for 15 minutes, and add 10 mL each of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry 2.2.24 using a solution, prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, Aₜ and Aₘ, of the subsequent solutions of the sample solution and the standard solution at 410 nm, respectively.
Amount (mg) of nitroglycerin (C$_3$H$_5$N$_3$O$_9$)  

\[ M_S = M_5 \times A_1/A_5 \times V/2000 \times 0.749 \]

\( M_S \): Amount (mg) of potassium nitrate

Calculate the average content from the contents of 10 tablets; it meets the requirements of the test when each content deviates from the average content by not more than 25%. When there is 1 tablet showing a deviation exceeding 25% and not exceeding 30%, determine the content of an additional 20 tablets in the same manner. Calculate the 30 deviations from the new average of all 30 tablets; it meets the requirements of the test when 1 tablet may deviate from the average content by between 25% and 30%, but no tablet deviates by more than 30%.

**Disintegration** 6.09  It meets the requirement, provided that the time limit of the test is 2 minutes, and the use of the disks is omitted.

**Assay** Weigh accurately and disintegrate, by soft pressing, not less than 20 Nitroglycerin Tablets. Weigh accurately a portion of the powder, equivalent to about 3.5 mg of nitroglycerin (C$_3$H$_5$N$_3$O$_9$), add exactly 50 mL of acetic acid (100), shake for 1 hour, filter, and use this filtrate as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 10 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, to each solution add 2 mL of salicylic acid TS, shake, allow to stand for 15 minutes, and add 10 mL of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2.24), using a solution of potassium nitrate to correct the volume at 20°C and at a pressure of 101.3 kPa weighs about 1.96 g.

**Identification** (1) A glowing splinter of wood held in Nitrous Oxide: it bursts into flame immediately.

(2) Transfer 1 mL each of Nitrous Oxide and nitrous oxide directly from metal cylinders with a pressure-reducing valve to gas measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography 2.02 according to the conditions of the Assay: the retention time of the main peak from Nitrous Oxide coincides with that of nitrous oxide.

**Purity** Maintain the containers of Nitrous Oxide between 18°C and 22°C for more than 6 hours before the test, and correct the volume at 20°C and at a pressure of 101.3 kPa.

(1) Acidity or alkalinity—To 400 mL of freshly boiled and cooled water add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS, and boil for 5 minutes. Transfer 50 mL of this solution to each of three Nessler tubes marked A, B and C. Add 0.10 mL of 0.01 mol/L hydrochloric acid VS to tube A, 0.20 mL of 0.01 mol/L hydrochloric acid VS to tube B, stopper each of the tubes, and cool. Pass 100 mL of Nitrous Oxide through the solution in tube A for 15 minutes, employing delivery tube with an orifice approximately 1 mm in diameter and extending to within 2 mm of the bottom of the Nessler tube: the color of the solution in tube A is not deeper orange-red than that of the solution in tube B and not deeper yellow-green than that of the solution in tube C.

(2) Carbon dioxide—Pass 1000 mL of Nitrous Oxide through 50 mL of barium hydroxide TS in a Nessler tube, in the same manner as directed in (1): any turbidity produced does not exceed that produced in the following control solution.

Control solution: To 50 mL of barium hydroxide TS in a Nessler tube add 1 mL of a solution of 0.1 g of sodium hydrogen carbonate in 100 mL of freshly boiled and cooled water.

(3) Oxidizing substances—Transfer 15 mL of potassium iodide-starch TS to each of two Nessler tubes marked A and B, add 1 drop of acetic acid (100) to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 2000 mL of Nitrous Oxide through solution A for 30 minutes in the same manner as directed in (1): the color of solution A is the same as that of the stoppered, untreated solution B.

(4) Potassium permanganate-reducing substance—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.10 mL of 0.02 mol/L potassium permanganate VS to each of the tubes, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the manner as directed in (1): the color of solution A is the same as that of solution B.

(5) Chloride 6.09—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.5 mL of silver nitrate TS to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the same manner as directed in (1): the turbidity of solution A is the same as that of solution B.

(6) Carbon monoxide—Introduce 5.0 mL of Nitrous Oxide into a gas-cylinder or a syringe for gas chromatography.
phy from a metal cylinder holding gas under pressure and fitted with a pressure-reducing valve, through a directly connected polyvinyl tube. Perform the test with this according to the Gas Chromatography <2.02> under the following conditions: no peak is observed at the same retention time as that of carbon monoxide.

Operating conditions—
Detector: A thermal-conductivity detector.
Column: A column about 3 mm in inside diameter and about 3 m in length, packed with 300 to 500 μm zeolite for gas chromatography (0.5 nm in pore size).
Column temperature: A constant temperature of about 50°C.
Carrier gas: Hydrogen or helium.
Flow rate: Adjust the flow rate so that the retention time of carbon monoxide is about 20 minutes.
Selection of column: To 0.1 mL each of carbon monoxide and air in a gas mixer add carrier gas to make 100 mL, and mix well. Proceed with 5.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of oxygen, nitrogen and carbon monoxide in this order.
Detection sensitivity: Adjust the sensitivity so that the peak height of carbon monoxide obtained from 5.0 mL of the mixed gas used in the selection of column is about 10 cm.

Assay Withdraw Nitrous Oxide as directed in the Purity.
Introduce 1.0 mL of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography from a metal cylinder under pressure through a pressure-reducing valve and a directly connected polyvinyl tube. Perform the test with this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area \( A_T \) of air. Separately, introduce 3.0 mL of nitrogen into a gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard mixed gas. Proceed with 1.0 mL of this mixture as directed in the case of Nitrous Oxide, and determine the peak area \( A_S \) of nitrogen in the same manner.

Amount (vol%) of \( N_2O = 100 - 3 \times \frac{A_T}{A_S} \)

Operating conditions—
Detector: A thermal-conductivity detector.
Column: A column about 3 mm in inside diameter and about 3 m in length, packed with silica gel for gas chromatography (300 to 500 μm in particle diameter).
Column temperature: A constant temperature of about 50°C.
Carrier gas: Hydrogen or helium.
Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 2 minutes.
Selection of column: To 3.0 mL of nitrogen in a gas mixer add Nitrous Oxide to make 100 mL, and mix well. Proceed with 1.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of nitrogen and nitrous oxide in this order.
System repeatability: Repeat the test five times with the standard mixed gas under the above operating conditions: the relative standard deviation of the peak area of nitrogen is not more than 2.0%.

Containers and storage Containers—Metal cylinders.
Storage—Not exceeding 40°C.

### Nizatidine

Nizatidine, when dried, contains not less than 98.0% and not more than 101.0% of \( C_{12}H_{21}N_5O_2S_2 \).

**Description** Nizatidine occurs as a white to pale yellowish white crystalline powder, and has a characteristic odor. It is soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (99.5).

**Identification**

(1) Determine the absorption spectrum of a solution of Nizatidine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nizatidine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nizatidine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Nizatidine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.02> 130 – 135°C (after drying).

**Purity**

(1) Heavy metals <1.07>—Proceed with 2.0 g of Nizatidine according to Method 4, and perform the test using 3 mL of sulfuric acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Nizatidine in 10 mL of a mixture of the mobile phase A and mobile phase B (19:6), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mixture of the mobile phase A and mobile phase B (19:6) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the area of the peaks other than nizatidine peak obtained from the sample solution is not larger than 1/5 times the nizatidine peak area from the standard solution. Furthermore, the total of the areas of peaks other than the nizatidine peak is not larger than the peak area of nizatidine from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Nizatidine Capsules / Official Monographs

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100). Mobile phase B: Methanol.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 3</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>3 – 20</td>
<td>76 → 50</td>
<td>24 → 50</td>
</tr>
<tr>
<td>20 – 45</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute.

Time span of measurement: About 3 times as long as the retention time of nizatidine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of the mobile phase A and mobile phase B (19:6) to make exactly 25 mL. Confirm that the peak area of nizatidine obtained from 50 μL of this solution is equivalent to 15 to 25% of that from 50 μL of the standard solution.

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nizatidine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nizatidine is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Nizatidine Capsules

ニザチジンカプセル

Nizatidine Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of nizatidine (C₁₂H₂₁N₅O₂S₂: 331.46).

Method of preparation—Prepare as directed under Capsules, with Nizatidine.

Identification—Take out the contents of Nizatidine Capsules, and powder. To a portion of the powder, equivalent to 50 mg of Nizatidine according to the labeled amount, add 50 mL of methanol, shake well, and filter. Pipet 1 mL of the filtrate, and add methanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry. It exhibits maxima between 239 nm and 244 nm, and between 323 nm and 327 nm.

Uniformity of dosage units—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents from 1 capsule of Nizatidine Capsules, add the mobile phase to make V mL so that each mL contains about 1.5 mg of nizatidine (C₁₂H₂₁N₅O₂S₂). After shaking vigorously for 10 minutes, centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution and add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of nizatidine (C₁₂H₂₁N₅O₂S₂) = \( \frac{M_S \times Q_S \times V}{10} \)

\( M_S \): Amount (mg) of Nizatidine RS

Internal standard solution—A solution of phenol in the mobile phase (1 in 100).

 Dissolution—When the test is performed at 50 revolutions per minute according to the Paddle method, using a sinker, using 900 mL of water as the dissolution medium, the
dissolution rate in 15 minutes of Nizatidine Capsules is not less than 80%.

Start the test with 1 capsule of Nizatidine Capsules, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V mL so that each mL contains about 10 μg of nizatidine (C12H21N5O2S2) according to the labeled amount. Use this solution as the sample solution. Separately, weigh accurately about 25 mg of Nizatidine RS, previously dried at 100°C for 1 hour, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A1 and A3, at 314 nm.

Dissolution rate (%) with respect to the labeled amount of nizatidine (C12H21N5O2S2) = M3 × A1/A3 × V'/V × 1/C × 36

M3: Amount (mg) of Nizatidine RS
C: Labeled amount (mg) of nizatidine (C12H21N5O2S2) in 1 capsule

Assay Take out the contents of not less than 10 Nizatidine Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of nizatidine (C12H21N5O2S2), add exactly 5 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q1 and Q3, of the peak area of nizatidine to that of the internal standard.

Amount (mg) of nizatidine (C12H21N5O2S2) = M3 × Q1/Q3 × 10

M3: Amount (mg) of Nizatidine RS

Internal standard solution—A solution of phenol in the mobile phase (1 in 100).

Operating conditions—
Detector: An ultraviolet absorption photometer (wave-length: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethyamine, and adjust to pH 7.5 with acetic acid (100). To this solution add 240 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of nizatidine is about 10 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and nizatidine are eluted in this order with the resolution between these peaks being not less than 3.
System repetitability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nizatidine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Noradrenaline

Norepinephrine

ノルアドレナリン

C11H17NO3: 169.18

4-[(1RS)-2-Amino-1-hydroxyethyl]benzene-1,2-diol [51-41-2]

Noradrenaline, when dried, contains not less than 98.0% of dl-norepinephrine (C11H15NO3).

Description Noradrenaline occurs as a white to light brown or slightly reddish brown, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water, and practically insoluble in ethanol (95%). It dissolves in dilute hydrochloric acid. It gradually changes to brown by air and by light.

Identification (1) Determine the absorption spectrum of a solution of Noradrenaline in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Noradrenaline, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Noradrenaline in 10 mL of 0.1 mol/L hydrochloric acid TS, and add water to make 100 mL: the solution is clear and colorless.

(2) Arterenone—Dissolve 50 mg of Noradrenaline in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL. Determine the absorbance of the solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.1.

(3) Adrenaline—Dissolve 10.0 mg of Noradrenaline in 2.0 mL of diluted acetic acid (100) (1 in 2). Pipet 1 mL of
Noradrenaline Injection / Official Monographs

**Noradrenaline Injection**

**Noradrenaline Hydrochloride Injection**

**Norepinephrine Hydrochloride Injection**

**Norepinephrine Injection**

Noradrenaline Injection is an aqueous solution for injection. It contains not less than 90.0% and not more than 110.0% of the labeled amount of dl-noradrenaline (C₈H₁₁NO₃; 169.18).

**Method of preparation** Dissolve Noradrenaline in 0.01 mol/L hydrochloric acid TS, and prepare as directed under Injections.

**Description** Norepinephrine Injection is a clear, colorless liquid. It gradually becomes a pale red color by light and by air. pH: 2.3 – 5.0

**Identification** Transfer a volume of Noradrenaline Injection, equivalent to 1 mg of Noradrenaline according to the labeled amount, add 1 mL of water to each tube. Add 10 mL of potassium hydrogen phthalate buffer solution, pH 3.5, to A, and 10 mL of phosphate buffer solution, pH 6.5, to B. To each of these solutions add 1.0 mL of iodine TS, allow to stand for 5 minutes, and add 2.0 mL of sodium thiosulfate TS: no color or a pale red color develops in test tube A, and a deep red-purple color develops in test tube B.

**Purity (1)** Arterenone—Measure a volume of Noradrenaline Injection, equivalent to 10 mg of Noradrenaline according to the labeled amount, add water to make exactly 20 mL, and determine the absorbance of this solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance is not more than 0.10.

(2) Adrenaline—Measure a volume of Noradrenaline Injection, equivalent to 5 mg of Noradrenaline according to the labeled amount, add 1 mL of diluted acetic acid (100) (1 in 2) and water to make exactly 10 mL, and proceed as directed in the Purity (3) under Noradrenaline.

**Bacterial endotoxins** <4.01> Less than 300 EU/mg.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Pipet a volume of Noradrenaline Injection, equivalent to about 5 mg of dl-noradrenaline (C₈H₁₁NO₃), add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Noradrenaline Bitartrate RS, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution, add 2 mL of potassium hydroxide TS, and shaken. Adjust the pH of the solution to 6.5 with 0.05 mol/L disodium hydrogenphosphate TS, add 10 mL of phosphate buffer solution, pH 6.5, and shake. Immediately after allowing to stand for 3 minutes, add sodium thiosulfate TS dropwise with swirling until a persistent blue color is produced. Add 2 mL of iodine TS, and shake. Adjust the pH of the solution to 6.5 with 0.05 mol/L disodium hydrogenphosphate TS, add 10 mL of phosphate buffer solution, pH 6.5, and shake. Immediately after allowing to stand for 5 minutes, add sodium thiosulfate TS dropwise until a red-purple color develops, then add water to make exactly 50 mL. Determine the absorbances, A₁ and A₄, of the subsequent solutions of the sample solution and the standard solution at 515 nm within 5 minutes as directed under Ultraviolet-visible Spectrophotometry <2.24>:

\[
\frac{M_s}{M_b} = \frac{A_1}{A_4} \times 0.502
\]

**Containers and storage** Containers—Hermetic containers, and colored containers may be used. Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.
Norethisterone

Norfloxacin

C_{20}H_{26}O_{2}: 298.42
17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one
[68-22-4]

Norethisterone, when dried, contains not less than 97.0% and not more than 103.0% of C_{20}H_{26}O_{2}.

**Description**  
Norethisterone occurs as a white to pale yellowish white crystalline powder. It has no odor.

It is sparingly soluble in ethanol (95), in acetone, and in tetrahydrofuran, slightly soluble in diethyl ether, and very slightly soluble in water.

It is affected by light.

**Identification (1)**  
To 2 mg of Norethisterone add 2 mL of sulfuric acid: the solution shows a red-brown color and a yellow-green fluorescence. Add 10 mL of water to this solution cautiously: a yellow color develops and a yellow-brown precipitate is formed.

(2) To 25 mg of Norethisterone add 3.5 mL of a solution of 0.05 g of hydroxyammonium chloride and 0.05 g of anhydrous sodium acetate trihydrate in 25 mL of methanol. Heat under a reflux condenser on a water bath for 5 hours, cool, and add 15 mL of water. Collect the precipitate formed, wash with 1 to 2 mL of water, recrystallize from methanol, and dry in a desiccator (in vacuum, silica gel) for 5 hours: the crystals melt at < 2.60°C between 112°C and 118°C.

**Optical rotation** [α]D^0 23: −32–−37° (after drying, 0.25 g, acetone, 25 mL, 100 mm).

**Melting point** < 2.60 203–209°C.

**Loss on drying** < 2.41  Not more than 0.5% (0.5 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** < 2.44  Not more than 0.1% (0.5 g).

**Assay**  
Weigh accurately about 0.2 g of Norethisterone, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate (1 in 20), and titrate < 2.50 with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 29.84 mg of C_{20}H_{26}O_{2}

**Containers and storage**  
Containers—Tight containers.

Storage—Light-resistant.

Norfloxacin

Norfloxacin, when dried, contains not less than 99.0% of C_{16}H_{18}F_{N_{3}}O_{3}.

**Description**  
Norfloxacin occurs as a white to pale yellow crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in ethanol (99.5) and in acetone, very slightly soluble in methanol, and practically insoluble in water.

It dissolves in dilute hydrochloric acid TS and in sodium hydroxide TS.

It is hygroscopic.

It is gradually colored by light.

**Identification (1)**  
Dissolve 0.01 g of Norfloxacin in a solution of sodium hydroxide (1 in 250) to make 100 mL. To 5 mL of this solution add a solution of sodium hydroxide (1 in 250) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry < 2.44>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve a suitable amount of Norfloxacin in a suitable amount of acetone, evaporate the acetone under reduced pressure, and dry the residue. Determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry < 2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity (1)**  
Sulfate < 1.14—Dissolve 1.0 g of Norfloxacin in 7 mL of 0.5 mol/L sodium hydroxide TS and 23 mL of water, and add 1 drop of phenolphthalein TS. Add gradually diluted hydrochloric acid (1 in 3) to the solution until the red color disappears, then add 0.5 mL of dilute hydrochloric acid, and cool in ice for 30 minutes. Filter through a glass filter (G4), and wash the residue with 10 mL of water. Combine the filtrate and the washing, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.50 mL of 0.005 mol/L sulfuric acid VS add 7 mL of 0.5 mol/L sodium hydroxide TS and 1 drop of phenolphthalein TS, add dilute hydrochloric acid (1 in 3) until the red color disappears, then add 1.5 mL of dilute hydrochloric acid, 1 or 2 drops of bromphenol blue TS and water to make 50 mL (not more than 0.024%).

(2) Heavy metals < 1.07—Proceed with 2.0 g of Norfloxacin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution
(not more than 15 ppm).

(3) Arsenic \(\textit{<1.1D}\) — Prepare the test solution with 1.0 g of Norfloxacin according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances — Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Norfloxacin in 50 mL of a mixture of methanol and acetone (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and acetone (1:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of methanol and acetone (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(\textit{<2.03}\). Spot 20 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography (5 – 7 \(\mu\)m in particle diameter). Develop with a mixture of methanol, toluene, diethylamine and water (20:20:10:7:4) to a distance of about 9 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm): the number of the spot other than the principal spot from the sample solution is not more than 2 and they are not more intense than the spot from the standard solution.

Loss on drying \(\textit{<2.4I}\) Not more than 1.0\% (1 g, 105°C, 2 hours).

Residue on ignition \(\textit{<2.4I}\) Not more than 0.1\% (1 g).

Assay Weigh accurately about 0.5 g of Norfloxacin, previously dried, dissolve in 50 mL of acetic acid (100), and titrate \(\textit{<2.5O}\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 31.93 \, \text{mg of C}_16\text{H}_{18}\text{FN}_3\text{O}_3
\]

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

\[\text{Norgestrel} \quad \text{ノルゲストレル} \]

\[
\text{C}_{21}\text{H}_{28}\text{O}_2: 312.45
\]

13-Ethyl-17-hydroxy-18,19-dinor-17\(\alpha\)-pregn-4-en-20-yn-3-one

\[6533-00-2\]

Norgestrel, when dried, contains not less than 98.0\% of \(\text{C}_{21}\text{H}_{28}\text{O}_2\).

Description Norgestrel occurs as white crystals or crystalline powder.

It is soluble in tetrahydrofuran and in chloroform, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 1 mg of Norgestrel in 2 mL of ethanol (95), and add 1 mL of sulfuric acid: a red-purple color develops. With this solution, examine under ultraviolet light (main wavelength: 365 nm): the solution shows a red-orange fluorescence.

(2) Determine the infrared absorption spectrum of Norgestrel, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(\textit{<2.2S}\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point \(\textit{<2.6D}\) 206 – 212°C.

Purity (1) Heavy metals \(\textit{<1.07}\) — Take 1.0 g of Norgestrel, heat gently to carbonize, cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed with this solution according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances — Dissolve 30 mg of Norgestrel in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(\textit{<2.03}\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography (5 – 7 \(\mu\)m in particle diameter). Develop with a mixture of dichloromethane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying \(\textit{<2.4I}\) Not more than 0.5\% (1 g, 105°C, 3 hours).

Residue on ignition \(\textit{<2.4I}\) Not more than 0.2\% (0.5 g).

Assay Weigh accurately about 0.2 g of Norgestrel, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate (1 in 20), and titrate \(\textit{<2.5O}\) with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L sodium hydroxide VS} = 31.25 \, \text{mg of C}_{21}\text{H}_{28}\text{O}_2
\]

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.
Norgestrel and Ethinylestradiol Tablets

ノルゲストレル・エチニルエストラジオール錠

Norgestrel and Ethinylestradiol Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of norgestrel (C_{21}H_{28}O_{2}: 312.45) and ethinylestradiol (C_{20}H_{24}O_{2}: 296.40).

Method of preparation Prepare as directed under Tablets, with Norgestrel and Ethinylestradiol.

Identification (1) Weigh a quantity of Norgestrel and Ethinylestradiol Tablets, equivalent to 10 mg of Norgestrel according to the labeled amount, previously powdered, add 10 mL of chloroform, shake for 10 minutes, and filter. To 2 mL of the filtrate add 6 mL of sodium hydroxide TS, shake vigorously, and centrifuge. Take 1 mL of the chloroform layer, evaporate on a water bath to dryness, dissolve the residue in 2 mL of ethanol (95%), and add 1 mL of sulfuric acid: a red-purple color develops. Examine under ultraviolet light (main wavelength: 365 nm): this solution shows a red-orange fluorescence (norgestrel).

(2) Take 1 mL of the filtrate obtained in (1), evaporate on a water bath to dryness, add 1 mL of boric acid-methanol buffer solution to the residue, shake, and cool in ice. Add 1 mL of ice-cold diazo TS, shake, add 1 mL of sodium hydroxide TS, and shake: a red-orange color develops (ethinylestradiol).

(3) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 10 mg of Norgestrel RS and 1 mg of Ethinylestradiol RS, respectively, in 10 mL of chloroform, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 20 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (368:32:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of p-toluene-sulfonate in ethanol (95%) (1 in 5) on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365 nm): two spots from the sample solution show the similar color tone and RF value to each spot from the standard solutions (1) and (2).

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Add 2 mL of diluted methanol (7 in 10) to 1 tablet of Norgestrel and Ethinylestradiol Tablets, add exactly 2 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 μm, and use this filtrate as the sample solution. Separately, weigh accurately quantities of Norgestrel RS and of Ethinylestradiol RS, equivalent to 100 times each of the labeled amounts, dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Calculate the ratios, \( Q_{Ta} \) and \( Q_{Tv} \), of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, \( Q_{Sa} \) and \( Q_{Sb} \), of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

\[
M_{Sa} = M_{Sb} = \frac{Q_{Ta}}{Q_{Tv}} \times 1/100 \\
M_{Sb} = M_{Sa} = \frac{Q_{Sa}}{Q_{Sb}} \times 1/100
\]

\( M_{Sa} \): Amount (mg) of Norgestrel RS
\( M_{Sb} \): Amount (mg) of Ethinylestradiol RS

Internal standard solution—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).

Operating conditions— Proceed as directed in the operating conditions in the Assay.

System suitability— Proceed as directed in the system suitability in the Assay.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Norgestrel and Ethinylestradiol Tablets is not less than 70%.

Start the test with 1 tablet of Norgestrel and Ethinylestradiol Tablets, withdraw not less than 50 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet exactly 1 mL of the subsequent filtrate, equivalent to about 17 μg of norgestrel (C_{21}H_{28}O_{2}) and about 1.7 μg of ethinylestradiol (C_{20}H_{24}O_{2}), transfer into a chromatography column [prepared by packing 0.36 g of octadecylsilanized silica gel for pretreatment (55 to 105 μm in particle diameter) in a tube about 1 cm in inside diameter]. After washing the column with 15 mL of water, elute with 3 mL of methanol, and evaporate the effluent in a water bath to dryness at about 40°C with the aid of a current air. Dissolve the residue in exactly 2 mL of diluted methanol (7 in 10), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Norgestrel RS and about 2.5 mg of Ethinylestradiol RS, dissolve in diluted methanol (7 in 10) to make exactly 10 mL, then pipet 3 mL of this solution, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine the peak areas, \( A_{Ta} \) and \( A_{Tv} \), of norgestrel and ethinylestradiol from the sample solution, and the peak areas, \( A_{Sa} \) and \( A_{Sb} \), of norgestrel and ethinylestradiol from the standard solution.

Dissolution rate (%) with respect to the labeled amount of norgestrel (C_{21}H_{28}O_{2})

\[
M_{Sa} = \frac{A_{Ta}}{A_{Tv}} \times 1/V \times 1/C_a \times 54
\]

Dissolution rate (%) with respect to the labeled amount of ethinylestradiol (C_{20}H_{24}O_{2})

\[
M_{Sb} = \frac{A_{Tv}}{A_{Sb}} \times 1/V \times 1/C_b \times 54
\]

\( M_{Sa} \): Amount (mg) of Norgestrel RS
Nortriptyline Hydrochloride / Official Monographs

**System suitability—**
Proceed as directed in the operating conditions in the Assay.

**Assay**
Weigh accurately not less than 20 Norgestrel and Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 mg of norgestrel (C₂₁H₂₈O₂), add 4 mL of diluted methanol (7 in 10), add exactly 4 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 μm, and use this filtrate as the sample solution. Separately, weigh accurately about 50 mg of Norgestrel RS and about 5 mg of Ethinylestradiol RS, and dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution, and use this solution as the standard solution.

Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions. Calculate the ratios, \( Q_{2a} \) and \( Q_{2b} \), of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, \( Q_{3a} \) and \( Q_{3b} \), of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

\[
M_{Sa} = \text{Amount (mg) of Norgestrel (C}_2\text{₁H}_2\text{₈O}_2) = M_{Sa} \times Q_{1a}/Q_{3a} \times 1/50
\]

\[
M_{Sb} = \text{Amount (mg) of Ethinylestradiol (C}_2\text{₀H}_2\text{₅O}_2) = M_{Sb} \times Q_{1b}/Q_{3b} \times 1/50
\]

**Operating conditions—**

- Detector: Norgestrel—An ultraviolet absorption spectrophotometer (wavelength: 241 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of acetonitrile and water (11:9).
- Flow rate: Adjust the flow rate so that the retention time of norgestrel is about 10 minutes.
- System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ethinylestradiol, norgestrel and the internal standard are eluted in this order, and the resolution between the peaks of norgestrel and the internal standard is not less than 8.
- System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethinylestradiol and norgestrel to that of the internal standard are not more than 1.0%, respectively.

**Containers and storage**
Containers—Tight containers.

### Nortriptyline Hydrochloride

**Chemical name:**
Nortriptyline hydrochloride. Nortriptyline hydrochloride is a white, odorless, crystalline powder. It is odorless, or has a faint, characteristic odor.

**Identification**
(1) To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 mL of bromine TS: the color of the test solution disappears.

(2) To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 to 2 drops of a solution of quinhydrone in methanol (1 in 10): a red color gradually develops.

(3) Determine the absorption spectrum of a solution of Nortriptyline Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry to examine that the absorbance at 299.84 nm is about 5.5.

(4) Determine the infrared absorption spectrum of Nortriptyline Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: it absorbs strongly at 299.84 nm and at other wave numbers.

**Purity**
(1) Clarity and color of solution—Dissolve 0.10 g of Nortriptyline Hydrochloride in 10 mL of water: the solution is clear and colorless to very light yellow.

(2) Heavy metals—Prepare with 1.0 g of Nortriptyline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Nortriptyline Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).
(4) Related substances—Dissolve 0.50 g of Nortriptyline Hydrochloride in 20 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\>\). Spot 4 mL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, methanol and diethylamine (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying \(<2.41\>\) Not more than 0.5\% (1 g, 105°C, 2 hours).

Residue on ignition \(<2.44\>\) Not more than 0.1\% (1 g).

Assay Weigh accurately about 0.5 g of Nortriptyline Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\>\). Spot 4 mL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, methanol and diethylamine (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Each mL of 0.1 mol/L perchloric acid VS = 29.98 mg of C\(_{19}\)H\(_{21}\)N.HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Noscapine

ノスカピン

\[
\text{C}_{22}\text{H}_{23}\text{NO}_{7}: 413.42 \\
(3S)-6,7-\text{Dimethoxy}-3-[(5R)-4-\text{methoxy}-6-\text{methyl}-5,6,7,8-\text{tetrahydro}[1,3]\text{dioxolo}[4,5-g]\text{isoquinolin-5-yl]}\text{isoobenzofuran-1(5H)-one} \\
[128-62-1]
\]

Noscapine, when dried, contains not less than 98.5\% of C\(_{22}\)H\(_{23}\)NO\(_7\).

Description Noscapine occurs as white crystals or crystalline powder. It is odorless and tasteless. It is very soluble in acetic acid (100), slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Noscapine in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Noscapine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry \(<2.25\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation \(<2.49\>\ [\alpha]^0_20^0 = +42° ± 48° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 nm).

Melting point \(<2.60\>\ 174 – 177°C.

Purity (1) Chloride \(<1.02\>\—Dissolve 0.7 g of Noscapine in 20 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution as follows: To 0.4 mL of 0.01 mol/L hydrochloric acid add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.02\%).

(2) Heavy metals \(<1.07\>—Proceed with 2.0 g of Noscapine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Morphine—Dissolve 10 mg of Noscapine in 1 mL of water and 5 mL of 1-nitroso-2-naphthol TS with shaking, add 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the solution with 10 mL of chloroform, centrifuge, and collect the aqueous layer: the solution so obtained has no more color than a pale red.

(4) Related substances—Dissolve 0.7 g of Noscapine in 50 mL of acetone, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add acetone to make exactly 50 mL. Pipet 5 mL of this solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.05\>\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28) (60:60:9:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute bismuth subnitrate-potassium iodide TS for spray on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying \(<2.41\>\ Not more than 0.5\% (2 g, 105°C, 4 hours).

Residue on ignition \(<2.44\>\ Not more than 0.1\% (1 g).

Assay Weigh accurately about 0.8 g of Noscapine, previously dried, dissolve in 30 mL of acetic acid (100) and titrate \(<2.50\>\ with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 41.34 mg of C\(_{22}\)H\(_{23}\)NO\(_7\).
Containers and storage  Containers—Well-closed containers.
Storage—Light-resistant.

Noscapine Hydrochloride Hydrate
Narcotine Hydrochloride

Noscapine Hydrochloride Hydrate, when dried, contains not less than 98.0% of noscapine hydrochloride \( \text{C}_{22}\text{H}_{23}\text{NO}_{7}\cdot\text{HCl} \): 449.88.

Description  Noscapine Hydrochloride Hydrate occurs as colorless or white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, in acetic acid (100), and in acetic anhydride, soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification  (1) To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of formaldehyde-sulfuric acid TS: a purple color, changing to yellow-brown, is produced.

(2) To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of a solution of disodium chlorotropate dihydrate (1 in 50), and evaporate to dryness. Dry the residue at 105 °C for 2 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.

Loss on drying  <2.4%  Not more than 9.0% (0.5 g, 120 °C, 4 hours).

Residue on ignition  <2.4%  Not more than 0.5% (1 g).

Assay  Weigh accurately about 0.5 g of Noscapine Hydrochloride Hydrate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

\[ \text{not more than } \frac{44.99}{50} \text{ mg of } \text{C}_{22}\text{H}_{23}\text{NO}_{7}\cdot\text{HCl} \]

Containers and storage  Containers—Well-closed containers.
Storage—Light-resistant.

Nystatin

Nystatin is a mixture of polyene macrolide substances having antifungal activity produced by the growth of Streptomyces noursei.

It contains not less than 4600 units (potency) per mg, calculated on the dried basis. The potency of Nystatin is expressed as the unit of nystatin \( \text{C}_{47}\text{H}_{75}\text{NO}_{17} \) : 926.09, and one unit corresponds to 0.27 \( \mu \)g of nystatin \( \text{C}_{47}\text{H}_{75}\text{NO}_{17} \).

Description  Nystatin occurs as a white to light yellow-brown powder.

It is soluble in formamide, sparingly soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

Identification  (1) Dissolve 1 mg of Nystatin in 5 mL of water and 1 mL of sodium hydroxide TS, heat for 2 minutes, and cool. To this solution add 3 mL of a solution of 4-aminoacetophenone in methanol (1 in 200) and 1 mL of hydrochloric acid: a red-purple color develops.

(2) To 10 mg of Nystatin add 50.25 mL of a mixture of diluted methanol (4 in 5) and sodium hydroxide TS (200:1). Heat at not exceeding 50°C to dissolve, then add diluted methanol (4 in 5) to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nystatin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity  Heavy metals <1.07%—Proceed with 1.0 g of Nystatin according to Method 4, and perform the test. Prepare the
Ofloxacin

Ofloxacin, when dried, contains not less than 99.0% and not more than 101.0% of ofloxacin (C_{18}H_{20}FN_{3}O_{4}).

**Description**

Ofloxacin occurs as pale yellowish white, crystals or crystalline powder. It is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (99.5).
mixture of water and acetonitrile (6:1) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ofloxacin demethyl substance and ofloxacin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ofloxacin is not more than 2.0%.

Loss on drying <2.44> Not less than 0.2% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Ofloxacin, previously dried, dissolve in 100 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of } 0.1 \text{ mol/L perchloric acid VS} = 36.14 \text{ mg of } \text{C}_18\text{H}_20\text{FN}_3\text{O}_4
\]

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Olive Oil

Oleum Olivae

オリブ油

Olive Oil is the fixed oil obtained by expression from the ripe fruit of Olea europaea Linné (Oleaceae).

Description Olive Oil is a light yellow oil. It has a faint odor, which is not rancid, and has a bland taste.

It is miscible with diethyl ether, with petroleum ether, and with carbon disulfide.

It is slightly soluble in ethanol (95).

The whole or a part of it congeals between 0°C and 6°C. Congealing point of the fatty acids: 17 – 26°C

Specific gravity <1.13> \(d_{20}^2 = 0.908 – 0.914\)

Acid value <1.13> Not more than 1.0.

Saponification value <1.13> 186 – 194

Unsaponifiable matters <1.13> Not more than 1.5%.

Iodine value <1.13> 79 – 88

Purity (1) Drying oil—Mix 2 mL of Olive Oil with 10 mL of diluted nitric acid (1 in 4), add 1 g of powdered sodium nitrite little by little with thorough shaking, and allow to stand in a cold place for 4 to 10 hours: the mixture congeals to a white solid.

(2) Peanut oil—Weigh exactly 1.0 g of Olive Oil, dissolve in 60 mL of sulfuric acid-hexane-methanol TS, boil for 2.5 hours on a water bath under a reflux condenser, cool, transfer to a separator, and add 100 mL of water. Wash the flask with 50 mL of petroleum ether, add the washing to the separator, shake, allow to stand, and separate the petroleum ether layer. Extract the water layer with another 50 mL of petroleum ether, and combine the petroleum ether layer with the former petroleum ether solution. Wash the petroleum ether solution repeatedly with 20-mL portions of water until the washings show no more acidity to methyl orange TS. Then add 5 g of anhydrous sodium sulfate, shake, filter, wash anhydrous sodium sulfate with two 10-mL portions of petroleum ether, filter the washings using the former separator, combine the filtrates, distil the petroleum ether on a water bath, passing nitrogen. Dissolve the residue in aceton to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 0.067 g of methyl behenate in aceton to make exactly 50 mL. Pipet 2 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure the peak heights, \(H_r\) and \(H_s\), of methyl behenate of respective solutions: \(H_r\) is not higher than \(H_s\).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter), coated with polyethylene glycol 20 mol/L in a ratio of 5%.

Column temperature: A constant temperature of about 220°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of methyl behenate is about 18 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of methyl behenate obtained from 2 μL of the standard solution is 5 to 10 mm.

Containers and storage Containers—Tight containers.

Omeprazole

オメプラゾール

\[
\text{C}_17\text{H}_{19}\text{N}_2\text{O}_5\text{S} : 345.42
\]

(RS)-5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-1H-benzoimidazole

[73590-58-6]

Omeprazole, when dried, contains not less than 99.0% and not more than 101.0% of \(\text{C}_17\text{H}_{19}\text{N}_2\text{O}_5\text{S}\).

Description Omeprazole occurs as a white to yellowish white crystalline powder.

It is freely soluble in \(N,\text{N}-\text{dimethylformamide}\), sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Omeprazole in \(N,\text{N}-\text{dimethylformamide}\) (1 in 25) shows no optical rotation.

It gradually turns yellowish white on exposure to light.

Melting point: about 150°C (with decomposition).

Identification (1) Add phosphate buffer solution, pH
7.4, to 1 mL of a solution of Omeprazole in ethanol (99.5) (1 in 1000) to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelength.

(2) Determine the infrared absorption spectrum of Omeprazole as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25> \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity**

(1) Clarity and color of solution—Dissolve 0.5 g of Omeprazole in 25 mL of \( N,N \)-dimethylformamide: the solution is clear and colorless or light yellow. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \): the absorbance at 420 nm is not more than 0.3.

(2) Heavy metals \( <1.07> \)—Proceed with 2.0 g of Omeprazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct the procedure soon after preparation of the sample solution. Dissolve 50 mg of Omeprazole in 50 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 \( \mu \)L of the sample solution as directed under Liquid Chromatography \( <2.01> \) according to the following conditions. Determine each of the peak areas of the sample solution by the automatic integration method, and calculate the amounts of them by the area percentage method: each of the amount of the peaks other than omeprazole is not more than 0.1%, and the total amount of the peaks other than omeprazole is not more than 0.5%.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilsilane silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.83 g of disodium hydrogen phosphate dodecahydrate and 0.21 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL. If necessary, adjust the pH to 7.6 with diluted phosphoric acid (1 in 100). Add 11 volumes of acetonitrile to 29 volumes of this solution.

Flow rate: Adjust the flow rate so that the retention time of omeprazole is about 8 minutes.

Time span of measurement: About 10 times as long as the retention time of omeprazole, beginning after the solvent peak.

**System suitability**—

Test for required detectability: Pipet 5 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 25 mL. Confirm that the peak area of omeprazole obtained from 10 \( \mu \)L of this solution is equivalent to 15 to 25% of that from 10 \( \mu \)L of the solution for system suitability test.

**System performance**

System performance: Dissolve 10 mg of Omeprazole and 25 mg of 1,2-dinitrobenzene in 5 mL of sodium borate solution (19 in 5000) and 95 mL of ethanol (99.5). When the procedure is run with 10 \( \mu \)L of this solution under the above conditions, omeprazole and 1,2-dinitrobenzene are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of omeprazole is not more than 2.0%.

**Loss on drying** \( <2.47> \) Not more than 0.2% (1 g, in Vacuum, phosphorus(V) oxide, 50°C; 2 hours).

**Residue on ignition** \( <2.44> \) Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.4 g of Omeprazole, previously dried, dissolve in 70 mL of \( N,N \)-dimethylformamide, and titrate \( <2.59> \) with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination using the same method on a solution consisting of 70 mL of \( N,N \)-dimethylformamide and 12 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 34.54 mg of \( C_{17}H_{19}N_{3}O_{3}S \).

**Containers and storage** Containers—Tight containers. Storage—Light-resistant, in a cold place.

---

**Powdered Opium**

**Opium Pulveratum**

アヘン末

Powdered Opium is a homogeneous powder of opium obtained from *Papaver somniferum* Linné (*Papaveraceae*). Starch or Lactose Hydrate may be added.

Powdered Opium contains not less than 9.5% and not more than 10.5% of morphine (\( C_{17}H_{19}NO_{3} \): 285.34).

**Description**

Powdered Opium occurs as a yellow-brown to dark brown powder.

**Identification**

(1) To 0.1 g of Powdered Opium add 5 mL of diluted ethanol (7 in 10), dissolve by treating with ultrasonic waves for 10 minutes, and add diluted ethanol (7 in 10) to make 10 mL. Filter this solution, and use the filtrate as the sample solution. Separately, dissolve 25 mg of Morphine Hydrochloride Hydrate, 12 mg of Codeine Phosphate Hydrate, 2 mg of Papaverine Hydrochloride, and 12 mg of Noscapine Hydrochloride Hydrate separately in 25 mL of diluted ethanol (7 in 10), and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3) and the standard solution (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.0> \) Spot 10 \( \mu \)L of each of the sample solution and the sample solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia water (28)
Diluted Opium Powder

アヘン散

Diluted Opium Powder contains not less than 0.90% and not more than 1.10% of morphine (C₁₇H₁₉NO₃: 285.34).

Method of preparation

<table>
<thead>
<tr>
<th>Powdered Opium</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch or a suitable diluent</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Powders, with the above ingredients. Lactose Hydrate should not be used.

Description Diluted Opium Powder occurs as a light brown powder.

Identification (1) Proceed with 1 g of Diluted Opium Powder as directed in the Identification (1) under Powdered Opium.

Identification (2) Proceed with 1 g of Diluted Opium Powder as directed in the Identification (2) under Powdered Opium.

Assay Place about 50 g of Diluted Opium Powder, accurately weighed, in a glass-stoppered flask, and stir with 250 mL of dilute ethanol in a water bath at 40°C for 1 hour. Filter the mixture through a glass filter (G3). Transfer the residue on the filter to the first glass-stoppered flask, and add 50 mL of dilute ethanol. Stir the mixture in a water bath at 40°C for 10 minutes, and filter through the same glass filter. Repeat the extraction with three 50-mL portions of dilute ethanol. Evaporate the combined filtrate in a mortar to dryness on a water bath. Add 10 mL of ethanol (99.5) to the residue, evaporate to dryness again, and, after cooling, triturate it with exactly 10 mL of water. Proceed with this solution as directed in Assay under Powdered Opium.

Each mL of 0.05 mol/L sulfuric acid VS = 28.53 mg of C₁₇H₁₉NO₃

Containers and storage Containers—Tight containers.

Opium Tincture

アヘンチンキ

Opium Tincture contains not less than 0.93 w/v% and not more than 1.07 w/v% of morphine (C₁₇H₁₉NO₃: 285.34).

Method of preparation

<table>
<thead>
<tr>
<th>Powdered Opium</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 vol% Ethanol</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Tinctures, with the above ingredients. May be prepared with an appropriate quantity of Ethanol and Purified Water or Purified Water in Containers in place of 35 vol% Ethanol.

Description Opium Tincture is a dark red-brown liquid.
It is affected by light.

**Identification (1)** To 1 mL of Opium Tincture add diluted ethanol (7 in 10) to make 10 mL, filter, and use the filtrate as the sample solution. Proceed as directed in the Identification (1) under Powedered Opium.

(2) Evaporate 1 mL of Opium Tincture to dryness on a water bath, and proceed with the residue as directed in the Identification (2) under Powedered Opium.

**Alcohol number** <1.01> Not less than 3.5 (Method 1).

**Assay** Evaporate 50 mL of Opium Tincture, accurately measured, on a water bath to dryness. Add 10 mL of ethanol (99.5) to the residue, evaporate to dryness again, cool, and triturate with exactly 10 mL of water. Proceed with this solution as directed in the Assay under Powdered Opium.

Each mL of 0.05 mol/L sulfuric acid VS = 28.53 mg of C_{17}H_{19}NO_3

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

### Opium Alkaloids Hydrochlorides

アヘンアルカロイド塩酸塩

Opium Alkaloids Hydrochlorides consist of the hydrochlorides of some of the main alkaloids obtained from opium.

It contains not less than 47.0% and not more than 52.0% of morphine (C_{17}H_{19}NO_3: 285.34), and not less than 35.0% and not more than 41.0% of other opium alkaloids.

**Description** Opium Alkaloids Hydrochlorides occur as a white to light brown powder.

It is soluble in water, and slightly soluble in ethanol (99.5). It is colored by light.

**Identification (1)** Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 10 mL of diluted ethanol (1 in 2), and use this solution as the sample solution. Separately, dissolve 60 mg of Morphine Hydrochloride Hydrate, 40 mg of Noscapine Hydrochloride Hydrate, 10 mg of Codeine Phosphate Hydrate and 10 mg of Papaverine Hydrochloride in 10 mL each of diluted ethanol (1 in 2), and use these solutions as the standard solutions (1), (2), (3) and (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28:20:20:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): each spot from the sample solution is the same in color tone and RF value with the corresponding spot from the standard solutions (1), (2), (3) and (4) (morphine, noscapine, codeine and papaverine).

(2) A solution of Opium Alkaloids Hydrochlorides (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

**pH** <2.54> Dissolve 1.0 g of Opium Alkaloids Hydrochlorides in 50 mL of water: the pH of the solution is between 3.0 and 4.0.

**Purity (1)** Clarity and color of solution—Dissolve 0.5 g of Opium Alkaloids Hydrochlorides in 10 mL of water: the solution is clear, and its absorbance <2.24> at 420 nm is not more than 0.20.

(2) Meconic acid—Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 2 mL of water, and pour into a polyethylene column 1 cm in inside diameter, packed with about 0.36 g of aminoopropylsilanized silica gel for pretreatment (55–105 μm in particle diameter) and previously washed through with 5 mL of water. Then, wash the column with 5 mL of water, 5 mL of methanol and 10 mL of 0.1 mol/L hydrochloric acid in this order, then elute with 2 mL of 1 mol/L hydrochloric acid, and use the eluate as the test solution. To the test solution add 2 mL of dilute sodium hydroxide TS and 1 drop of iron (III) chloride TS: no red color develops.

**Loss on drying** <2.41> Not more than 6.0% (0.5 g, 120°C, 8 hours).

**Residue on ignition** <2.44> Not more than 0.5% (0.5 g).

**Assay** Weigh accurately about 0.1 g of Opium Alkaloids Hydrochlorides, and dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of morphine hydrochloride for assay, dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of morphine, codeine, papaverine, thebaine, narceine and noscapine, A_{T1}, A_{T2}, A_{T3}, A_{T4}, A_{T5} and A_{T6}, from the sample solution, and the peak area of morphine, A_{S}, from the standard solution.

Amount (mg) of morphine (C_{17}H_{19}NO_3) = M_5 \times A_{T1}/A_{S} \times 0.887

Amount (mg) of other opium alkaloids = M_6 \times [(A_{T2} + 0.29A_{T4} + 0.20A_{T3} + 0.19A_{T5} + A_{T6})/A_{S}] \times 0.887

M_5: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

The relative retention time of codeine, papaverine, thebaine, narceine and noscapine with respect to morphine obtained under the following operating conditions are as follows.

<table>
<thead>
<tr>
<th>Component</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>codeine</td>
<td>1.1</td>
</tr>
<tr>
<td>papaverine</td>
<td>1.9</td>
</tr>
<tr>
<td>thebaine</td>
<td>2.5</td>
</tr>
<tr>
<td>narceine</td>
<td>2.8</td>
</tr>
<tr>
<td>noscapine</td>
<td>3.6</td>
</tr>
</tbody>
</table>

**Operating conditions**—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about
40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: Dissolve 60 mg of Morphine Hydrochloride Hydrate, 10 mg of Codeine Phosphate Hydrate, 10 mg of Papaverine Hydrochloride and 40 mg of Noscapine Hydrochloride Hydrate in water to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, morphine, codeine, papaverine and noscapine are eluted in this order with the complete separation between these peaks and with the resolution between the peaks of morphine and codeine being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of morphine is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Storage—Light-resistant.

Opium Alkaloids Hydrochlorides Injection

アヘンアルカロイド塩酸塩注射液

Opium Alkaloids Hydrochlorides Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine (C_{17}H_{19}NO_{3}: 285.34).

Method of preparation

Opium Alkaloids Hydrochlorides 20 g

Water for Injection or Sterile Water for Injection in Containers a sufficient quantity

To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description—Opium Alkaloids Hydrochlorides Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification—To 1 mL of Opium Alkaloids Hydrochlorides Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution, and proceed as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

Extractable volume <6.05> It meets the requirement.

Assay— Pipet 2 mL of Opium Alkaloids Hydrochlorides Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, and dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of morphine to that of the internal standard.

$$\text{Amount (mg) of morphine (C}_{17}\text{H}_{19}\text{NO}_{3}) = M_S \times Q_T / Q_S \times 0.887$$

$M_S$: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.
Opium Alkaloids and Atropine Injection

アヘンアルカロイド・アトロピン注射液

Opium Alkaloids and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine \((C_{17}H_{21}NO_3)\): 285.34, and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate \([C_{17}H_{23}NO_3]_2\cdot H_2SO_4\cdot H_2O: 694.84\].

**Method of preparation**

| Opium Alkaloids Hydrochlorides | 20 g |
| Atropine Sulfate Hydrate | 0.3 g |
| Water for Injection or Sterile Water | a sufficient quantity |

Prepare as directed under Injections, with the above ingredients.

**Description** Opium Alkaloids and Atropine Injection is a colorless or light brown, clear liquid. It is affected by light.

pH: 2.5 - 3.5

**Identification** (1) To 1 mL of Opium Alkaloids and Atropine Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 2 mL of Opium Alkaloids and Atropine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Atropine Sulfate RS in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\>\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.2 \(Rf\) value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same \(Rf\) value (atropine).

**Extractable volume** \(<5.05\>\) It meets the requirements.

**Assay** (1) Morphine—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\>\) according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of morphine to that of the internal standard.

\[
Q_T = \frac{M_S \times Q_T}{Q_S} \times 0.887
\]

\(M_S\): Amount (mg) of morphine \((C_{17}H_{21}NO_3)\)

Internal standard solution—A solution of ethylefrine hydrochloride (1 in 500).

**Operating conditions**


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 \(\mu\)L of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Atropine sulfate hydrate—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 2 mL of the internal standard solution, and add 10 mL of diluted dilute hydrochloric acid (1 in 10). Shake this solution with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloromethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate RS (determine separately the loss on drying \(<2.41\>\) under the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL.

Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use this solution as the standard solution. Perform the test with 2 \(\mu\)L each of the sample solution and standard solution as directed under Gas Chromatography \(<2.02\>\) according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of atropine to that of the internal standard.
the peak area of atropine to that of the internal standard.

\[
\text{Amount (mg) of atropine sulfate hydrate} = M_S \times \frac{Q_S}{Q_M} \times \frac{1}{50} \times 1.027
\]

\(M_S\): Amount (mg) of Atropine Sulfate RS, calculated on the dried basis.

**Internal standard solution**—A solution of homatropine hydrobromide (1 in 4000).

**Operating conditions**—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenylmethyl silicone polymer for gas chromatography.
Column temperature: A constant temperature of about 210°C.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust the flow rate so that the retention time of atropine is about 5 minutes.

**System suitability**—
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and atropine are eluted in this order with the resolution between these peaks being not less than 3.
System reproducibility: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 2.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

**Opium Alkaloids and Scopolamine Injection**

アヘンアルカロイド・スコポラミン注射液

Opium Alkaloids and Scopolamine Injection is an aqueous solution for injection.

It contains not less than 1.80 w/v% and not more than 2.20 w/v% of morphine (C_{17}H_{19}NO_3; 285.34) and not less than 0.054 w/v% and not more than 0.066 w/v% of scopolamine hydrobromide hydrate (C_{17}H_{23}NO_3·HBr·3H_2O; 438.31).

**Method of preparation**

| Opium Alkaloids Hydrochlorides | 40 g |
| Scopolamine Hydrobromide Hydrate | 0.6 g |
| Water for Injection or Sterile Water | a sufficient quantity |

To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

**Description** Opium Alkaloids and Scopolamine Injection is a clear, colorless to light brown liquid.

It is affected by light.

**pH:** 2.5 - 3.5

**Identification (1)** To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide RS in 100 mL of water. To 2 mL of this solution add 2 mL of ammonia TS, proceed with this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28:200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate: a spot of about 0.7 Rf value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same Rf value (scopolamine).

**Extractable volume** <6.05> It meets the requirements.

**Assay (1)** Morphine—Pipet 1 mL of Opium Alkaloids and Scopolamine Injection, add 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.05> according to the following conditions, and calculate the ratios, \(Q_M\) and \(Q_S\), of the peak area of morphine to that of the internal standard.

\[
\text{Amount (mg) of morphine (C_{17}H_{20}NO_3)} = M_S \times \frac{Q_S}{Q_M} \times 0.887
\]

\(M_S\): Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis.

**Internal standard solution**—A solution of etilefrin hydrochloride (1 in 500).

**Operating conditions**—
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.
Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Scopolamine hydrobromide hydrate—Pipet 2 mL of Opium Alkaloids and Scopolamine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted hydrochloric acid (1 in 10), and shake with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scopolamine Hydrobromide RS (determine separately the loss on drying ≤4%) under the same conditions as Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use thus obtained solution as the standard solution. Perform the test with 2 mL each of the sample solution and standard solution as directed under Gas Chromatography ≤0.02% according to the following conditions, and calculate the ratios, Q<sub>m</sub> and Q<sub<s>o</sub></sub>, of the peak area of scopolamine to that of the internal standard.

Amount (mg) of scopolamine hydrobromide hydrate

\[ (C_{17}H_{21}NO_{4} \cdot HBr \cdot 3H_2O) \times M_c \times Q_o / Q_m \times 1/50 \times 1.141 \]

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenylmethyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and scopolamine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

**Weak Opium Alkaloids and Scopolamine Injection**

弱アヘンアルカロイド・スコポラミン注射液

Weak Opium Alkaloids and Scopolamine Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine (C<sub>17</sub>H<sub>19</sub>NO<sub>3</sub> 285.34) and not less than 0.027 w/v% and not more than 0.033 w/v% of scopolamine hydrobromide hydrate (C<sub>17</sub>H<sub>21</sub>NO<sub>4</sub>·HBr·3H<sub>2</sub>O: 438.31).

**Method of preparation**

| Opium Alkaloids Hydrochlorides | 20 g |
| Scopolamine Hydrobromide Hydrate | 0.3 g |
| Water for Injection or Sterile Water | a sufficient quantity |

Prepared as directed under Injections, with the above ingredients.

**Description** Weak Opium Alkaloids and Scopolamine Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

**Identification** (1) To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 2 mL of Weak Opium Alkaloids and Scopolamine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide RS in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ≤0.02%. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28)
Extractable volume *<6.0> It meets the requirements.

Assay (1) Morphone—Pipet 2 mL of Weak Opium Alkaloids and Scopolamine Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.0> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_s$, of the peak area of morphine to that of the internal standard.

\[
\text{Amount (mg) of morphine} = M_5 \times Q_T \times Q_s \times 0.887
\]

$M_5$: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

**Internal standard solution**—A solution of etilefrin hydrochloride (1 in 500).

**Operating conditions**—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

**System suitability**—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Scopolamine hydrobromide hydrate—Pipet 4 mL of Weak Opium Alkaloids and Scopolamine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10), and shake with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scopolamine Hydrobromide RS (separately determine the loss on drying *<2.4> under the same conditions as Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use so obtained solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography *<2.0> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_s$, of the peak area of scopolamine to that of the internal standard.

\[
M_5 = \frac{M_s \times Q_T \times Q_s}{1/50 \times 1.141}
\]

$M_5$: Amount (mg) of Scopolamine Hydrobromide RS, calculated on the dried basis

**Internal standard solution**—A solution of homatropine hydrobromide (1 in 4000).

**Operating conditions**—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenylmethyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

**System suitability**—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and scopolamine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

**Containers and storage**—Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

**Orange Oil**

*Oleum Aurantiit*

オレンジ油

Orange Oil is the essential oil obtained by expression from the peel of the edible fruit of *Citrus* species (*Rutaceae*).

**Description** Orange Oil is a yellow to yellow-brown liquid.
It has a characteristic, aromatic odor, and a slightly bitter taste.

It is miscible with an equal volume of ethanol (95) with turbidity.

Refractive index \(<2.45\) \(n_0^{20}: 1.472 – 1.474\)

Optical rotation \(<2.49\) \(\alpha_0^{20}: +85 – +99^\circ\) (100 mm).

Specific gravity \(<1.13\) \(d_0^{20}: 0.842 – 0.848\)

Purity Heavy metals \(<1.07\)—Proceed with 1.0 g of Orange Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

**Orciprenaline Sulfate**

オルシプレナリン硫酸塩

\[
(C_{11}H_{17}NO_3)_2\cdot H_2SO_4;\quad 520.59
\]

\(5\-[(1RS)-1-Hydroxy-2\-(1\-methylethyl)amino]ethyl)benzene-1,3\-diol hemisulfate\) [5874-97-5]

Orciprenaline Sulfate contains not less than 98.5% of \((C_{11}H_{17}NO_3)_2\cdot H_2SO_4\), calculated on the dried basis.

**Description** Orciprenaline Sulfate occurs as white crystals or crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Orciprenaline Sulfate (1 in 20) shows no optical rotation.

Melting point: about 220°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Orciprenaline Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Orciprenaline Sulfate (1 in 100) responds to the Qualitative Tests \(<1.69\>\) for sulfate.

**pH** \(<2.54\>\) Dissolve 1.0 g of Orciprenaline Sulfate in 10 mL of water: the pH of this solution is between 4.0 and 5.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Orciprenaline Sulfate in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3 mL of Matching Fluid T add 1 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals \(<1.07\>\)—Proceed with 2.0 g of Orciprenaline Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Orciprenalone—Dissolve 0.200 g of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\): the absorbance at 328 nm is not more than 0.075.

**Loss on drying** \(<2.47\>\) Not more than 1.5% (1 g, in vacuum, 105°C, 4 hours).

**Residue on ignition** \(<2.44\>\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.7 g of Orciprenaline Sulfate, dissolve in 100 mL of acetic acid (100) by warming on a water bath, and titrate \(<2.59\>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 52.06 mg of \((C_{11}H_{17}NO_3)_2\cdot H_2SO_4\)

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

**Oxapium Iodide**

オキサピウムヨウ化物

\[C_{22}H_{34}INO_2: 471.42\]

1-(2-Cyclohexyl-2-phenyl-1,3-dioxolan-4-ylmethyl)-1-methylpiperidinium iodide [6577-41-9]

Oxapium Iodide, when dried, contains not less than 98.5% of \(C_{22}H_{34}INO_2\).

**Description** Oxapium Iodide occurs as a white, crystalline powder.

It is soluble in acetonitrile, in methanol and in ethanol (95), slightly soluble in water, in acetic anhydride and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Oxapium Iodide in methanol (1 in 100) does not show optical rotation.

**Identification** (1) Determine the infrared absorption spectrum of Oxapium Iodide, previously dried, as directed in the paste method under Infrared Spectrophotometry \(<2.25\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.1 g of Oxapium Iodide in 10 mL of methanol, and add 2 mL of dilute nitric acid and 2 mL of silver
assay
Weigh accurately about 0.7 g of Oxapium Iodide, residue on ignition (4 hours).

PURITY (1) Heavy metals <1.07>—Proceed with 1.0 g of Oxapium Iodide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.05 g of Oxapium Iodide in 100 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of oxapium from the sample solution is not larger than the area of the peak of oxapium from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of 20°C to 30°C.
Mobile phase: To 57 mL of acetic acid (100) and 139 mL of triethylamine add water to make 1000 mL. To 50 mL of this solution add 500 mL of acetonitrile, 10 mL of dilute acetic acid and 440 mL of water.
Flow rate: Adjust the flow rate so that the retention time of oxapium is about 4 minutes.
Selection of column: Dissolve 0.05 g of Oxapium Iodide and 3 mg of benzophenone in 100 mL of the mobile phase. Proceed with 20 µL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of oxapium and benzophenone in this order with the resolution between these peaks being not less than 5.
Detection sensitivity: Adjust the detection sensitivity so that the peak height of oxapium obtained from 50 µL of the standard solution and standard solution as directed under Liquid Chromatography <2.01> is not more than 1.0 mL.

Time span of measurement: About 6 times as long as the retention time of oxapium beginning after the peak of iodide ion.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Oxapium Iodide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (9:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration, platinum electrode). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 47.14 mg of C₂₂H₃₂INO₇.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Oxaprozin オキサプロジン

C₁₈H₁₅NO₃: 293.32
3-(4,5-Diphenyloxazol-2-yl)propanoic acid [21256-18-8]

Oxaprozin, when dried, contains not less than 98.5% of C₁₈H₁₅NO₃.

Description Oxaprozin occurs as a white to yellowish white crystalline powder. It is sparingly soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually affected by light.

Identification Determine the infrared absorption spectrum of Oxaprozin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> E₁%₁₅m (285 nm): 455 – 495 (after drying, 10 mg, methanol, 1000 mL).

Melting point <2.60> 161 – 165°C.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Oxaprozin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 2.0 g of Oxaprozin according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Oxaprozin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 5 mL, 3 mL and 1 mL of this solution, add methanol to each to make exactly 10 mL, and use these solutions as the standard solutions (2), (3) and (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 µL each of the sample solution and standard solutions (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the total intensity of the spots other than the principal spot from the sample solution is not more than 1.0% calculated on the basis of intensities of the spots from the standard solutions (1), (2), (3) and (4).

Loss on drying <2.41> Not more than 0.3% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).
**Assay**  Weigh accurately about 0.5 g of Oxaprazin, previously dried, dissolve in 50 mL of ethanol (95), and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 29.33 mg of C18H17NO3

**Containers and storage**  Containers—Tight containers.

**Storage**  Light-resistant.

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**Oxazolam**

オキサゾラム

\[
\text{C}_{18}\text{H}_{17}\text{ClN}_{2}\text{O}_{2}: \text{328.79}
\]

10-Chloro-2-methyl-11b-phenyl-2,3,7,11b-tetrahydro[1,3]oxazolo[3,2-d][1,4]benzodiazepin-6(5H)-one

[24143-17-7]

Oxazolam, when dried, contains not less than 99.0% of C18H17ClN2O2.

**Description**  Oxazolam occurs as white crystals or crystalline powder.

It is odorless and tasteless.

It is freely soluble in acetic acid (100), soluble in 1,4-dioxane and in dichloromethane, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It gradually changes in color by light.

Melting point: about 187°C (with decomposition).

**Identification**

(1)  Dissolve 0.01 g of Oxazolam in 5 mL of dilute hydrochloric acid by heating in a water bath for 10 minutes. After cooling, 1 mL of this solution responds to the Qualitative Tests for primary aromatic amines.

(3)  Place 2 g of Oxazolam in a 200-mL flask, add 50 mL of ethanol (95) and 25 mL of 6 mol/L hydrochloric acid TS, and boil under a reflux condenser for 5 hours. After cooling, neutralize with a solution of sodium hydroxide (1 in 4), and extract with 30 mL of dichloromethane. Dehydrate with 3 g of anhydrous sodium sulfate, filter, and evaporate the dichloromethane of the filtrate. Dissolve the residue in 20 mL of methanol by heating on a water bath, and cool immediately in an ice bath. Collect the crystals, and dry in vacuum at 60°C for 1 hour: the crystals melt between 96°C and 100°C.

(4)  Determine the absorption spectrum of a solution of Oxazolam in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24 >\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave-lengths.

(5)  Proceed with Oxazolam as directed under Flame Coloration Test \( <1.06 >\) (2), and perform the test: a green color appears.

**Absorbance**  \( <2.24 >\) \( E^\%_\text{cm} \ (246 \text{ nm}) \): 410 – 430 (after drying, 1 mg, ethanol (95), 100 mL).

**Purity**

(1)  Chloride \( <1.03 >\)—To 1.0 g of Oxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of this filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2)  Heavy metals \( <1.07 >\)—Proceed with 1.0 g of Oxazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3)  Arsenic \( <1.11 >\)—Place 1.0 g of Oxazolam in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat gently. Repeat the addition of 2 to 3 mL of nitric acid at times, and continue to heat until a colorless to light yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate monohydrate solution, heat the solution until dense white fumes are evolved, and evaporate to a volume of 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

**Related substances**—Dissolve 0.05 g of Oxazolam in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.03 >\). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately air-dry, develop the plate with a mixture of toluene and acetone (8:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**  \( <2.41 >\)  Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition**  \( <2.44 >\)  Not more than 0.1% (1 g).

**Assay**  Weigh accurately about 0.65 g of Oxazolam, previously dried, dissolve in 100 mL of a mixture of acetic acid (100) and 1,4-dioxane (1:1). Titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.88 mg of C18H17ClN2O2

**Containers and storage**  Containers—Tight containers.

**Storage**  Light-resistant.
**Oxethazaine**

**Oxetacaine**

オキセサゼイン

C₂₈H₄₁N₃O₃: 467.64
2,2′-(2-Hydroxyethylimino)bis[N-(1,1-dimethyl-2-phenylethyl)-N-methylacetamide]

[126-27-2]

Oxethazaine, when dried, contains not less than 98.5% of C₂₈H₄₁N₃O₃.

**Description** Oxethazaine occurs as a white to pale yellowish white, crystalline powder.

**Identification** (1) Determine the absorption spectrum of a solution of Oxethazaine in ethanol (95) (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Oxethazaine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point** <2.69> 101 – 104°C.

**Purity** (1) Chloride <1.07>—Dissolve 1.0 g of Oxethazaine in 20 mL of ethanol (95), add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Oxethazaine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.40 g of Oxethazaine in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.63>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropyl ether, tetrahydrofuran, methanol and ammonia solution (28:24:10:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(4) 2-Aminoethanol—To 1.0 g of Oxethazaine add methanol to make exactly 10 mL, then add 0.1 mL of a solution of 1-fluoro-2,4-dinitrobenezene in methanol (1 in 25), shake well, and heat at 60°C for 20 minutes: the solution has no more color than the following control solution.

Control solution: To 0.10 g of 2-aminoethanol add methanol to make exactly 200 mL, pipet 1 mL of this solution, and add methanol to make exactly 10 mL. Proceed as directed above.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.9 g of Oxethazaine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 46.76 mg of C₂₈H₄₁N₃O₃

**Containers and storage** Containers—Tight containers.

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**Oxprenolol Hydrochloride**

オクスプレノロール塩酸塩

C₁₅H₂₃NO₃.HCl: 301.81
(2RS)-1-[2-(Allyloxy)phenoxy]-3-(1-methylethyl)aminopropan-2-ol monohydrochloride [6452-73-9]

Oxprenolol Hydrochloride, when dried, contains not less than 98.5% of C₁₅H₂₃NO₃.HCl.

**Description** Oxprenolol Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

**Identification** (1) To 2 mL of a solution of Oxprenolol Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a blue-purple color develops. To this solution add 1 mL of diethyl ether, shake well, and allow to stand: a red-purple color develops in the diethyl ether layer, and a blue-purple color develops in the water layer.

(2) To 3 mL of a solution of Oxprenolol Hydrochloride (1 in 150) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the infrared absorption spectrum of Oxprenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Oxprenolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.
Oxybuprocaaine Hydrochloride

Benoxinate Hydrochloride

オキシブプロカイン塩酸塩

\[
\text{C}_7\text{H}_{15}\text{N}_2\text{O}_3\cdot\text{HCl}: \text{344.88}
\]

2-(Diethylamino)ethyl 4-aminoo-3-butyloxysalicylic monohydrochloride

[5987-82-6]

Oxybuprocaaine Hydrochloride, when dried, contains not less than 99.0% of \( \text{C}_7\text{H}_{15}\text{N}_2\text{O}_3\cdot\text{HCl} \).

**Description**

Oxybuprocaaine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a saline taste. It exhibits anesthetic properties when placed on the tongue.

It is very soluble in water, freely soluble in ethanol (95%) and in chloroform, and practically insoluble in diethyl ether.

The pH of a solution of Oxybuprocaaine Hydrochloride (in 10) is between 5.0 and 6.0.

It is gradually colored by light.

**Identification**

(1) Dissolve 0.01 g of Oxybuprocaaine Hydrochloride in 1 mL of dilute hydrochloric acid and 4 mL of water. This solution responds to the Qualitative Tests for primary aromatic amines.

(2) Dissolve 0.1 g of Oxybuprocaaine Hydrochloride in 8 mL of water, and add 3 mL of ammonium thiocyanate TS: an oily substance is produced. Rub the inner surface of the container with a glass rod: white crystals are formed. Collect the crystals so obtained, recrystallize from water, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 5 hours: the crystals melt \( \text{120°C} \) between 103°C and 106°C.

(3) Determine the absorption spectrum of a solution of Oxybuprocaaine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( \text{2.24} \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Oxybuprocaaine Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

**Melting point** \( \text{158 – 162°C} \).

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of Oxybuprocaaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals \( \text{<1.07} \)—Proceed with 1.0 g of Oxybuprocaaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.25 g of Oxybuprocaaine Hydrochloride in 1 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL of water, and add 3 mL of ammonium thiocyanate TS: a white precipitate is produced.

**Residue on ignition** \( \text{<0.1} \) (1 g).

**Assay**

Weigh accurately about 0.6 g of Oxprenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} \quad = \quad 30.18 \text{ mg of C}_7\text{H}_3\text{N}_2\text{O}_3\cdot\text{HCl}
\]

**Containers and storage**

Containers—Tight containers.

**JP XVI** Official Monographs / Oxprenolol Hydrochloride 1195

**pH** \( \text{<2.54} \)

Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

**Melting point** \( \text{<2.60} \)

107 – 110°C.

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals \( \text{<1.07} \)—Proceed with 2.0 g of Oxprenolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic \( \text{<1.11} \)—Prepare the test solution with 1.0 g of Oxprenolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Oxprenolol Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 4 mL of the sample solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{<2.03} \).

Spot the spot from the standard solution.

A solution of chloroform and methanol (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \( \text{<2.41} \)

Not more than 0.5% (1 g, 80°C, 3 hours).

**Residue on ignition** \( \text{<2.44} \)

Not more than 0.1% (1 g).

**Melting point** \( \text{<2.60} \)

107 – 110°C.

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals \( \text{<1.07} \)—Proceed with 2.0 g of Oxprenolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic \( \text{<1.11} \)—Prepare the test solution with 1.0 g of Oxprenolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Oxprenolol Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 4 mL of the sample solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{<2.03} \).

Spot 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate in a developing chamber saturated with ammonia vapor with a mixture of chloroform and methanol (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \( \text{<2.41} \)

Not more than 0.5% (1 g, 80°C, 3 hours).

**Residue on ignition** \( \text{<2.44} \)

Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.6 g of Oxprenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} \quad = \quad 30.18 \text{ mg of C}_7\text{H}_3\text{N}_2\text{O}_3\cdot\text{HCl}
\]

**Containers and storage**

Containers—Tight containers.
Assay

Weigh accurately about 0.6 g of Oxybuprocaine Hydrochloride Hydrate and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS is equivalent to 34.49 mg of C\textsubscript{17}H\textsubscript{28}N\textsubscript{2}O\textsubscript{3}.HCl.

Containers and storage

Containers—Well-closed containers.

Storage—Light-resistant.

Oxycodone Hydrochloride Hydrate

オキシコドン塩酸塩水和物

C\textsubscript{18}H\textsubscript{21}NO\textsubscript{4}.HCl.3H\textsubscript{2}O: 405.87
(5R)-4,5-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one monohydrochloride trihydrate [124-90-3, anhydride]

Oxycodone Hydrochloride Hydrate contains not less than 98.0% of C\textsubscript{18}H\textsubscript{21}NO\textsubscript{4}.HCl (mol. wt.: 351.83), calculated on the anhydrous basis.

Description

Oxycodone Hydrochloride Hydrate occurs as a white, crystalline powder.

It is freely soluble in water, in methanol and in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution dissolved 1.0 g of Oxycodone Hydrochloride Hydrate in 10 mL of water is between 3.8 and 5.8.

It is affected by light.

Identification

(1) Determine the absorption spectrum of a solution of Oxycodone Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.2>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Oxycodone Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Oxycodone Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.0> (2) for chloride.

Optical rotation

([α]\textsubscript{D}²⁰) = −140° – −149° (0.5 g, calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity

(1) Clarity and color of solution—Dissolve 0.5 g of Oxycodone Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Morphine—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. To this solution add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, add 10 mL of chloroform, shake, centrifuge, and separate the water layer: the color of the solution is not more intense than a pale red.

(3) Codeine—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and warm: no blue color is produced. Add 1 drop of nitric acid: no red color develops.

(4) Thebaine—Dissolve 0.10 g of Oxycodone Hydrochloride Hydrate in 2 mL of diluted hydrochloric acid (1 in 10), and heat the solution in a water bath for 25 minutes. After cooling, add 0.5 mL of 4-aminoantipyrine hydrochloride TS and 0.5 mL of a solution of potassium hexacyanoferrate (III) (1 in 100), and shake. Then shake the solution with 2 mL of ammonia TS and 3 mL of chloroform: no red color develops in the chloroform layer.

Water

<2.48> 12 – 15% (0.2 g, volumetric titration, direct titration).

Residue on ignition

<2.44> Not more than 0.1% (0.5 g).

Assay

Weigh accurately about 0.5 g of Oxycodone Hydrochloride Hydrate, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS is equivalent to 35.18 mg of C\textsubscript{18}H\textsubscript{21}NO\textsubscript{4}.HCl.

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.
**Compound Oxycodeone Injection**

**Compound Hycodenone Injection**

複方オキシコドン注射液

Compound Oxycodeone Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodeone hydrochloride hydrate \((C_{18}H_{21}NO_4\cdot HCl\cdot 3H_2O: 405.87)\), and not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate \((C_{12}H_{15}NO_3\cdot HCl\cdot H_2O: 275.73)\).

**Method of preparation**

<table>
<thead>
<tr>
<th>Oxycodeone Hydrochloride Hydrate</th>
<th>8 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocotarnine Hydrochloride Hydrate</td>
<td>2 g</td>
</tr>
<tr>
<td>Water for Injection or Sterile Water</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

Prepare as directed under Injections, with the above ingredients.

**Description** Compound Oxycodeone Injection is a clear, colorless to pale yellow liquid.

It is affected by light.

**pH** 2.5–4.0

**Identification** (1) To 1 mL of Compound Oxycodeone Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodeone).

(2) Evaporate 1 mL of Compound Oxycodeone Injection on a water bath. Dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

(3) Evaporate 1 mL of Compound Oxycodeone Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

**Extractable volume** <6.05> It meets the requirement.

**Assay** Pipet 2 mL of Compound Oxycodeone Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodeone hydrochloride for assay and about 0.1 g of hydrocotarnine hydrochloride for assay previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions. Calculate the ratios, \(Q_{10a}\) and \(Q_{10b}\), of the peak area of oxycodeone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, \(Q_{5a}\) and \(Q_{5b}\), of the peak area of oxycodeone and hydrocotarnine to that of the internal standard from the standard solution.

Amount (mg) of oxycodeone hydrochloride hydrate
\[
\text{Amount (mg)} = M_{5a} \times Q_{10a} / Q_{5a} \times 1/25 \times 1.154
\]

Amount (mg) of hydrocotarnine hydrochloride hydrate
\[
\text{Amount (mg)} = M_{5b} \times Q_{10b} / Q_{5b} \times 1/25 \times 1.070
\]

**Compound Oxycodeone and Atropine Injection**

**Hycoato Injection**

複方オキシコドン・アトロピン注射液

Compound Oxycodeone and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodeone hydrochloride hydrate \((C_{18}H_{21}NO_4\cdot HCl\cdot 3H_2O: 405.87)\), not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate \((C_{12}H_{15}NO_3\cdot HCl\cdot H_2O: 275.73)\), and not less than 0.027 w/v% of atropine sulfate hydrate \([(C_{17}H_{23}NO_3)_2\cdot H_2SO_4\cdot H_2O: 694.83]\).
Method of preparation

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxycodone Hydrochloride Hydrate</td>
<td>8 g</td>
</tr>
<tr>
<td>Hydrocotarnine Hydrochloride Hydrate</td>
<td>2 g</td>
</tr>
<tr>
<td>Atropine Sulfate Hydrate</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Water for Injection or Sterile Water</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

Prepare as directed under Injections, with the above ingredients.

**Description**

Compound Oxycodone and Atropine Injection is a colorless or pale yellow, clear liquid.

It is affected by light.

pH: 2.5 - 4.0

**Identification (1)**

To 1 mL of Compound Oxycodone and Atropine Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

(2) Evaporate 1 mL of Compound Oxycodone and Atropine Injection on a water bath, and dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

(3) Evaporate 1 mL of Compound Oxycodone and Atropine Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

(4) To 1 mL of Compound Oxycodone and Atropine Injection add 0.5 mL of 2,4-dinitrophenylhydrazine-ethanol TS, and allow to stand for 1 hour. Centrifuge, and add acetone to the supernatant liquid until no more precipitate is produced. Allow to stand for 20 minutes, and centrifuge. To the supernatant liquid add potassium hydroxide TS until the solution is light purple. Shake the liquid with 5 mL of dichloromethane, and separate the dichloromethane layer. Take 0.5 mL of the dichloromethane layer, and evaporate to dryness on a water bath. Add 5 drops of fuming nitric acid to the residue, and evaporate to dryness on a water bath. Cool, dissolve the residue in 1 mL of N,N-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color is produced (atropine).

**Extractable volume**

It meets the requirement.

**Assay (1)**

Oxycodone hydrochloride hydrate and hydrocotarnine hydrochloride hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride for assay and about 0.1 g of hydrocotarnine hydrochloride for assay previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.02> according to the following conditions. Calculate the ratios, Q_Ta and Q_Sb, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, Q_Sa and Q_Sb, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the standard solution.

Amount (mg) of oxycodone hydrochloride hydrate

\[ \text{Amount (mg)} = M_S \times \frac{Q_{Ta}}{Q_{Sa}} \times \frac{1}{25} \times 1.154 \]

Amount (mg) of hydrocotarnine hydrochloride hydrate

\[ \text{Amount (mg)} = M_S \times \frac{Q_{Ta}}{Q_{Sa}} \times \frac{1}{25} \times 1.070 \]

**Internal standard solution**

Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

**Operating conditions**


Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogenphosphate TS add 0.05 mol/L sodium dihydrogenphosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of oxycodone hydrochloride is about 8 minutes.

Selection of column: Proceed with 10 µL of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotarnine in this order with complete separation of these peaks.

(2) Atropine sulfate hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10) and 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane layer through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloromethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate RS (separately determine the loss on drying <2.41> under the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use so obtained solution as the standard solution. Perform the test with 2 µL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_Ta and Q_Sb, of the peak area of atropine to that of the internal standards.

Amount (mg) of atropine sulfate hydrate

\[ \text{Amount (mg)} = M_S \times \frac{Q_T}{Q_S} \times \frac{1}{50} \times 1.027 \]

M_S: Amount (mg) of Atropine Sulfate RS, calculated on
the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column about 3 mm in inside diameter and about 1.5 m in length, packed with 180- to 250-μm siliceous earth for gas chromatography coated with 1 to 3% of 50% phenyl-methylsilicone polymer.
Column temperature: A constant temperature of about 210°C.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust the flow rate so that the retention time of atropine is about 5 minutes.
Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and atropine in this order with the resolution between these peaks being not less than 3.

Containers and storage—Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Oxydol

オキシドール

Oxydol contains not less than 2.5 w/v% and not more than 3.5 w/v% of hydrogen peroxide (H₂O₂: 34.01). It contains suitable stabilizers.

Description
Oxydol occurs as a clear, colorless liquid. It is odorless or has an odor resembling that of ozone. It gradually decomposes upon standing or upon vigorous agitation. It rapidly decomposes when in contact with oxidizing substances as well as reducing substances. It, when alkalized, decomposes with effervescence. It is affected by light.

pH: 3.0 – 5.0
Specific gravity d₃₀°: about 1.01

Identification
1 mL of Oxydol responds to the Qualitative Tests <1.09> for peroxide.

Purity
(1) Acidity—To 25.0 mL of Oxydol add 2 drops of phenolphthalein TS and 2.5 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.
(2) Heavy metals <1.07>—To 5.0 mL of Oxydol add 20 mL of water and 2 mL of ammonia TS, evaporate on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid by heating, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2 mL of dilute acetic acid, 2.5 mL of Standard Lead Solution and water to make 50 mL (not more than 5 ppm).
(3) Arsenic <1.11>—To 1.0 mL of Oxydol add 1 mL of ammonia TS, evaporate on a water bath to dryness, take the residue, prepare the test solution according to Method 1, and perform the test (not more than 2 ppm).
(4) Organic stabilizer—Extract 100 mL of Oxydol with 50-mL, 25-mL and 25-mL portions of a mixture of chloroform and diethyl ether (3:2) successively, combine the extracts in a tared vessel, and evaporate the combined extract on a water bath. Dry the residue over silica gel to constant mass: the mass of the residue is not more than 50 mg.
(5) Nonvolatile residue—Evaporate 20.0 mL of Oxydol on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 20 mg.

Assay
Pipe 1.0 mL of Oxydol, transfer it to a flask containing 10 mL of water and 10 mL of dilute sulfuric acid, and titrate with 0.02 mol/L potassium permanganate VS.

Each mL of 0.02 mol/L potassium permanganate VS = 1.701 mg of H₂O₂

Containers and storage—Containers—Tight containers.
Storage—Light-resistant, and not exceeding 30°C.

Oxygen

酸素

O₂: 32.00

Oxygen is oxygen produced by the air liquefaction separation method.

It contains not less than 99.5 v/v% of O₂.

Description
Oxygen is a colorless gas under atmospheric pressure, and is odorless.
1 mL of Oxygen dissolves in 32 mL of water, and in 7 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.
1000 mL of Oxygen at 0°C and at a pressure of 101.3 kPa weighs 1.429 g.

Identification
Transfer 1 mL each of Oxygen and oxygen directly from cylinders with a pressure-reducing valve to gas-measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography <2.02> according to the following conditions: the retention time of principal peak obtained from Oxygen is the same as that of the peak obtained from oxygen.

Operating conditions—
Proceed as directed in the operating conditions in the Purity.

Purity
Nitrogen—Transfer 1.0 mL of Oxygen directly from cylinder with a pressure-reducing valve to gas-measuring tube or syringe for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with this gas as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area A₁ of nitrogen.
Introduce 0.50 mL of nitrogen into the gas mixer, draw carrier gas into the mixer to make exactly 100 mL, allow to mix thoroughly and use this gas as the standard mixed gas. Perform the test in the same manner with 1.0 mL of this mixture as directed above, and determine the peak area A₅ of nitrogen: A₁ is not larger than A₅.

Operating conditions—
Detector: A thermal-conductivity detector.
Column: A column 3 mm in inside diameter and 3 m in length, packed with zeolite for gas chromatography 250- to
355-µm in particle diameter (a porosity of 0.5 nm).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 5 minutes.

System suitability—

System performance: Introduce 0.5 mL of nitrogen into a gas mixer, add Oxygen to make 100 mL, and mix thoroughly. When the test is run with 1.0 mL of the mixture under the above operating conditions, oxygen and nitrogen are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 1.0 mL of the standard mixed gas under the above operating conditions, the relative standard deviation of the peak area of nitrogen is not more than 2.0%.

Assay

(i) Apparatus—The apparatus is shown diagrammatically in the accompanying figure. A is a 100-mL gas buret having a two-way stopcock a, b – c, d – e and e – f are graduated in 0.1 mL, and c – d is graduated in 2 mL. A is properly connected with a leveling tube B by a thick rubber tube. Fill ammonium chloride-ammonia TS up to the middle of A and B. Place in the absorption ball g of the gas pipette C a coil of copper wire, not more than 2 mm in diameter, which extends to the uppermost portion of the bulb, add 125 mL of ammonium chloride-ammonia TS, and stopper with a rubber stopper i. Connect C with A using the thick rubber tube.

(ii) Procedure—Open a, set B downward and draw the liquid in g to the stopcock opening a. Then close a. Open a to the intake tube h, and fill A and h with ammonium chloride-ammonia TS by lifting B. Close a, connect h with a container of Oxygen, open a, set B downward and measure accurately 100 mL of Oxygen. Open a toward C, and transfer the Oxygen to g by lifting B. Close a, and rock C gently for 5 minutes. Open a, draw the residual gas back into A by setting B downward, and measure the volume of the residual gas. Repeat the procedure until the volume of residual gas is constant, and designate this as \( V \) (mL). With fresh ammonium chloride-ammonia TS in C, repeat the procedure at least four times, and measure the volume of residual gas. Calculate the volume of Oxygen and \( V \) in the following formula on the basis of the gas volume at 20°C and at 101.3 kPa.

\[
\text{Volume (mL) of oxygen (O}_2\text{)} = \text{volume of Oxygen (mL)} - V \text{ (mL)}
\]

Containers and storage

Containers—Cylinders.

Storage—Not exceeding 40°C.

Oxymetholone

オキシメトロン

\[\text{C}_{21}\text{H}_{32}\text{O}_3: 332.48} \]

17β-Hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one

[434-07-1]

Oxymetholone, when dried, contains not less than 97.0% and not more than 103.0% of \( \text{C}_{21}\text{H}_{32}\text{O}_3\).

Description

Oxymetholone occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in methanol, in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored and decomposed by light.

Identification

(1) Dissolve 2 mg of Oxymetholone in 1 mL of ethanol (95), and add 1 drop of iron (III) chloride TS: a purple color develops.

(2) Dissolve 0.01 g of Oxymetholone in methanol to make 50 mL. To 5 mL of the solution add 5 mL of sodium hydroxide-methanol TS and methanol to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Oxymetholone as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation \(<2.49\rangle\ [\alpha]^{20}_D: +34 – +38° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point \(<2.60\rangle 175 – 182°C.

Purity

(1) Clarity and color of solution—Dissolve 0.5 g of Oxymetholone in 25 mL of 1,4-dioxane: the solution is clear, and shows a colorless to pale yellow color.
(2) Related substances—Dissolve 50 mg of Oxymetholone in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\). Spot 10 \(\mu L\) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the spot. Develop immediately the plate with a mixture of toluene and ethanol (99.5) (49:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid solution on the plate, and heat at 100°C for 3 to 5 minutes: any spot other than the principal spot and starting point obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying \(<2.41\>Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition \(<2.44\>Not more than 0.1% (0.5 g).

Assay Weigh accurately about 40 mg of Oxymetholone, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add methanol to make exactly 50 mL. To exactly measured 5 mL of this solution add 5 mL of sodium hydroxide-methanol TS and methanol to make exactly 50 mL. Determine the absorbance at 220 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\), using a solution, prepared by adding methanol to 5 mL of sodium hydroxide-methanol TS to make 50 mL, as the blank.

\[
\text{Amount (mg) of C}_{21}H_{32}O_3 = A/541 \times 50,000
\]

Containers and storage—Containers—Tight containers. Storage—Light-resistant.

**Oxytetracycline Hydrochloride**

オキシテトラサイクリン塩酸塩

\[
\text{C}_{22}H_{28}N_{2}O_9\cdot \text{HCl: 496.89}
\]

(4S,4aR,5S,5aR,6S,12aS)-4-Dimethylamino-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride

\[2058-46-0\]

Oxytetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of *Streptomyces rimosus*.

It contains not less than 880 \(\mu g\) (potency) and not more than 945 \(\mu g\) (potency) per mg, calculated on the dried basis. The potency of Oxytetracycline Hydrochloride is expressed as mass (potency) of oxytetracycline \((\text{C}_{22}H_{27}N_{2}O_8): 460.43\).

Description Oxytetracycline Hydrochloride occurs as yellow, crystals or crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

**Identification** (1) Determine the absorption spectrum of a solution of Oxytetracycline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Oxytetracycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 20 mg of Oxytetracycline Hydrochloride in 3 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

**Optical rotation** \(<2.49\>\ [\alpha]_b^{[b]} = -188 - 200°\) (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid, 25 mL, 100 mm).

Purity (1) Heavy metals \(<1.07\>—Proceed with 0.5 g of Oxytetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(2) Related substances—Dissolve 20 mg of Oxytetracycline Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 4-epi oxytetracycline in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 4-epi oxytetracycline stock solution. Separately, dissolve 20 mg of tetracycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as tetracycline hydrochloride stock solution. Separately, dissolve 8 mg of \(\beta\)-apo oxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as \(\beta\)-apo oxytetracycline stock solution. Pipet 1 mL of 4-epi oxytetracycline stock solution, 4 mL of tetracycline hydrochloride stock solution and 40 mL of \(\beta\)-apo oxytetracycline stock solution, add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution.

Perform the test with exactly 20 \(\mu L\) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07\>\) according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of 4-epi oxytetracycline and tetracycline obtained from the sample solution are not larger than each of the peak area from the standard solution, and the total area of the peaks, \(\alpha\)-apo oxytetracycline having the relative retention time of about 2.1 with respect to oxytetracycline, \(\beta\)-apo oxytetracycline and the peaks, which appear between \(\alpha\)-apo oxytetracycline and \(\beta\)-apo oxytetracycline, is not larger than the peak area of \(\beta\)-apo oxytetracycline from the standard solution. The peak area of 2-acetyl-2-decarboxy oxytetracycline, which appears after the principal peak, obtained from the sample solution is not larger than 4 times the peak area of 4-epi oxytetracycline from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene.
copolymers for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase A: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 100 mL of a solution of tetrabutylammonium hydrogen sulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 30 g of t-butanol and water to make 1000 mL.

Mobile phase B: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 50 mL of a solution of tetrabutylammonium hydrogen sulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 100 g of t-butanol and water to make 1000 mL.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 20</td>
<td>70 → 10</td>
<td>30 → 90</td>
</tr>
<tr>
<td>20 – 35</td>
<td>10 → 20</td>
<td>90 → 80</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL/min.

Time span of measurement: About 3.5 times as long as the retention time of oxytetracycline beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of 4-epoxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 4 mL of this solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peaks of 4-epoxytetracycline obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.

System performance: Dissolve 8 mg of α-apo-epoxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as α-apo-epoxytetracycline stock solution. Mix 3 mL of the sample solution, 2 mL of 4-epoxytetracycline stock solution, 6 mL of tetracycline hydrochloride stock solution, 6 mL of β-apo-epoxytetracycline stock solution and 6 mL of β-apo-epoxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the theoretical plates and the symmetrical coefficient of the peak of oxytetracycline are not less than 1000 and not more than 2.0.

System repeatability: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytetracycline is not more than 1.3.

System repeatability: Pipet 1 mL of 4-epoxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of 4-epoxytetracycline is not more than 2.0%.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Weigh accurately an amount of Oxytetracycline Hydrochloride and Oxytetracycline Hydrochloride RS, equivalent to about 50 mg (potency), and dissolve each in diluted hydrochloric acid (1 in 100) to make exactly 50 mL. Pipet 5 mL of these solutions, add diluted methanol (3 in 20) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.91> according to the following conditions, and determine the peak areas, A1 and A3, of oxytetracycline.

Amount [μg (potency)] of oxytetracycline (C22H24N2O9)

\[ M_S = M_A \times A_T / A_S \times 1000 \]

\( M_S \): Amount [mg (potency)] of Oxytetracycline Hydrochloride RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with strongly acidic ion exchange resin for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 3.402 g of potassium dihydrogen phosphate and 9.306 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 700 mL of water, add 300 mL of methanol, and adjust the pH to 4.5 with dilute hydrochloric acid.

Flow rate: Adjust the flow rate so that the retention time of oxytetracycline is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the theoretical plates and the symmetrical coefficient of the peak of oxytetracycline are not less than 1000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytetracycline is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Storage—Light-resistant.
Oxytocin

オキシトシン

\[
\text{C}_{33}\text{H}_{30}\text{N}_{12}\text{O}_{12}\text{S}_{2}: 1007.19
\]

Oxytocin is a synthetic peptide having the property of causing the contraction of uterine smooth muscle.

It contains not less than 540 oxytocin Units and not more than 600 oxytocin Units per mg, calculated on the dehydrated and de-acetic acid basis.

**Description**

Oxytocin occurs as a white powder. It is very soluble in water, and freely soluble in ethanol (99.5%). It dissolves in hydrochloric acid TS.

The pH of a solution prepared by dissolving 0.10 g of Oxytocin in 10 mL of freshly boiled and cooled water is between 4.0 and 6.0.

It is hygroscopic.

**Identification**

Determine the absorption spectrum of a solution of Oxytocin (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Constituent amino acids**

Put about 1 mg of Oxytocin in a test tube for hydrolysis, add 6 mol/L hydrochloric acid TS to dissolve, replace the air in the tube with nitrogen, seal the tube under reduced pressure, and heat at 110 to 115°C for 16 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, add 2 mL of 0.02 mol/L hydrochloric acid TS to dissolve the residue, and use this solution as the sample solution. Separately, weigh accurately about 27 mg of L-aspartic acid, about 24 mg of L-threonine, about 21 mg of L-serine, about 29 mg of L-glutamic acid, about 23 mg of L-proline, about 15 mg of glycine, about 18 mg of L-alanine, about 23 mg of L-valine, about 48 mg of L-cystine, about 30 mg of methionine, about 26 mg of L-isoleucine, about 26 mg of L-leucine, about 36 mg of L-lysine, about 33 mg of L-isoleucine, about 33 mg of L-lysine hydrochloride, about 42 mg of L-histidine hydrochloride monohydrate, and about 42 mg of L-arginine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride monohydrate.

**System suitability**

- **Mobile phase:** Prepare mobile phases A, B and C according to the following table.

```
<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid monohydrate</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>6.10 g</td>
</tr>
<tr>
<td>Trisodium citrate dihydrate</td>
<td>6.19 g</td>
<td>7.74 g</td>
<td>26.67 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>54.35 g</td>
</tr>
<tr>
<td>Ethanol (99.5)</td>
<td>260.0 mL</td>
<td>20.0 mL</td>
<td>—</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>—</td>
<td>—</td>
<td>5.0 mL</td>
</tr>
<tr>
<td>Thiodiglycol</td>
<td>5.0 mL</td>
<td>5.0 mL</td>
<td>—</td>
</tr>
<tr>
<td>Lauromacrogol solution (1 in 4)</td>
<td>4.0 mL</td>
<td>4.0 mL</td>
<td>4.0 mL</td>
</tr>
<tr>
<td>Caprylic acid</td>
<td>0.1 mL</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
</tr>
<tr>
<td>Water</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
</tr>
<tr>
<td>Total amount</td>
<td>2000 mL</td>
<td>1000 mL</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>
```

**Flowing of the mobile phase:** Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

```
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<tr>
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<th>Mobile phase C (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 9</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9 – 25</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>25 – 61</td>
<td>0</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>61 – 80</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>
```

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for more than 10 minutes while passing nitrogen, and use this solution as Solution A. Separately, to 1957 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for more than 10 minutes while passing nitrogen, and use this solution as Solution B. Mix Solution A and Solution B before use.

Flow rate of mobile phase: About 0.26 mL per minute.

Flow rate of reaction reagent: About 0.3 mL per minute.

**System suitability—**

- **Detector:** A visible spectrophotometer (wavelength: 440 nm and 570 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (sodium type) composed with a sulfonated polystyrene copolymer (3 µm in particle diameter).
- **Column temperature:** A constant temperature of about 57°C.
- **Chemical reaction bath temperature:** A constant temperature of about 130°C.
- **Color developing time:** About 1 minute.

**Operating conditions—**

- Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

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<td>0</td>
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<td>100</td>
<td>0</td>
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<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>61 – 80</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
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Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for more than 10 minutes while passing nitrogen, and use this solution as Solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for more than 30 minutes while passing nitrogen, and use this solution as Solution B. Mix Solution A and Solution B before use.

Flow rate of mobile phase: About 0.26 mL per minute.

Flow rate of reaction reagent: About 0.3 mL per minute.

**System suitability—**

- **Detector:** A visible spectrophotometer (wavelength: 440 nm and 570 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (sodium type) composed with a sulfonated polystyrene copolymer (3 µm in particle diameter).
- **Column temperature:** A constant temperature of about 57°C.
- **Chemical reaction bath temperature:** A constant temperature of about 130°C.
- **Color developing time:** About 1 minute.

**Operating conditions—**

- Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

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<td>61 – 80</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>
```
peaks of threonine and serine, glycine and alanine, and isoleucine and leucine being not less than 1.5, 1.4 and 1.2, respectively.

System repeatability: When the test is repeated 3 times with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviations of the peak area of acetic acid, proline, valine and arginine are not more than 2.0\%, respectively.

**Purity (1)** Acetic acid—Weigh accurately about 15 mg of Oxytocin, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of acetic acid (100), add the internal standard solution to make exactly 100 mL. Pipet 2 mL of this solution, add the internal standard solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of acetic acid to that of the internal standard: the amount of acetic acid is not less than 6.0\% and not more than 10.0\%.

\[
\text{Amount (\%)} = \frac{M_S}{M_T} \times \frac{Q_T}{Q_S} \times 1/10
\]

\( M_S \): Amount (mg) of acetic acid (100)  
\( M_T \): Amount (mg) of the sample

**Internal standard solution**—A solution of propionic acid in the mobile phase (1 in 10,000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecysilsilazaned silica gel for liquid chromatography (5 \( \mu \text{m} \) in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 0.7 mL of phosphoric acid add 900 mL of water, adjust the pH to 3.0 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 3 minutes.

**System suitability**—
System performance: When the procedure is run with 10 \( \mu \text{L} \) of the standard solution under the above operating conditions, acetic acid and propionic acid are eluted in this order with the resolution between these peaks being not less than 14.

System repeatability: When the test is repeated 6 times with 10 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acetic acid to that of the internal standard is not more than 2.0\%.

(2) Related substances—Dissolve 25 mg of Oxytocin in 100 mL of the mobile phase A, and use this solution as the sample solution. Perform the test with 50 \( \mu \text{L} \) of the sample solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak other than Oxytocin is not more than 1.5\%, and the total of them is not more than 5.0\%.

**Operating conditions**—
Detector, column, column temperature, mobile phase, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of oxytocin.

**System suitability**—
Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of oxytocin obtained from 50 \( \mu \text{L} \) of this solution is equivalent to 5 to 15\% of that from 50 \( \mu \text{L} \) of the solution for system suitability test.

System performance: Dissolve an adequate amount of oxytocin and vasopressin in the mobile phase A, so that each mL contains about 0.1 mg each of them. When the procedure is run with 50 \( \mu \text{L} \) of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 50 \( \mu \text{L} \) of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0\%.

**Water** \(<2.48>\) Not more than 5.0\% (50 mg, coulometric titration).

**Assay** Weigh accurately an amount of Oxytocin, equivalent to about 13,000 Units, dissolve in the mobile phase A to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 1 bottle of the Oxytocin RS in the mobile phase A to make a known concentration solution containing each mL contains about 130 Units, and use this solution as the standard solution. Perform the test with exactly 25 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of oxytocin.

Units per mg of Oxytocin, calculated on the dehydrated and de-acetic acid basis

\[
M_S/\frac{M_T}{100} \times A_T/A_S
\]

\( M_S \): Units per mL of the standard solution  
\( M_T \): Amount (mg) of sample, calculated on the dehydrated and de-acetic acid basis

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecysilsilazaned silica gel for liquid chromatography (5 \( \mu \text{m} \) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.

Mobile phase B: A mixture of water and acetonitrile (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.
Oxytocin Injection

オキシトシン注射液

Oxytocin Injection is an aqueous solution for injection. It contains not less than 90.0% and not more than 110.0% of the labeled oxytocin Units.

Method of preparation Prepare as directed under Injections, with Oxytocin.

Description Oxytocin Injection is a colorless, clear liquid.

pH 2.5 – 4.5

Bacterial endotoxins 4.01 Less than 10 EU/oxytocin Unit.

Extractable volume 6.05 It meets the requirement.

Foreign insoluble matter 6.06 Perform the test according to the Method 1: it meets the requirement.

Insoluble particulate matter 6.07 It meets the requirement.

Sterility 4.06 Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a portion of Oxytocin Injection according to the labeled Units, dilute with the diluent so that each mL contains about 1 Unit, and use this solution as the sample solution. Separately, dissolve 1 bottle of Oxytocin RS in the mobile phase A to make exactly 20 mL. Pipet a suitable volume of this solution, dilute with the diluent to make a known concentration solution so that each mL contains about 1 Unit, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.07 according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of oxytocin.

\[
M_S = \frac{A_S}{A_T} / b \times a
\]

\( M_S \): Units per mL of the standard solution
\( a \): Volume (mL) of sample
\( b \): Total volume of the sample solution prepared by diluting with the diluent

Diluent: Dissolve 5 g of chlorobutanol, 1.1 g of sodium acetate trihydrate, 5 g of acetic acid (100) and 6 mL of ethanol (99.5) in water to make 1000 mL.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.
Mobile phase B: A mixture of water and acetonitrile (1:1).
Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>70 → 40</td>
<td>30 → 60</td>
</tr>
<tr>
<td>30 – 30.1</td>
<td>40 → 70</td>
<td>60 → 30</td>
</tr>
<tr>
<td>30.1 – 45</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

Flow rate: About 1.0 mL per minute.
System suitability—
System performance: Dissolve 2 mg each of oxytocin and vasopressin in 20 mL of the mobile phase A. When the procedure is run with 25 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—At 2 to 8°C.
Ozagrel Sodium

オザグレルナトリウム

\[ \text{C}_{13}\text{H}_{11}\text{N}_{2}\text{NaO}_{2} \times 250.23 \]

Monosodium \((2E)-3\text{-[4-\text{(1H-imidazolyl-1-ylmethyl)phenyl]prop-2-enoate}}\)

[189224-26-8]

Ozagrel Sodium, when dried, contains not less than 98.0% and not more than 102.0% of \(\text{C}_{13}\text{H}_{11}\text{N}_{2}\text{NaO}_{2}\).

**Description**

Ozagrel Sodium occurs as white crystals or crystalline powder.

It is freely soluble in water, soluble in methanol, and practically insoluble in ethanol (99.5).

**Identification**

1. Determine the absorption spectrum of a solution of Ozagrel Sodium (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ozagrel Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Ozagrel Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ozagrel Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

3. A solution of Ozagrel Sodium (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt.

**pH**

The pH of a solution prepared by dissolving 0.5 g of Ozagrel Sodium in 10 mL of water is between 9.5 and 10.5.

**Purity**

Clarity and color of solution—Dissolve 0.5 g of Ozagrel Sodium in 10 mL of water: the solution is clear and colorless.

Chloride <1.03>—Dissolve 2.0 g of Ozagrel Sodium in 30 mL of water, add 1 mL of acetic acid (100) and water to make 50 mL, shake, and allow to stand for 30 minutes. Filter the solution, discard the first 5 mL of the filtrate, and to 25 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.01 mol/L hydrochloric acid VS add 0.5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.012%).

Heavy metals <1.07>—Proceed with 2.0 g of Ozagrel Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Related substances—Dissolve 50 mg of Ozagrel Sodium in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 5 \(\mu\)L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: each of the amount other than ozagrel is not more than 0.2%, and the total amount other than ozagrel is not more than 0.5%.

**Operating conditions**

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Time span of measurement: About 2 times as long as the retention time of ozagrel, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 200 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ozagrel obtained from 5 \(\mu\)L of this solution is equivalent to 15 to 25% of that from 5 \(\mu\)L of the solution for system suitability test.

System performance: When the procedure is run with 5 \(\mu\)L of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ozagrel are not less than 6000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 \(\mu\)L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ozagrel is not more than 2.0%.

**Loss on drying** <2.41>—Not more than 0.5% (1 g, 105°C, 4 hours).

**Assay**

Weigh accurately about 25 mg each of Ozagrel Sodium and Ozagrel Sodium RS, both previously dried, and dissolve each in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 1 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_t\) and \(Q_s\), of the peak area of ozagrel to that of the internal standard.

\[ M_S = \text{Amount (mg) of Ozagrel Sodium RS} \]

\[ M_5 = \text{Amount (mg) of Ozagrel Sodium} \times \frac{Q_t}{Q_s} \]

**Internal standard solution**—A solution of benzoic acid in methanol (1 in 100).

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium acetate (3 in 1000) and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of ozagrel is about 10 minutes.

**System suitability**

System performance: When the procedure is run with 1 \(\mu\)L of the standard solution under the above operating condi-
Ozagrel Sodium for Injection

注射用オザグレルナトリウム

Ozagrel Sodium for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of ozagrel sodium \((C_{13}H_{11}N_{2}NaO_{2} \times 250.23)\).

**Method of preparation** Prepare as directed under Injections, with Ozagrel Sodium.

**Description** Ozagrel Sodium for Injection occurs as white masses or powder.

**Identification** Dissolve an amount of Ozagrel Sodium for Injection, equivalent to 40 mg of Ozagrel Sodium according to the labeled amount, in water to make 40 mL. To 1 mL of this solution add water to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(2.24\): it exhibits a maximum between 269 nm and 273 nm.

**pH** Being specified separately.

**Purity** Related substances—Dissolve an amount of Ozagrel Sodium for Injection, equivalent to 0.20 g of Ozagrel Sodium according to the labeled amount, in the mobile phase to make 100 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Purity (4) under Ozagrel Sodium.

**Bacterial endotoxins** \(\leqslant 4.01\) Less than 3.7 EU/mg.

**Uniformity of dosage units** \(\leqslant 4.02\) It meets the requirement of the Mass variation test.

**Foreign insoluble matter** \(\leqslant 6.06\) Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** \(\leqslant 6.07\) It meets the requirement.

**Sterility** \(\leqslant 4.06\) Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Dissolve an amount of Ozagrel Sodium for Injection, equivalent to about 0.4 g of ozagrel sodium \((C_{13}H_{11}N_{2}NaO_{2})\), in water to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and 5 mL of water, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ozagrel Sodium RS, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay under Ozagrel Sodium.

\[
\text{Amount (mg) of ozagrel sodium (C}_{13}\text{H}_{11}\text{N}_{2}\text{NaO}_{2}\) = M_s \times Q_t/Q_s \times 16
\]

\(M_s\): Amount (mg) of Ozagrel Sodium RS

**Containers and storage** Containers—Tight containers.

**Storage**—Light-resistant.

**Pancreatin**

パンクレアチン

Pancreatin is a substance containing enzymes prepared from the pancreas of edible animals, mostly the hog, and has amylolytic, proteolytic and lipolytic activities.

It contains not less than 2800 starch saccharifying activity units, not less than 28,000 proteolytic activity units, and not less than 960 lipolytic activity units per g.

It is usually diluted with suitable excipients.

**Description** Pancreatin occurs as a white to light yellow powder. It has a characteristic odor.

**Purity** (1) Rancidity—Pancreatin has no unpleasant or rancid odor and is tasteless.

(2) Fat—Add 20 mL of diethyl ether to 1.0 g of Pancreatin, extract with occasional shaking for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, combine the washing with the filtrate, evaporate the diethyl ether, and dry the residue at 105°C for 2 hours: the mass of the residue does not exceed 20 mg.

**Loss on drying** \(\leqslant 2.41\) Not more than 4.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

**Residue on ignition** \(\leqslant 2.44\) Not more than 5% (1 g).

**Assay** (1) Starch digestive activity \(\leqslant 4.03\)

(i) Substrate solution—Use potato starch TS for amylolytic activity test, prepared by adding 10 mL of phosphate buffer solution for pancreatin instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 100 mL. Pipet 10 mL of this solution, and add ice-cold water to make exactly 100 mL.


(2) Protein digestive activity \(\leqslant 4.03\)

(i) Substrate solution—Use the substrate solution 2 described in (2) Assay for protein digestive activity under Digestion Test after adjusting the pH to 8.5.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir,
and add ice-cold water to make exactly 200 mL.

(iii) Procedure—Proceed as directed in (2) Assay for protein digestive activity under Digestion Test, using trichloroacetic acid TS B as the precipitation reagent.

(3) Fat digestive activity \(<4.03\>

(i) Emulsifier—Prepare with 18 g of polyvinyl alcohol I and 2 g of polyvinyl alcohol II as directed in (3) Assay for fat digestive activity under Digestion Test.

(ii) Substrate solution—Use the substrate solution described in (3) Assay for fat digestive activity under the Digestion Test.

(iii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 100 mL.

(iv) Procedure—Proceed as directed in (3) Assay for fat digestive activity under Digestion Test, using phosphate buffer solution, pH 8.0, as the buffer solution.

Containers and storage Containers—Tight containers.

Storage—Not exceeding 30°C.

### Pancuronium Bromide

パノクロニウム臭化物

\[
\text{C}_{35}\text{H}_{60}\text{Br}_{2}\text{N}_{2}\text{O}_{4}: 732.67
\]

1,1'-\(3\alpha,17\beta\)-Diacetoxy-5\(\alpha\)-androstan-2\(\beta\),16\(\beta\)-diyl)bis(1-methylpiperidinium) dibromide

[15500-66-0]

Pancuronium Bromide contains not less than 98.0% and not more than 102.0% of \(\text{C}_{35}\text{H}_{60}\text{Br}_{2}\text{N}_{2}\text{O}_{4}\), calculated on the dehydrated basis.

**Description** Pancuronium Bromide occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95) and in acetic anhydride.

It is hygroscopic.

**Identification** (1) Determine the infrared absorption spectrum of Pancuronium Bromide as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25>\) and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Pancuronium Bromide (1 in 100) responds to the Qualitative Tests \(<1.09>\) (1) for bromide.

**Optical rotation** \(<2.49>\) \([\alpha]_{D}^0 + 38 \pm +42^\circ\) (0.75 g calculated on the dehydrated basis, water, 25 mL, 100 mm).

**pH** \(<2.50>\) The pH of a solution of Pancuronium Bromide (1 in 100) is between 4.5 and 6.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Pancuronium Bromide in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 50 mg of Pancuronium Bromide in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution (1). Separately, weigh exactly 5 mg of pancuronium bromide for thin-layer chromatography, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03>\).

Spot 2 \(\mu\)L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, acetonitrile and a solution of sodium iodide (1 in 5) (17:2:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly with a solution of sodium nitrite in methanol (1 in 100) on the plate, allow to stand for 2 minutes, and spray evenly with potassium bismuth iodide TS on the plate: a spot from the sample solution, corresponding to that from the standard solution (2), has no more color than that from the standard solution (2), and the spots other than the principal spot and the above mentioned spot from the sample solution have no more color than the spot from the standard solution (1).

**Water** \(<2.48>\) Not more than 8.0% (0.3 g, volumetric titration, direct titration).

**Residue on ignition** \(<2.44>\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Pancuronium Bromide, dissolve in 50 mL of acetic anhydride by warming, and titrate \(<2.50>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

\[= 36.63 \text{ mg of } \text{C}_{35}\text{H}_{60}\text{Br}_{2}\text{N}_{2}\text{O}_{4}\]

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

### Panipenem

パニペネム

\[
\text{C}_{15}\text{H}_{21}\text{N}_{3}\text{O}_{4}\text{S}: 339.41
\]

\((5\text{R},6\text{S})\)-6-((1\text{R})-1-Hydroxyethyl)-3-\((3\text{S})-1-(1\text{-iminoethyl})pyrrololidin-3-ylsulfanyl\)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

[87726-17-8]

Panipenem contains not less than 90.0% (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis and corrected on the amount of the residual solvent. The potency of Panipenem is expressed as mass (potency) of panipenem (\(\text{C}_{15}\text{H}_{21}\text{N}_{3}\text{O}_{4}\text{S}\)).

**Description** Panipenem occurs as a white to light yellow, crystalline powder or mass.
It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

It is hygroscopic.

It deliquesces in the presence of moisture.

**Identification** (1) Dissolve 0.02 g of Panipenem in 2 mL of water, add 1 mL of hydroxylationmonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Determine the absorption spectrum of a solution of Panipenem in 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>:

- Exhibits a maximum between 296 nm and 300 nm.
- Exhibits absorption at the wave numbers of about 1760 cm$^{-1}$, 1676 cm$^{-1}$, 1632 cm$^{-1}$, 1588 cm$^{-1}$, 1384 cm$^{-1}$, and 1249 cm$^{-1}$.

**Absorbance**<2.24> $E_{1}%_{10.6} (289 	ext{ nm})$: 280 – 310 (50 mg calculated on the anhydrous and desolvent basis, 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0, 2500 mL).

**Optical rotation** <2.49> $[\alpha]_D$: +55 – +65° (0.1 g, calculated on the anhydrous and corrected on the amount of the residual solvent, 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0, 10 mL, 100 mm).

**pH** <2.54> Dissolve 0.5 g of Panipenem in 10 mL of water: the pH of the solution is between 4.5 and 6.5.

**Purity** (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Panipenem according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Residual solvents <2.46>—Weigh accurately about 0.2 g of Panipenem, transfer to a 20-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution and 2 mL of water to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, pipet 15 mL of ethanol (99.5) and 3 mL of acetone, add water to make exactly 200 mL. Pipet 1 mL and 2 mL of this solution, and add water to them to make exactly 20 mL. Transfer exactly 2 mL each of these solutions to a 20-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution, seal tightly a rubber stopper with aluminum cap, and use these solutions as the standard solution (1) and the standard solution (2). Shake gently the sample solution and the standard solutions (1) and (2) in a water bath at a constant room temperature, and allow to stand for 30 minutes. Perform the test with 1 mL of the gas in each container as directed under Gas Chromatography <2.02> according to the following conditions. Calculate the ratios, $Q_{S1}$ and $Q_{S2}$, of the peak area of ethanol and acetonol to that of the internal standard from the sample solution, the ratios, $Q_{S1}$ and $Q_{S3}$, of the peak area of ethanol and acetonol to that of the internal standard from the standard solution (1), and the ratios, $Q_{S2}$ and $Q_{SB2}$, of the peak area of ethanol and acetonol to that of the internal standard from the standard solution (2). Calculate the amount of the ethanol and acetone by the following formula: ethanol is not more than 5.0% and acetone is not more than 1.0%.

**Amount (%) of ethanol in Panipenem**

\[
\text{Amount (g) of ethanol in Panipenem} = 15 \times 0.79 \times (Q_{S1} + Q_{SB2} - 2Q_{S3})/2(Q_{S2} - Q_{S3}) \times 1/1000 \times 100/M
\]

**Amount (%) of acetone in Panipenem**

\[
\text{Amount (g) of acetone in Panipenem} = 3 \times 0.79 \times (Q_{S1} + Q_{SB2} - 2Q_{S3})/2(Q_{S2} - Q_{S3}) \times 1/1000 \times 100/M
\]

**Internal standard solution**—A solution of 1-propanol (1 in 400).

**Operating conditions**—

- Detector: Hydrogen flame-ionization detector.

- Column: A glass column 1 mm in inside diameter and 40 m in length, coated with porous polymer beads for gas chromatography.

- Column temperature: A constant temperature of about 140°C.

- Carrier gas: Helium.

- Flow rate: Adjust the flow rate so that the retention time of 1-propanol is about 6 minutes.

**System suitability**—

- System performance: When the procedure is run with 1 mL of the gas of the standard solution (2) under the above operating conditions, ethanol, acetone and the internal standard are eluted in this order with the resolution between ethanol and acetone being not less than 4. System repeatability: When the test is repeated 6 times with 1 mL of the gas of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 5.0%.

(4) Related substances—Being specified separately.

**Water** <2.48> Weigh accurately about 0.5 g of Panipenem, transfer to a 15-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, weigh accurately 2 g of water, and add the internal standard solution to make exactly 100 mL. Pipet 5 mL and 10 mL of this solution, add the internal standard solution to make exactly 20 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with 1 mL of the sample solution and standard solutions (1) and (2) as directed under Gas Chromatography <2.02> according to the following condition, and calculate the ratios, $Q_{S1}$, $Q_{S3}$, and $Q_{S2}$ of the peak area of water to that of the internal standard. Calculate the amount of water by the following formula: water is not more than 5.0%.

**Amount of water (%)**

\[
\text{Amount of water (g)} = M_1/M_2 \times (Q_{S1} + Q_{S3} - 2Q_{S2})/2(Q_{S2} - Q_{S3}) \times 1/1000 \times 100
\]

**$M_1$: Amount (g) of water**

**$M_2$: Amount (g) of Panipenem**
**Internal standard solution**—A solution of acetonitrile in methanol (1 in 100).

**Operating conditions**—

Detector: A thermal conductivity detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 125°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of acetonitrile is about 8 minutes.

**System suitability**—

System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, water, methanol, and the internal standard are eluted in this order with the resolution between water and internal standard being not less than 10.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of panipenem to that of the internal standard is not more than 5.0%.

**Residue on ignition** Being specified separately.

**Bacterial endotoxins** <4.0/l Less than 0.15 EU/mg (potency).

**Assay** Weigh accurately an amount of Panipenem and Panipenem RS, equivalent to about 0.1 g (potency), dissolve separately in 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test within 30 minutes after preparation of the solutions with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Qs and Qt, of the peak area of panipenem to that of the internal standard.

\[
Q_s = M_s \times Q_t / Q_s \times 1000
\]

where

- \(M_s\): Amount [mg (potency)] of Panipenem RS
- \(Q_t\): Amount [μg (potency)] of Panipenem

**Internal standard solution**—A solution of sodium p-styrenesulfonate in 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 1000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silicone polymer coated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 8.0 and acetonitrile (50:1).

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 12 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, panipenem and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of panipenem to that of the internal standard is not more than 2.0%.

**Containers and storage** Containers—Tight containers.

**Pantethine**

パンテチン

\[\text{C}_{22}\text{H}_{42}\text{N}_{4}\text{O}_{8}\text{S}_{2}: 554.72\]

\[\text{Bis}(2\text{-}[3\text{-}\{2\text{R}\}-2,4\text{-dihydroxy}-3\text{-}\text{dimethylbutanoylamino}\text{propanoylamino}\text{ethyl}] \text{ disulfide} [16816-67-4]\]

Pantethine is an aqueous solution containing 80% of pantethine.

Pantethine contains not less than 98.0% of pantethine (C_{22}H_{42}N_{4}O_{8}S_{2}), calculated on the anhydrous basis.

**Description** Pantethine is a clear, colorless to pale yellow viscous liquid.

It is miscible with water, with methanol and with ethanol (95). It is decomposed by light.

**Identification** (1) To 0.7 g of Pantethine add 5 mL of sodium hydroxide TS, shake, and add 1 to 2 drops of copper (II) sulfate TS: a blue-purple color develops.

(2) To 0.7 g of Pantethine add 3 mL of water, shake, add 0.1 g of copper powder and 2 mL of acetic acid (100), and boil for 2 to 3 minutes. After cooling, add 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red-purple color develops.

(3) To 1.0 g of Pantethine add 500 mL of water, and shake. To 5 mL of this solution add 3 mL of 1 mol/L hydrochloric acid TS, and heat on a water bath for 30 minutes. After cooling, add 7 mL of a solution of hydroxylammonium chloride in sodium hydroxide TS (3 in 140), and allow to stand for 5 minutes. Add 3 drops of 2,4-dinitrophenol TS, and add 1 mol/L hydrochloric acid TS dropwise until the solution has no color, and then add 1 mL of iron (III) chloride TS: a red-purple color develops.

**Optical rotation** <2.49 [α]_D^25: +15.0° - +18.0° (1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).
**Purity (1)** Heavy metals \(< 0.07\)—Proceed with 2.0 g of Pantethine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.1\)—Prepare the test solution with 2.0 g of Pantethine according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.6 g of Pantethine in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.07\). Spot 2 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with 2-butanone saturated with water to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand for about 10 minutes in iodide vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(4) Mercapto compounds—To 1.5 g of Pantethine add 20 mL of water, shake, add 1 drop of ammonia TS and 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red color is not developed.

**Water** \(<2.48\) 18 - 22% (0.2 g, volumetric titration, direct titration).

**Residue on Ignition** \(<2.49\) Not more than 0.1% (2 g).

**Assay** Weigh accurately about 0.3 g of Pantethine, add water to make exactly 20 mL. Transfer exactly 5 mL of this solution in an iodine bottle, and add exactly 25 mL of 0.05 mol/L bromine VS and 100 mL of water. Add 5 mL of diluted sulfuric acid (1 in 5) rapidly, stopper tightly immediately, and warm at 40 to 50°C for 15 minutes with occasional shaking. After cooling, carefully add 5 mL of a solution of potassium iodide (2 in 5), then immediately stopper tightly, shake, add 100 mL of water and titrate \(<2.50\) the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination.

\[
\text{Each mL of 0.05 mol/L bromine VS} \\
= 5.547 \text{ mg of C}_{22}\text{H}_{42}\text{N}_{4}\text{O}_{8}\text{S}_{2}
\]

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding 10°C.

---

### Papaverine Hydrochloride

パバベリン塩酸塩

\[
\text{C}_{20}\text{H}_{21}\text{NO}_{4}\text{HCl}: 375.85 \\
6,7-\text{Dimethoxy}-1-(3,4-\text{dimethoxybenzyl})isouquinoline monohydrochloride [61-25-6]
\]

Papaverine Hydrochloride, when dried, contains not less than 98.5% of \(\text{C}_{20}\text{H}_{21}\text{NO}_{4}\text{HCl}\).

**Description** Papaverine Hydrochloride occurs as white crystals or crystalline powder.

It is sparingly soluble in water and in acetic acid (100), slightly soluble in ethanol (95), and practically insoluble in acetic anhydride and in diethyl ether.

The pH of a solution of Papaverine Hydrochloride (1 in 50) is between 3.0 and 4.0.

**Identification (1)** To 1 mg of Papaverine Hydrochloride add 1 drops of formaldehyde-sulfuric acid TS: a colorless to light yellow-green color is produced, and it gradually changes to deep red, then to brown.

(2) Dissolve 0.02 g of Papaverine Hydrochloride in 1 mL of water, and add 3 drops of sodium acetate TS: a white precipitate is produced.

(3) Dissolve 1 mg of Papaverine Hydrochloride in 3 mL of acetic anhydride and 5 drops of sulfuric acid, heat in a water bath for 1 minute, and examine under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

(4) Dissolve 0.1 g of Papaverine Hydrochloride in 10 mL of water, make alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and filter. Evaporate the filtrate on a water bath, and dry the residue at 105°C for 3 hours: the residue so obtained melts \(<2.60\) between 145°C and 148°C.

(5) Alkalify a solution of Papaverine Hydrochloride (1 in 50) with ammonia TS, and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to Qualitative Tests \(<1.09\) (2) for chloride.

**Purity (1)** Clarity and color of solution—Dissolve 0.10 g of Papaverine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Morphine—Dissolve 10 mg of Papaverine Hydrochloride in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrate (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.
Papaverine Hydrochloride Injection

Papaverine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of papaverine hydrochloride (C_{20}H_{21}NO_4.HCl: 375.85).

Method of preparation Prepare as directed under Injections, with Papaverine Hydrochloride.

Description Papaverine Hydrochloride Injection is a clear, colorless liquid.

pH: 3.0–5.0

Identification (1) To 1 mL of Papaverine Hydrochloride Injection add 3 drops of sodium acetate TS: a white precipitate is produced.

(2) Dilute a volume of Papaverine Hydrochloride Injection, equivalent to 0.1 g of papaverine hydrochloride (C_{20}H_{21}NO_4.HCl), with water to 50 mL, and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 37.59 mg of C_{20}H_{21}NO_4.HCl

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Paraffin

Paraffin is a mixture of solid hydrocarbons obtained from petroleum.

Description Paraffin occurs as a colorless or white, more or less transparent, crystalline mass. It is odorless and tasteless.

It is sparingly soluble in diethyl ether and practically insoluble in water, in ethanol (95) and in ethanol (99.5).

Specific gravity d_{20}^{20} about 0.92 (proceed as directed in 4.2. in 4. Specific gravity under Fats and Fatty Oils Test《1.13》).

Identification (1) Heat Paraffin strongly in a porcelain dish, and ignite: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 g of Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Melting point <2.60> 50–75°C (Method 2).

Purity (1) Acidity or alkalinity—Boil 10.0 g of Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS in a water bath for 5 minutes, and shake vigorously: a red color is not produced. Add 0.20 mL of 0.02 mol/L sodium hydroxide VS to this solution, and shake: a red color is not produced.

Add 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 18.79 mg of C_{30}H_{62}NO_4.HCl

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.
of Paraffin according to Method 3, and perform the test (not more than 2 ppm).

(4) Sulfur compounds—To 4.0 g of Paraffin add 2 mL of ethanol (99.5), further add 2 drops of a clear saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and heat for 10 minutes at 70°C with occasional shaking: no dark brown color develops in the aqueous layer.

(5) Readily carbonizable substances—Melt 5.0 g of Paraffin placed in a Nessler tube at a temperature near the melting point. Add 5 mL of sulfuric acid for readily carbonizable substances, and warm at 70°C for 5 minutes in a water bath. Remove the tube from the water bath, immediately shake vigorously and vertically for 3 seconds, and warm for 1 minute in a water bath at 70°C. Repeat this procedure five times: the color of the sulfuric acid layer is not darker than that of the following control solution.

Control solution: Add 1.5 mL of Cobalt (II) Chloride CS, 0.5 mL of Copper (II) Sulfate CS and 5 mL of liquid paraffin to 3.0 mL of Iron (III) Chloride CS, and shake vigorously.

Containers and storage Containers—Well-closed containers.

Liquid Paraffin
流動パラフィン

Liquid Paraffin is a mixture of liquid hydrocarbons obtained from petroleum.

Tocopherols of a suitable form may be added at a concentration not exceeding 0.001% as a stabilizer.

Description Liquid Paraffin is a colorless, transparent, oily liquid, nearly free from fluorescence. It is odorless and tasteless.

It is freely soluble in diethyl ether, very slightly soluble in ethanol (99.5), and practically insoluble in water and in ethanol (95%).

Boiling point: above 300°C.

Identification (1) Heat Liquid Paraffin strongly in a porcelain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 of Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Specific gravity <2.56> \( \rho_{20^\circ} : 0.860 - 0.890 \)

Viscosity <2.55> Not less than 37 mm²/s (Method 1, 37.8°C).

Purity (1) Odor—Transfer a suitable amount of Liquid Paraffin to a small beaker, and heat on a water bath: a foreign odor is not perceptible.

(2) Acidity or alkalinity—Shake vigorously 10 mL of Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals <1.07>—Ignite 2.0 g of Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Arsenic <1.17>—Prepare the test solution with 1.0 g of Liquid Paraffin, according to Method 3 except that after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5), add 1.5 mL of hydrogen peroxide (30), fire to burn, and perform the test (not more than 0.2 ppm).

(5) Solid paraffin—Transfer 50 mL of Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(6) Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

(7) Polycyclic aromatic hydrocarbons—Take 25 mL of Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution obtained as the sample solution. Transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to another 50-mL separator, shake vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution thus obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

(8) Readily carbonizable substances—Transfer 5 mL of Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the Liquid Paraffin layer remains unchanged in color, and the sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Iron (III) Chloride CS
Light Liquid Paraffin

Light Liquid Paraffin is a mixture of liquid hydrocarbons obtained from petroleum.

To cophorels of a suitable form may be added at a concentration not exceeding 0.001% as a stabilizer.

Description Light Liquid Paraffin is a clear, colorless oily liquid, nearly free from fluorescence. It is odorless and tasteless.

It is freely soluble in diethyl ether, and practically insoluble in water and in ethanol (95%).

Boiling point: above 300°C.

Identification (1) Heat Light Liquid Paraffin strongly in a porcelain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 of Light Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Specific gravity <2.56> \( \rho \pm 0.830 \sim 0.870 \)

Viscosity <2.53> Less than 37 mm²/s (Method 1, 37.8°C).

Purity (1) Odor—Transfer a suitable amount of Light Liquid Paraffin to a small beaker, and heat on a water bath: no foreign odor is perceptible.

(2) Acidity or alkalinity—Shake vigorously 10 mL of Light Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals <1.07>—Ignite 2.0 g of Light Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Light Liquid Paraffin according to Method 3, and perform the test (not more than 2 ppm).

(5) Solid paraffin—Transfer 50 mL of Light Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(6) Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Light Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

(7) Polycyclic aromatic hydrocarbons—Take 25 mL of Light Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as the sample solution. Separately, transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to a 50-mL separator, add 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

(8) Readily carbonizable substances—Transfer 5 mL of Light Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the liquid paraffin layer remains unchanged in color, and sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Iron (III) Chloride CS with 1.5 mL of Cobalt (II) Chloride CS and 0.50 mL of Copper (II) Sulfate CS.

Containers and storage Containers—Tight containers.

Paraformaldehyde

Paraformaldehyde contains not less than 95.0% of \( \text{CH}_2\text{O}_n \). The poly(oxyethylene) \( [30525-89-4] \)

Paraformaldehyde occurs as a white powder. It has a slight odor of formaldehyde, but a very strong irritating odor is perceptible when it is heated.

It is practically insoluble in water, in ethanol but in diethyl ether.

It dissolves in hot water, in hot dilute hydrochloric acid, in
sodium hydroxide TS and in ammonia TS.

It sublimes at about 100°C.

Identification (1) Dissolve 0.1 g of Paraformaldehyde in 5 mL of ammonia TS, add 5 mL of silver nitrate TS, shake, and add 3 mL of a solution of sodium hydroxide (1 in 10): a mirror of metallic silver is immediately formed on the sides of the container.

(2) Add a solution of 0.04 g of salicylic acid in 5 mL of sulfuric acid to 0.02 g of Paraformaldehyde, and warm slowly: a persistent, dark red color is produced.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Paraformaldehyde in 10 mL of ammonia TS: the solution is clear and colorless.

(2) Acidity or alkalinity—To 0.5 g of Paraformaldehyde add 10 mL of water, shake vigorously for 1 minute, and filter: the filtrate is neutral.

(3) Chloride <1.03>—Dissolve 1.5 g of Paraformaldehyde in 75 mL of water and 7.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize with diluted nitric acid (3 in 10), and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 7.5 mL of sodium carbonate TS, a volume of diluted nitric acid (3 in 10) required for neutralization of the sample, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.006%).

(4) Sulfate <1.14>—Dissolve 1.5 g of Paraformaldehyde in 45 mL of water and 4.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize the diluted hydrochloric acid (3 in 5), and boil for 5 minutes. After cooling, add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 4.5 mL of sodium carbonate TS add an equal volume of diluted hydrochloric acid (3 in 5) for the neutralization of the sample and 15 mL of water, and boil for 5 minutes. After cooling, add 0.35 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.011%).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve about 50 mg of Paraformaldehyde, accurately weighed, in 10 mL of potassium hydroxide TS in an iodine flask. Add 40 mL of water and an exactly measured 50 mL of 0.05 mol/L iodine VS, stopper, and allow to stand for 5 minutes. Then add 5 mL of dilute hydrochloric acid, stopper immediately, allow to stand for 15 minutes, and titrate <2.30> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 1.501 mg of CH₂O

Containers and storage Containers—Tight containers.

Dental Paraformaldehyde Paste

歯科用パラホルムパスタ

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraformaldehyde, finely powdered</td>
<td>35 g</td>
</tr>
<tr>
<td>Procaine Hydrochloride, finely powdered</td>
<td>35 g</td>
</tr>
<tr>
<td>Hydrous Lanolin</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 100 g

Prepare as directed under Ointments, with the above ingredients.

Description Dental Paraformaldehyde Paste is yellowish white in color. It has a characteristic odor.

Identification (1) To 0.15 g of Dental Paraformaldehyde Paste add 20 mL of diethyl ether and 20 mL of 0.5 mol/L sodium hydroxide TS, shake well, separate the water layer, and dilute with water to make 100 mL. To 1 mL of this solution add 10 mL of acetylacetone TS, and heat on a water bath for 10 minutes: a yellow color is produced (paraformaldehyde).

(2) To the diethyl ether layer obtained in (1) add 5 mL of dilute hydrochloric acid and 20 mL of water, shake well, and separate the water layer: the solution responds to Qualitative Tests <1.09> for primary aromatic amines (procaine hydrochloride).

(3) To 0.15 g of Dental Paraformaldehyde Paste add 25 mL of diethyl ether and 25 mL of water, shake, separate the water layer, filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g of procaine hydrochloride in 5 mL of water, and use this solution as standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): spots from the sample solution and standard solution show the same Rf value.

Containers and storage Containers—Tight containers.
Parnaparin Sodium

パルナパリンナトリウム

Parnaparin Sodium is a low-molecular heparin sodium obtained by depolymerization, with hydrogen peroxide and with copper (II) acetate, of heparins sodium from the healthy edible porcine intestinal mucosa. The mass-average molecular mass ranges between 4500 and 6400.

The potency is not less than 70 low-molecular-mass-heparin units and not more than 95 low-molecular-mass-heparin units of anti-factor Xa activity per milligram calculated with reference of the dried substance.

**Description** Parnaparin Sodium occurs as a white or light yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

**Identification (1)** Mix 0.1 mL of a solution of Parnaparin Sodium (1 in 20) and 10 mL of a solution of tritoluidine blue O (1 in 100,000), and shake the mixture: the blue color of solution immediately changes to purple.

(2) A solution of Parnaparin Sodium (1 in 20) responds to Qualitative Tests <1.09> for sodium salt.

**pH** <2.54> Dissolve 0.1 g of Parnaparin Sodium in 10 mL of water: the pH of this solution is between 6.0 and 8.0.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Parnaparin Sodium in 10 mL of water: the solution is clear and colorless or pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Parnaparin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <2.41> Not more than 8.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

**Molecular mass** Calculate the molecular mass of Parnaparin Sodium by the following methods: The mass-average molecular mass ranges between 4500 and 6400.

(i) Creation of calibration curve—Weigh 20 mg of low-molecular mass heparin for calibration of molecular mass, and dissolve it in 2.0 mL of the mobile phase as the standard solution. Perform the test with 50 μL of the standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine the peak height, $H_{UV}$, in chromatogram obtained by the ultraviolet absorption photometer, and determine the peak height, $H_R$, in chromatogram obtained by the differential refractometer. Calculate the ratio of $H_{UV}$ to $H_R$, $H_{UV}/H_R$, at each peak. Assume the molecular mass in the 4th peak from the low-molecular mass in chromatogram obtained by the ultraviolet absorption photometer as 2400, and make the calculation of the standard coefficient from dividing 2400 by the $H_R/H_{UV}$ at the corresponding peak. Make the calculation to multiply the $H_R/H_{UV}$ at each peak by the standard coefficient, and determine the molecular mass of each peak by the calculation. Prepare the calculation curve by plotting the logarithm of molecular masses at each peak on the vertical axis and the retention time on the chromatogram obtained by the differential refractometer on the horizontal axis.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm) and a differential refractometer.

Column: Connect two stainless steel columns which are 7.5 mm in inside diameter and 30 cm in length, and are packed with porous silica gel for liquid chromatography; one column, the molecular mass of limited size exclusion is about 500,000; the other, the molecular mass of limited size exclusion is about 100,000. Connect a pump, the about 500,000-molecular mass of limited size exclusion column, the about 100,000-molecular mass of limited size exclusion column, the ultraviolet absorption photometer and the differential refractometer in this order.

**Column temperature**; A constant temperature of about 40°C.

Mobile phase: Dissolve 28.4 g of sodium sulfate anhydride in 1000 mL of water, and 5.0 with 0.05 mol/L sulfuric acid TS.

Flow rate: 0.5 mL per minute.

**System suitability**—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, confirm that more than ten peaks in chromatogram obtained as directed under either the Ultraviolet-visible Spectrophotometry, or the Differential Refractometry are observed.

System repeatability: When the tests repeated 6 times with 50 μL of the standard solution under the above operating conditions, relative standard deviation of the 4th peak height in chromatogram ($H_{UV}$ and $H_R$) is not more than 3.0%.

(ii) Determination of molecular mass—Dissolve the 20 mg of Parnaparin Sodium with 2.0 mL of mobile phase, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions. Divide the main peak observed between 30 min and 45 min to 30 sec-interval fractions, and determine the strength of differential refractometer of each 30 sec-interval fraction. Determine the molecular mass of each fraction using the calibration curve and the retention time of each fraction. Determine the mean of molecular mass in the entire peak using the strength of differential refractometer and the molecular mass in every fractions.

Mean molecular mass of parnaparin sodium

$$\frac{\sum (n_i \cdot M_i)}{\sum n_i}$$
\( n \): The differential refractometer strength of fraction i in the main peak of chromatogram
\( M_f \): Molecular mass of fraction i in main peak

**Operating conditions—**

Detector: A differential refractometer.

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in (i) Creation of calibration curve.

**System suitability—**

Proceed as directed in (i) Creation of calibration curve.

**Distribution of molecular mass**

The molecular mass of Parnaparin Sodium is calculated as directed in the determination of molecular mass and the distribution of molecular mass is calculated by the following equation: the molecular mass of not less than 80% parnaparin sodium is between 1500 and 10,000.

\[
\text{Distribution of molecular mass (\%)} = \left( \frac{\Sigma n_i}{\Sigma n_0} \right) \times 100
\]

\( n_i \): The differential refractometer strength of fraction i in the main peak of chromatogram
\( \Sigma n_i \): Sum of differential refractometer strength in the each fraction between 1500 and 10,000 molecular mass in the main peak

**The degree of sulfate ester**

Dissolve 0.5 g of Parnaparin Sodium with 10 mL water. Treat the solution with 5 mL of a strongly basic ion exchange resin, and subsequently with 10 mL of a strongly acidic ion exchange resin. Dilute the solution with water to 50 mL, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Calculate the degree of sulfate ester of Parnaparin Sodium from the equivalence point by the following equation; it is between 2.0 and 2.4.

The degree of sulfate ester

\[
= \frac{\text{the first equivalence point (mL)} - \text{the second equivalence point (mL)}}{\text{first equivalence point (mL)}}
\]

**Total nitrogen**

Weigh accurately about 0.10 g of Parnaparin Sodium which is dried, and perform the test as directed under Nitrogen Determination (\textless;0.05): it contains not less than 1.9% and not more than 2.3% of nitrogen (N:14.01).

**Anti-factor IIa activity**

Determine the potency of anti-factor IIa activity of Parnaparin Sodium according to the following method, it contains not less than 35 and not more than 60 low-molecular-mass-heparin unit per milligram calculated with reference to the dried substance.

(i) Standard solution

Dissolve Low-molecular Mass Heparin RS with isotonic sodium chloride solution to make solutions which contain 0.1, 0.2 and 0.3 low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL, respectively.

(ii) Sample solution

Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it with isotonic sodium chloride solution to adjust the solution which contains 4 \( \mu \)g parnaparin sodium in 1 mL.

(iii) Procedure

To each plastic tube add 0.10 mL of the sample solution and the standard solution, separately. To each tube add 0.10 mL of human normal plasma and mix, and incubate at 37 ± 1°C accurately for 1 minute. Next, to each test tube add 0.10 mL of activated thromboplastin-time assay solution, which is pre-warmed at 37 ± 1°C, and after the mixing incubate accurately for 5 minutes at 37 ± 1°C. Then, to each tube add 0.10 mL of sodium calcium solution (277 in 100,000) which is pre-warmed at 37 ± 1°C, mix, start a stop watch simultaneously, and permit to stand at the same temperature. Determine the time for the first appearance of fibrin clot.

(iv) Calculation

Determine the low-molecular-mass-heparin unit (anti-factor IIa activity) of the sample solution from calibration curve obtained plots of clotting times for each standard solution; calculate the low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium as following equation.

The low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium

\[
= \frac{\text{the low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL of sample solution}}{\text{b/a}}
\]

\( a \): Amount (mg) of Parnaparin Sodium
\( b \): The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution

**The ratio of anti-factor Xa activity to anti-factor IIa activity**

Divide the anti-factor Xa activity, obtained in the Assay, by the anti-factor IIa activity which has been obtained from the test according to the method of anti-factor IIa activity; the ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.5 and 2.5.

**Assay**

(i) Standard solution

Dissolve Low-molecular Mass Heparin RS in isotonic sodium chloride solution to make solutions which contain 0.4, 0.6 and 0.8 low-molecular-mass-heparin units (anti-factor Xa activity) in 1 mL, respectively.

(ii) Sample solution

Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it in isotonic sodium chloride solution to make a solution which contains 7 \( \mu \)g parnaparin sodium in 1 mL.

(iii) Procedure

To each plastic tube add 0.10 mL of either the sample solution or the standard solution, separately. Subsequently to the every tubes add 0.70 mL of diluted acetic acid (100) (1 in 2). Determine the absorbance of the mixing incubate accurately for 5 minutes at 37 ± 1°C. Then, to each tube add 0.10 mL of chromogenic synthetic substrate solution (3 in 4000) and mix it, and subsequently incubate accurately for 3 minutes at 37 ± 1°C. Next, to each tube add 0.10 mL of factor Xa TS and mix it, permit to stand 37 ± 1°C accurately for 30 seconds, and immediately add 0.20 mL of chromogenic synthetic substrate solution (3 in 4000) and mix it, and subsequently incubate accurately for 5 min at 37 ± 1°C. To each test tube add 0.30 mL of diluted acetic acid (100) solution (1 in 2) to stop the reaction. Separately, to plastic tube add 0.10 mL of isotonic sodium chloride solution, 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin III TS, and 0.10 mL of normal human plasma, and mix them. To another plastic tube transfer 0.20 mL of these solutions, separately, and incubate for accurate 3 minutes at 37 ± 1°C. Next, to each tube add 0.10 mL of factor Xa TS and mix it, permit to stand 37 ± 1°C accurately for 30 seconds, and immediately add 0.20 mL of chromogenic synthetic substrate solution (3 in 4000) and mix it, and subsequently incubate accurately for 5 min at 37 ± 1°C. To each test tube add 0.30 mL of diluted acetic acid (100) solution (1 in 2) to stop the reaction. Separately, to plastic tube add 0.10 mL of isotonic sodium chloride solution, 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin III TS, and 0.10 mL of normal human plasma to every tubes, and mix well. To another plastic tube transfer 0.2 mL of the solution, separately, and add both 0.30 mL of water and 0.30 mL of diluted acetic acid (100) (1 in 2). Determine the absorbance of both the sample solution and the standard solution at 405 nm as directed under Ultraviolet-visible Spectrophotometry (<2.24) using a solution obtained from this solution as the blank.

(iv) Calculation method

Determine the low-molecular-mass unit (anti-factor Xa activity) of the sample solution
using the calibration curve prepared from the absorbance of the standard solutions and their logarithmic concentrations, and calculate the low-molecular-mass unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium.

Low-molecular-mass-heparin unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium

\[ \text{activity} = \frac{a}{b} \]

where:

- \( a \): Amount (mg) of Parnaparin Sodium
- \( b \): The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution

**Container and Storage**

- Container—Well-closed containers.

**Peanut Oil**

*Oleum Arachidis*

ラッカセイ油

Peanut Oil is the fixed oil obtained from the seeds of *Arachis hypogaea* Linné (*Leguminosae)*.

**Description**

- Peanut Oil is a pale yellow, clear oil. It is odorless or has a slight odor. It has a mild taste.
- It is miscible with diethyl ether and with petroleum ether.
- It is slightly soluble in ethanol (95).
- Specific gravity: \( d^15 = 0.909 \sim 0.916 \)
- Congealing point of the fatty acids: 22 – 33°C

**Identification**

- Saponify 5 g of Peanut Oil by boiling with 2.5 mL of sodium hydroxide solution (3 in 10) and 12.5 mL of ethanol (95). Evaporate the ethanol, dissolve the residue in 50 mL of hot water, and add dilute hydrochloric acid in excess until the free fatty acids separate as an oily layer. Cool the mixture, remove the separated fatty acids, and dissolve them in 75 mL of diethyl ether. To the diethyl ether solution add a solution of 4 g of lead (II) acetate trihydrate in 40 mL of ethanol (95), and allow the mixture to stand for 18 hours. Filter the supernatant liquid, transfer the precipitate to the filter with the aid of diethyl ether, and filter by suction. Place the precipitate in a beaker, heat it with 40 mL of dilute hydrochloric acid and 20 mL of water until the oily layer is entirely clear, cool, and decant the water layer. Boil the fatty acids with 50 mL of dilute hydrochloric acid (1 in 100). When the solution prepared by dissolving 0.5 g of Parnaparin Sodium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 20 ppm).

**Purity**

(1) Clarity and color of solution—A solution obtained by dissolving 0.5 g of Parnaparin Potassium in 10 mL of water is clear and colorless.

(2) Heavy metals —Proceed with 0.5 g of Parnaparin Potassium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 20 ppm).

**Related substances**—Dissolve 50 mg of Parnaparin Potassium in 50 mL of a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 100 mL. To exactly 2.5 mL of this solution add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and standard solution as
directed under Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than pemirolast obtained from the sample solution is not larger than the peak area of pemirolast from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 9 times as long as the retention time of pemirolast.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 25 mL. Confirm that the peak area of pemirolast obtained with 10 μL of this solution is equivalent to 15 to 25% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pemirolast is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Water Not more than 0.5% (0.1 g, coulometric titration).

Assay Weigh accurately about 50 mg each of Pemirolast Potassium and Pemirolast Potassium RS (separately determine the water in the same manner as Pemirolast Potassium), dissolve in a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 50 mL. Pipet 5 mL of each of these solutions, add exactly 5 mL of the internal standard solution to each, then add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q₁ and Q₅, of the peak area of pemirolast to that of the internal standard.

\[
\text{Amount (mg) of } C_{10}H_{7}KN_{6}O = M_5 \times \frac{Q_1}{Q_5}
\]

\[
M_5: \text{Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis}
\]

Internal standard solution—A solution of ethyl aminobenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, methanol and acetic acid (100) (30:20:1).

Flow rate: Adjust the flow rate so that the retention time of pemirolast is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pemirolast and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pemirolast to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Pemirolast Potassium for Syrup

シロップ用ペミロラストカリウム

Pemirolast Potassium for Syrup is a preparation for syrup, which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pemirolast potassium (C₁₀H₇KN₆O: 266.30).

Method of preparation Prepare as directed under Preparations for Syrups, with Pemirolast Potassium.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 255 nm and 259 nm and between 355 nm and 359 nm.

pH Being specified separately.

Uniformity of dosage units Perform the test according to the following method: Pemirolast Potassium for Syrup in single-unit containers meet the requirement of the Content uniformity test.

Dissolve the total amount of the content of 1 container of Pemirolast Potassium for Syrup in water to make exactly V mL so that each mL contains about 50 μg of pemirolast potassium (C₁₀H₇KN₆O). Pipet 10 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of pemirolast potassium (C₁₀H₇KN₆O)} = M_5 \times \frac{A_T}{A_S} \times \frac{V}{400}
\]

\[
M_5: \text{Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis}
\]

Assay Powder Pemirolast Potassium for Syrup. Weigh accurately a portion of the powder, equivalent to about 5 mg of pemirolast potassium (C₁₀H₇KN₆O), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pemirolast Potassium RS in the same manner as Pemirolast Potassium, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this
solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_1 \) and \( A_5 \), at 357 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.24 \).

\[
\text{Amount (mg) of pemirolast potassium (C}_{10}\text{H}_{7}\text{KN}_{6}\text{O}) = M_S \times A_1/A_S \times 1/4 \\
M_S: \text{Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis}
\]

Containers and storage  Containers—Tight containers. Storage—Light-resistant.

**Pemirolast Potassium Tablets**

ペミロラストカリウム錠

Pemirolast Potassium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pemirolast potassium (\( \text{C}_{10}\text{H}_{7}\text{KN}_{6}\text{O} \): 266.30).

**Method of preparation**  Prepare as directed under Tablets, with Pemirolast Potassium.

**Identification**  Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.24 \): it exhibits maxima between 255 nm and 259 nm, and between 355 nm and 359 nm.

**Uniformity of dosage units**  \( \leq 6.02 \)  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Pemirolast Potassium Tablets add 50 mL of water for 5 mg of pemirolast potassium (\( \text{C}_{10}\text{H}_{7}\text{KN}_{6}\text{O} \)), and shake to disintegrate the tablet completely. Then, add water to make exactly \( V \) mL so that each mL contains about 50 \( \mu \)g of pemirolast potassium (\( \text{C}_{10}\text{H}_{7}\text{KN}_{6}\text{O} \)), and filter. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of pemirolast potassium (C}_{10}\text{H}_{7}\text{KN}_{6}\text{O}) = M_S \times A_1/A_S \times V/400 \\
M_S: \text{Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis}
\]

Dissolution  \( \leq 6.10 \)  When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 5.0 as the dissolution medium, the dissolution rate in 45 minutes of a 5-mg tablet is not less than 75%, and that in 60 minutes of a 10-mg tablet is not less than 70%.

Start the test with 1 tablet of Pemirolast Potassium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \)m. Discard the first 10 mL of the filtrate, pipet \( V' \) mL of the subsequent filtrate, and add the dissolution medium to make exactly \( V' \) mL so that each mL contains about 5.6 \( \mu \)g of pemirolast potassium (\( \text{C}_{10}\text{H}_{7}\text{KN}_{6}\text{O} \)) according to the labeled amount. Pipet 4 mL of this solution, add exactly 2 mL of diluted potassium hydroxide TS (1 in 10), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Pemirolast Potassium RS (separately determine the water \( \leq 2.48 \) in the same manner as Pemirolast Potassium), dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL. Pipet 4 mL of this solution, add exactly 2 mL of diluted potassium hydroxide TS (1 in 10), and use this solution as the standard solution. Then, proceed as directed in the Assay.

\[
\text{Dissolution rate (%) with respect to the labeled amount of pemirolast potassium (C}_{10}\text{H}_{7}\text{KN}_{6}\text{O}) = M_S \times A_1/A_S \times V'/V \times 1/C \times 18 \\
M_S: \text{Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis} \\
C: \text{Labeled amount (mg) of pemirolast potassium (C}_{10}\text{H}_{7}\text{KN}_{6}\text{O}) in 1 tablet}
\]

**Assay**  Accurately weigh the mass of not less than 20 Pemirolast Potassium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of pemirolast potassium (\( \text{C}_{10}\text{H}_{7}\text{KN}_{6}\text{O} \)), add 50 mL of water, shake thoroughly for 20 minutes, then add water to make exactly 100 mL. Filter, discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pemirolast Potassium RS (separately determine the water \( \leq 2.48 \) in the same manner as Pemirolast Potassium), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_1 \) sand \( A_5 \), at 357 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.24 \), using water as the blank.

\[
\text{Amount (mg) of pemirolast potassium (C}_{10}\text{H}_{7}\text{KN}_{6}\text{O}) = M_S \times A_1/A_S \times V'/V \\
M_S: \text{Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis}
\]

Containers and storage  Containers—Tight containers. Storage—Light-resistant.
Penbutolol Sulfate
ペンブトロール硫酸塩

\[
\begin{align*}
\text{(C}_{18}\text{H}_{29}\text{NO}_{2})_2\text{H}_2\text{SO}_4: 680.94} \\
\text{(2S)-(2-Cyclopentylphenox)-1-(1,1-dimethyllethyl)aminopropan-2-ol hemisulfate [38363-32-5]} \\
\end{align*}
\]

Penbutolol Sulfate, when dried, contains not less than 98.5\% of \((\text{C}_{18}\text{H}_{29}\text{NO}_{2})_2\text{H}_2\text{SO}_4\).

**Description** Penbutolol Sulfate occurs as a white crystalline powder.

It is very soluble in acetic acid (100), freely soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in acetic anhydride and in diethyl ether.

**Identification (1)** Determine the absorption spectrum of a solution of Penbutolol Sulfate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** \(<2.49>\) [\(\alpha\)]: \(-23 - -25^\circ\) (after drying, 0.2 g, methanol, 20 mL; 100 mm).

**Melting point** \(<2.60>\) 213 – 217°C

**Purity (1)** Heavy metals \(<1.07>\)—Proceed with 2.0 g of Penbutolol Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Arsenic** \(<1.11>\)—Prepare the test solution with 1.0 g of Penbutolol Sulfate according to Method 4, and perform the test (not more than 2 ppm).

**Related substances**—Dissolve 0.8 g of Penbutolol Sulfate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Prepare the test with these solutions as directed under Thin-layer Chromatography \(<2.05>\). Dot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, ethanol (95) and ammonia solution (28) (85:12:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.41>\) Not more than 0.5\% (0.5 g, 105°C, 3 hours).

**Residue on ignition** \(<2.44>\) Not more than 0.2\% (1 g).

**Assay** Weigh accurately about 0.8 g of Penbutolol Sulfate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate \(<2.50>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS \(= 68.09\) mg of \((\text{C}_{18}\text{H}_{29}\text{NO}_{2})_2\text{H}_2\text{SO}_4\).

**Containers and storage** Containers—Well-closed containers.

Pentazocine
ペンタゾシン

\[
\begin{align*}
\text{C}_{19}\text{H}_{27}\text{NO}: 285.42} \\
\text{(2RS,6RS,11RS)-6,11-Dimethyl-3-(3-methylbut-2-en-1-yl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzoazocin-8-ol [359-83-1]} \\
\end{align*}
\]

Penazocine, when dried, contains not less than 99.0\% of \(\text{C}_{19}\text{H}_{27}\text{NO}\).

**Description** Pentazocine occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in chloroform, soluble in ethanol (95), sparingly soluble in diethyl ether and practically insoluble in water.

**Identification (1)** To 1 mg of Pentazocine add 0.5 mL of formaldehyde-sulfuric acid TS: a deep red color is produced, and it changes to grayish brown immediately.

**Dissolve 5 mg of Pentazocine in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and heat in a water bath for 2 minutes: the color of the solution changes from light yellow to deep yellow. Shake the solution with 1 drop of nitric acid: the solution remains yellow in color.

**Determine the absorption spectrum of a solution of Pentazocine in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Absorbance** \(<2.24>\) \(E_{1cm}^1\) (278 nm): 67.5 – 71.5 (after drying, 0.1 g, 0.01 mol/L hydrochloric acid TS, 1000 mL).
Pentobarbital Calcium / Official Monographs

**Melting point** 2.60° 150 – 158°C

**Purity** (1) Clarity and color of solution—Dissolve 0.10 g of Pentazocine in 20 mL of 0.1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Heavy metals 1.07—Proceed with 1.0 g of Pentazocine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic 1.17—Prepare the test solution with 1.0 g of Pentazocine according to Method 3, and perform the test with a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Pentazocine in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.05. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and isopropylamine (94:3:3) to a distance of about 13 cm, and air-dry the plate. Allow to stand for 5 minutes in iodine vapor: any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** 2.41—Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 5 hours).

**Residue on ignition** 2.44—Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.5 g of Pentazocine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate 2.50 with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 28.54 mg of C₁₉H₂₇NO = 28.54 mg of C₂₂H₃₄CaN₄O₆

Containers and storage Containers—Well-closed containers.

**Pentobarbital Calcium**

ペントバルビタールカルシウム

![Chemical Structure](image)

C₂₂H₃₄CaN₄O₆· 490.61
Monocalcium bis[(5-ethyl-5-{[(1S)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydroprymidin-2-olate] [76-74-4, Pentobarbital]

Pentobarbital Calcium contains not less than 98.0% and not more than 102.0% of C₂₂H₃₄CaN₄O₆, calculated on the dried basis.

**Description** Pentobarbital Calcium occurs as a white powder.

It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in acetone.

A solution of Pentobarbital Calcium (1 in 100) shows no optical rotation.

**Identification** (1) Determine the infrared absorption spectrum of Pentobarbital Calcium as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 1 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, shake with 5 mL of dilute hydrochloric acid and 10 mL of water, allow to cool, and filter. To the filtrate add 1 drop of methyl red TS, and add ammonia TS until a slight yellow color develops: the solution responds to Qualitative Tests 1.099 (1), (2) and (3) for calcium salt.

**Purity** (1) Chloride 1.07—To 1.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 2.5 mL of dilute nitric acid, dissolve by warming with shaking, cool, add water to make 50 mL, shake well, and filter. Discard the first 10 mL of the filtrate, and to 15 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 1.5 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.035%).

(2) Heavy metals 1.07—To 2.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, cool, add water to make 80 mL, shake well, and filter. Discard the first 10 mL of the filtrate, to 40 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 2.5 mL of ethanol (95) add 2.5 mL of dilute hydrochloric acid and water to make 50 mL. Add 1 drop of phenolphthalein TS, add dropwise ammonium TS until a pale red color develops, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 2.5 mL of ethanol (95) add 2.5 mL of dilute hydrochloric acid and water to make 30 mL. Add 1 drop of phenolphthalein TS, add dropwise ammonium TS until a pale red color develops, then add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Pentobarbital Calcium in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.05. according to the following conditions, and determine the areas of each peak by the automatic integration method: the area of any peak other than the peak of pentobarbital from the sample solution is not larger than 3/10 times the peak area of pentobarbital from the standard solution, and the total of these peak areas is not larger than the peak area of pentobarbital from the standard solution.

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the
retention time of pentobarbital beginning after the solvent peak.

**System suitability**—

Test for required detection: Pipet 2 mL of the standard solution, add water to make exactly 20 mL, and confirm that the peak area of pentobarbital obtained from 20 μL of this solution is equivalent to 5 to 15% of that from 20 μL of the standard solution.

System performance: Proceed as directed in the system performance in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pentobarbital is not more than 5%.

**Loss on drying</2.4>** Not more than 7.0% (1 g, 105°C, 5 hours).

**Assay**—Weigh accurately about 20 mg of Pentobarbital Calci um, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 18 mg of Pentobarbital RS, previously dried at 105°C for 2 hours, dissolve in 10 mL of acetonitrile, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0> according to the following conditions, and calculate the ratios, Q₁ and Q₃, of the peak area of pentobarbital to that of the internal standard.

\[
M₂: \text{Amount (mg) of Pentobarbital RS} = M₃ \times \frac{Q₁}{Q₃} \times 1.084
\]

\[
M₃: \text{Amount (mg) of C₄₂H₅₄CaN₄O₆}
\]

**Internal standard solution**—Dissolve 0.2 g of isopropyl parahydroxybenzoate in 20 mL of acetonitrile, and add water to make 100 mL.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust to pH 4.0 with diluted phosphoric acid (1 in 10). To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pentobarbital is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pentobarbital and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pentobarbital to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Well-closed containers.

### Pentoxyverine Citrate

#### Carbetapentane Citrate

#### Carbetapentene Citrate

ペントキシベリンクエン酸塩

C₂₀H₃₁NO₃.C₆H₈O₇: 525.59
2-[2-(Diethylamino)ethoxy]ethyl 1-phenylcyclopentanecarboxylate monocitrate [23142-01-0]

Pentoxyverine Citrate, when dried, contains not less than 98.5% of C₂₀H₃₁NO₃.C₆H₈O₇.

**Description**—Pentoxyverine Citrate occurs as a white, crystalline powder.

It is very soluble in acetic acid (100), freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

**Identification**—(1) Dissolve 0.1 g of Pentoxyverine Citrate in 10 mL of water, and add 10 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Pentoxyverine Citrate, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pentoxyverine Citrate (1 in 10) responds to Qualitative Tests <1.09> (1) and (2) for citrate.

**Melting point</2.60> 92 – 95°C

**Purity**—(1) Clarity and color of solution—Dissolve 1.0 g of Pentoxyverine Citrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals</1.07>—Proceed with 2.0 g of Pentoxyverine Citrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic</1.11>—Prepare the test solution with 1.0 g of Pentoxyverine Citrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Pentoxyverine Citrate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 15 μL each of the sample solution and standard solution on a
Peplomycin Sulfate

Peplomycin Sulfate is the sulfate of a substance having antitumor activity produced by the growth of *Streptomyces verticillus*.

It contains not less than 865 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Peplomycin Sulfate is expressed as mass (potency) of peplomycin (C_{61}H_{88}N_{18}O_{21}S_{2}: 1473.59).

Description Peplomycin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95%).

It is hygroscopic.

Identification (1) To 4 mg of Peplomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Peplomycin Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 10 mg each of Peplomycin Sulfate and Peplomycin Sulfate RS in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL of each of these solutions as directed under Liquid Chromatography (2.015) according to the following conditions: the retention time of the principal peak obtained from the sample solution is the same as that from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase stock solution, mobile phase A, mobile phase B, flow of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3).

(3) A solution of Peplomycin Sulfate (1 in 200) responds to Qualitative Tests (1.09) (1) and (2) for sulfate.

Optical rotation 2.49 [α]_{D} = −2.2 ± 5° (0.1 g calculated on the dried basis, 0.1 mol/L phosphate buffer solution, pH 5.3, 10 mL, 100 mm).

pH 2.54 The pH of a solution obtained by dissolving 0.10 g of Peplomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 80 mg of Peplomycin Sulfate in 4 mL of water: the solution is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Peplomycin Sulfate in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to 5.0 mL of Standard Copper Stock Solution add diluted nitric acid (1 in 100) to make exactly 100 mL. To 3.0 mL of this solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry (2.23) according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: Copper hollow cathode lamp.

Wavelength: 324.8 nm.

(3) Related substances—Dissolve about 10 mg of Peplomycin Sulfate in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography (2.017) according to the following conditions. Determine the areas of the peaks, appeared after the peak of copper sulfate, by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than peplomy-
cin is not more than 7.0%.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 60</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>60 – 75</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 1.2 mL per minute.

Time span of measurement: As long as 20 minutes after elution of peplomycin beginning after the peak of copper sulfate.

**System suitability**—
Test for required detectability: Measure exactly 1 mL of the sample solution, add water to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of peplomycin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of peplomycin are not less than 30,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of peplomycin is not more than 2.0%.

**Loss on drying** <2.47> Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Handle the sample avoiding absorption of moisture.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Mycobacterium smegmatis* ATCC 607
(ii) Agar media for base and seed layer, and for transferring test organism

<table>
<thead>
<tr>
<th></th>
<th>Glycerin</th>
<th>Peptone</th>
<th>Meat extract</th>
<th>Sodium chloride</th>
<th>Agar</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.0 g</td>
<td>10.0 g</td>
<td>10.0 g</td>
<td>3.0 g</td>
<td>15.0</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(iii) Liquid medium for suspending test organism

<table>
<thead>
<tr>
<th></th>
<th>Glycerin</th>
<th>Peptone</th>
<th>Meat extract</th>
<th>Sodium chloride</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.0 g</td>
<td>10.0 g</td>
<td>10.0 g</td>
<td>3.0 g</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(iv) Preparation of agar medium of seeded layer—Inoculate the test organism onto the slant of the agar medium for transferring test organism, and incubate the slant at 27°C for 40 to 48 hours.

Inoculate the subcultured test organism into 100 mL of the liquid medium for suspending test organism, inoculate at 25 to 27°C for 5 days while shaking, and use this suspension as the suspension of the test organism. Keep the suspension of the test organism at a temperature of not exceeding 5°C and use within 14 days. Add 0.5 mL of the suspension of the test organism in 100 mL of the Agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the agar medium of seeded layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 1.7. Preparation of cylinder-agar plates with the exception of the amounts of the agar medium for base layer and the agar medium of seeded layer to put in the Petri dish, which are 5.0 mL and 8.0 mL, respectively.

(vi) Standard solutions—Weigh accurately an amount of Peplomycin Sulfate RS, equivalent to about 20 mg (potency), and use this solution as the high concentration standard solution and the low concentration standard solution, respectively.

Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C, and use within 15 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the high concentration standard solution and the low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Peplomycin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL.

**Containers and storage** Containers—Tight containers.
Peplomycin Sulfate for Injection

Peplomycin Sulfate for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled amount of peplomycin \((C_{61}H_{88}N_{18}O_{21}S_{2}: 1473.59)\).

Method of preparation Prepare as directed under Injections, with Peplomycin Sulfate.

Description Peplomycin Sulfate for Injection occurs as white light masses or powder.

Identification Take an amount of Peplomycin Sulfate for Injection, equivalent to 10 mg (potency) of Peplomycin Sulfate according to the labeled amount, and dissolve in 15 \(\mu\)L of Copper (II) sulfate TS and water to make 2 mL. Apply this solution to the column (prepared by filling a 15 mm inside diameter and 15 cm long chromatography tube with 15 mL of strongly basic ion exchange resin (Cl type) for column chromatography \((75 – 150\ \text{mm} \times 15\ \text{cm})\) and run off. Then wash the column using water at 2.5 mL per minute, collect about 30 mL of the effluent. Add water to the effluent to make 250 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \((\lambda_{\text{max}} 222, 243\ \text{nm})\): it exhibits maxima between 242 nm and 246 nm, and between 291 nm and 295 nm. Further determine the absorbances \(A_1\) and \(A_2\), at 243 nm and 293 nm, respectively: the ratio \(A_1/A_2\) is 1.20 to 1.30.

Osmotic pressure ratio Being specified separately.

pH \(<2.5\) The pH of a solution prepared by dissolving an amount of Peplomycin Sulfate for Injection, equivalent to 50 mg (potency) of Peplomycin Sulfate according to the labeled amount, in 10 mL of water is 4.5 to 6.0.

Purity Clarity and color of solution—A solution prepared by dissolving an amount of Peplomycin Sulfate for Injection, equivalent to 10 mg (potency) of Peplomycin Sulfate according to the labeled amount, in 10 mL of water is clear and colorless.

Loss on drying \(<2.4\) Not more than 4.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Perform the sampling preventing from moisture absorption.

Bacterial endotoxins \(<0.01\) Less than 1.5 EU/mg (potency).

Uniformity of dosage units \(<6.0\) It meets the requirement of the Mass variation test.

Foreign insoluble matter \(<6.0\) Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter \(<6.0\) It meets the requirement.

Sterility \(<4.0\) Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics \(<4.0\) according to the following conditions.

(i) Test organism, culture medium, liquid medium for suspending test organisms, preparation of seeded agar layer, preparation of cylinder-agar plate and the standard solutions—Proceed as directed in the Assay under Peplomycin Sulfate.

(ii) Sample solutions—Weigh accurately the mass of the contents of not less than 10 containers of Peplomycin Sulfate for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Peplomycin Sulfate, dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8, to make exactly 100 mL. Measure exactly a suitable quantity of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 so that each mL contains 4 \(\mu\)g (potency) and 2 \(\mu\)g (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Perphenazine

ペルフェナジン

\[C_{21}H_{26}ClN_3OS: \text{403.97}\]

2-[4-[3-(2-Chloro-10H-phenothiazin-10-yl)propyl]piperazin-1-yl]ethanol

[i] Perphenazine, when dried, contains not less than 98.5% of \(C_{21}H_{36}ClIN_3OS\).

Description Perphenazine occurs as white to light yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in methanol and in ethanol (95), soluble in acetic acid (100), sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Identification (1) Dissolve 5 mg of Perphenazine in 5 mL of sulfuric acid: a red color, changing to deep red-purple upon warming, is produced.

(2) Dissolve 0.2 g of Perphenazine in 2 mL of methanol, add this solution to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and allow to stand for 4 hours. Collect the crystals, wash with a small volume of methanol, and dry at 105°C for 1 hour: the crystals so obtained melt \(\langle 260\rangle\) between 237°C and 244°C (with decomposition).

(3) Determine the absorption spectrum of a solution of Perphenazine in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry \((\lambda_{\text{max}} 222\)\), and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Perphenazine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the
same wavelengths. Separately, to 10 mL of the solution add 10 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Perphenazine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Perphenazine as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.66> 95 – 100°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Perphenazine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Perform the test in the current of nitrogen in light-resistant containers under the protection from sunlight. Dissolve 0.10 g of Perphenazine in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.67>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and 1 mol/L ammonia TS (5:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the sample solution is not more intense than that from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 65°C, 4 hours).

Residue on ignition <2.43> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Perphenazine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-purple to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 20.20 mg of C_{21}H_{26}ClN_{3}O_{5}S

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Perphenazine Tablets

ペルフェナジン錠

Perphenazine Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of perphenazine (C_{21}H_{26}ClN_{3}O_{5}S: 403.97).

Method of preparation Prepare as directed under Tablets, with Perphenazine.

Identification (1) Shake well a quantity of powdered Perphenazine Tablets, equivalent to 25 mg of Perphenazine according to the labeled amount, with 10 mL of methanol, and filter. Evaporate 2 mL of the filtrate on a water bath to dryness. With the residue, proceed as directed in the Identification (1) under Perphenazine.

(2) Add 5 mL of the filtrate obtained in the Identification (1) to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and proceed as directed in the Identification (2) under Perphenazine.

(3) Determine the absorption spectrum of the filtrate obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 309 nm and 313 nm. Add 30 mL of methanol to another 10 mL of the filtrate, and determine the absorption spectrum: it exhibits a maximum between 256 nm and 260 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 Perphenazine Tablet by shaking with 5 mL of water, shake well with 70 mL of methanol, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add methanol to make exactly 250 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Separately, weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum over phosphorus (V) oxide at 65°C for 4 hours, dissolve in methanol to make exactly 250 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_{1} and A_{2}, of the sample solution and standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of perphenazine (C_{21}H_{26}ClN_{3}O_{5}S) = M_{s} \times A_{1} / A_{2} \times V' / V' \times 1 / 25

M_{5}: Amount (mg) of Perphenazine RS

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 90 minutes of Perphenazine Tablets is not less than 70%.

Start the test with 1 tablet of Perphenazine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum with phosphorus (V) oxide at 65°C for 4 hours, dissolve in 5 mL of 0.1 mol/L hydrochloric acid TS, and add the dissolution medium to make exactly 250 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_{1} and A_{2}, of the sample solution and standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Perphenazine Tablets in 90 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of perphenazine (C_{21}H_{26}ClN_{3}O_{5}S) = M_{s} \times A_{1} / A_{2} \times 1 / C \times 36

M_{5}: Amount (mg) of Perphenazine RS
Perphenazine Maleate

Perphenazine Maleate, when dried, contains not less than 98.0% of \( \text{C}_{21}\text{H}_{26}\text{ClN}_{3}\text{OS} \cdot 2\text{C}_{4}\text{H}_{4}\text{O}_{4} \).

**Description** Perphenazine Maleate occurs as a white to light yellow powder. It is odorless.

It is sparingly soluble in acetic acid (100), slightly soluble in water and in ethanol (95), and practically insoluble in chloroform.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Melting point: about 175°C (with decomposition).

**Identification**

1. Dissolve 8 mg of Perphenazine Maleate in 5 mL of sulfuric acid: a red color is produced, which becomes deep-red-purple on warming.

2. Dissolve 0.3 g of Perphenazine Maleate in 3 mL of dilute hydrochloric acid, add 2 mL of water and 3 mL of ammonia solution (28), shake, and extract with three 10-mL portions of chloroform. Reserve the aqueous layer and use for test (5). Evaporate the combined chloroform extracts on a water bath to dryness, dissolve the residue in 20 mL of methanol, and pour into 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25). Allow to stand for 4 hours, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour; the crystals melt \(< 220°C\) between 237°C and 244°C (with decomposition).

3. Determine the absorption spectrum of a solution of Perphenazine Maleate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry \(< 2.24\)\), and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to 10 mL of the solution add 30 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \(< 2.24\)\), and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

4. Perform the test with Perphenazine Maleate as directed under Flame Coloration Test \(< 1.04\> (2): a green color appears.

5. Evaporate the aqueous layer reserved in (2) to dryness. To the residue add 1 mL of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate in a water bath at about 35°C with the aid of a current of air: the residue melts \(< 2.60°C\) between 128°C and 136°C.

**Purity**

(1) Heavy metals \(< 1.07\>—Proceed with 2.0 g of perphenazine maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(< 1.11\>—Prepare the test solution with 1.0 g of Perphenazine Maleate according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying** \(< 2.41\> — Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** \(< 2.44\> — Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Perphenazine Maleate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate \(< 2.50\> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction. Each mL of 0.1 mol/L perchloric acid VS is equivalent to 31.81 mg of \( \text{C}_{21}\text{H}_{26}\text{ClN}_{3}\text{OS} \cdot 2\text{C}_{4}\text{H}_{4}\text{O}_{4} \).

**Containers and storage** Containers—Well-closed containers. Storage—Light-resistant.
**Identification** (1) Shake a quantity of powdered Perphenazine Maleate Tablets, equivalent to 0.04 g of Perphenazine Maleate according to the labeled amount, with 3 mL of dilute hydrochloric acid and 30 mL of water, centrifuge, filter the supernatant liquid, add 3 mL of ammonia solution to the filtrate, and extract with three 10-mL portions of chloroform. [Reserve the aqueous layer, and use for test (4).] Wash the combined chloroform extracts with two 5-mL portions of water, and separate the chloroform layer. Evaporate 6 mL of the chloroform solution on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Perphenazine Maleate.

(2) Evaporate 20 mL of the chloroform solution obtained in (1) on a water bath to dryness, dissolve the residue in 20 mL of methanol, and filter, if necessary. Warm the filtrate, add 5 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), allow to stand for 4 hours, and proceed as directed in the Identification (2) under Perphenazine Maleate.

(3) To 2 mL of the filtrate obtained in the Assay add water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 253 nm and 257 nm and between 303 nm and 313 nm.

(4) Filter, if necessary, the aqueous layer reserved in (1), evaporate the filtrate to make about 5 mL, add 2 mL of dilute sulfuric acid, and extract with two 10-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate on a water bath to dryness, dissolve the residue in 5 mL of sulfuric acid TS, and add 1 to 2 drops of potassium permanganate TS: the red color of potassium permanganate TS fades immediately.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 tablet of Perphenazine Maleate Tablets by shaking with 15 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously with 30 mL of methanol, add water to make exactly 100 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add water to make exactly 5 mL of a solution containing about 6 μg of perphenazine maleate \((C_{21}H_{26}ClN_3OS.2C_4H_4O_4)\) in each mL, and use this solution as the sample solution. Separately, weigh accurately 30 mg of perphenazine maleate for assay, previously dried at 105 °C for 3 hours, dissolve in 15 mL of 0.1 mol/L hydrochloric acid TS and 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 3 mL of 0.1 mol/L hydrochloric acid TS, 10 mL of methanol and water to make exactly 250 mL, and use this solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), of the sample solution and the standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

\[
\text{Amount (mg) of perphenazine maleate} = M_S \times \frac{A_T}{A_S} \times \frac{V}{V} \times \frac{1}{50}
\]

\(M_S:\) Amount (mg) of perphenazine maleate for assay

**Dissolution** <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Perphenazine Maleate Tablets is not less than 70%.

Conduct this procedure without exposure to light. Start the test with 1 tablet of Perphenazine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add the dissolution medium to make exactly \(V\) mL so that each mL contains about 3.5 μg of perphenazine maleate \((C_{21}H_{26}ClN_3OS.2C_4H_4O_4)\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of perphenazine maleate for assay, previously dried at 105 °C for 3 hours, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, and add the dissolution medium to make exactly 200 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>:

\[
\text{Dissolution rate (%) with respect to the labeled amount} = M_S \times \frac{A_T}{A_S} \times \frac{V}{V} \times \frac{1}{C} \times 45/4
\]

**Assay** Weigh accurately and powder not less than 20 Perphenazine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 40 mg of perphenazine maleate \((C_{21}H_{26}ClN_3OS.2C_4H_4O_4)\), shake well with 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, measure exactly 5 mL of the subsequent filtrate, add water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of perphenazine maleate for assay, previously dried at 105 °C for 3 hours, dissolve in 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the standard solution. Determine the absorbances, \(A_T\) and \(A_S\), of the sample solution and the standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

\[
\text{Amount (mg) of perphenazine maleate} = M_S \times \frac{A_T}{A_S}
\]

\(M_S:\) Amount (mg) of perphenazine maleate for assay

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.
Adsorbed Purified Pertussis Vaccine

Adsorbed Purified Pertussis Vaccine is a liquid for injection prepared by adding an aluminum salt to a liquid containing the protective antigen of Bordetella pertussis to make the antigen insoluble.

It conforms to the requirements of Adsorbed Purified Pertussis Vaccine in the Minimum Requirements for Biological Products.

Description Adsorbed Purified Pertussis Vaccine forms a homogeneous, white turbidity on shaking.

Pethidine Hydrochloride

Operidine

Pethidine Hydrochloride, when dried, contains not less than 98.0% of C₁₅H₂₁NO₂.HCl.

Description Pethidine Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water and in acetic acid (100), freely soluble in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution dissolved 1.0 g of Pethidine Hydrochloride in 20 mL of water is between 3.8 and 5.8.

Identification (1) Determine the absorption spectrum of a solution of Pethidine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pethidine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25> \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pethidine Hydrochloride (1 in 50) responds to Qualitative Tests \( <1.09> (2) \) for chloride.

Melting point \( <2.60> \) 187 – 189°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pethidine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate \( <1.14> \) —Perform the test with 0.20 g of Pethidine Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

(3) Related substances—Dissolve 0.05 g of Pethidine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01> \) according to the following conditions. Determine each peak area obtained from both solutions by the automatic integration method: the total area of the peaks other than pethidine from the sample solution is not larger than the peak area of pethidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of pethidine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of pethidine obtained from 20 \( \mu \)L of this solution is equivalent to 5 to 15% of that from 20 \( \mu \)L of the standard solution.

System performance: To 2 mL each of the sample solution and a solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 50,000) add the mobile phase to make 10 mL. When the procedure is run with 20 \( \mu \)L of this solution according to the above operating conditions, pethidine and isoamyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pethidine is not more than 2.0%.

Loss on drying \( <2.41> \) Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition \( <2.44> \) Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Pethidine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate \( <2.50> \) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.
Bacterial endotoxins

It exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Method of preparation

Prepare as directed under Injection, with Pethidine Hydrochloride.

Description

Pethidine Hydrochloride Injection is a clear, colorless liquid.

Identification

Take a volume of Pethidine Hydrochloride Injection equivalent to 0.1 g of Pethidine Hydrochloride according to the labeled amount, and add water to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Bacterial endotoxins

Less than 6.0 EU/mg.

Extractable volume

It meets the requirement.

Foreign insoluble matter

Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter

It meets the requirement.

Sterility

Perform the test according to the Membrane filtration method: it meets the requirement.

Assay

Measure exactly a volume of Pethidine Hydrochloride Injection, equivalent to about 0.1 g of pethidine hydrochloride (C₁₅H₂₁NO₂·HCl: 283.79), and add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pethidine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL of the sample solution and standard solution as directed under Liquid Chromatography: according to the following conditions, and calculate the ratios, Q₁ and Q₅, of the peak area of pethidine to that of the internal standard.

Amount (mg) of pethidine hydrochloride (C₁₅H₂₁NO₂·HCl)

= Mₛ × Q₁/Q₅

Mₛ: Amount (mg) of pethidine hydrochloride for assay

Internal standard solution—A solution of isomyl parahydroxybenzoate in the mobile phase (1 in 12,500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pethidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pethidine to that of the internal standard is not more than 1.0%.

Containers and storage

Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Hydrophilic Petrolatum

Method of preparation

White Beeswax

80 g

Stearyl Alcohol or Cetanol

30 g

Cholesterol

30 g

White Petrolatum

a sufficient quantity

To make 1000 g

Melt and mix Stearyl Alcohol or Cetanol, White Beeswax and White Petrolatum on a water bath. Add Cholesterol, and melt completely by stirring. Stop warming, and stir until the mixture congeals.

Description

Hydrophilic Petrolatum is white in color. It has a slight, characteristic odor.

When mixed with an equal volume of water, it retains the consistency of ointment.

Containers and storage

Containers—Tight containers.
White Petrolatum

White Petrolatum is a decolorized and purified mixture of hydrocarbons obtained from petroleum.

**Description** White Petrolatum is a white to pale yellow, homogeneous, unctuous mass. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in ethanol (99.5).

It dissolves in diethyl ether making a clear liquid or producing slight insoluble substances.

It becomes a clear liquid when warmed.

**Melting point** <2.60> 38 – 60°C (Method 3).

**Purity** (1) Color—Melt White Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: Add 3.4 mL of water to 1.6 mL of Iron (III) Chloride CS.

(2) Acidity or alkalinity—To 35.0 g of White Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the White Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of White Petrolatum according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of White Petrolatum, according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(5) Sulfur compound—To 4.0 g of White Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

(6) Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, and then 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

(7) Fats and fatty oils or resins—To 10.0 g of White Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.

**Residue on ignition** <2.44> Not more than 0.05% (2 g).

**Containers and storage** Containers—Tight containers.

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Yellow Petrolatum

Yellow Petrolatum is a purified mixture of hydrocarbons obtained from petroleum.

**Description** Yellow Petrolatum occurs as a yellow, homogeneous, unctuous mass. It is odorless and tasteless.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It dissolves in diethyl ether, in petroleum benzine and in turpentine oil, making a clear liquid or producing slight insoluble substances.

It becomes a yellow, clear liquid with slight fluorescence when warmed.

**Melting point** <2.60> 38 – 60°C (Method 3).

**Purity** (1) Color—Melt Yellow Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: To 3.8 mL of Iron (III) Chloride CS add 1.2 mL of Cobalt (II) Chloride CS.

(2) Acidity or alkalinity—To 35.0 g of Yellow Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the Yellow Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Yellow Petrolatum according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Yellow Petrolatum, according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(5) Sulfur compound—To 4.0 g of Yellow Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

(6) Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, and then 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

(7) Fats and fatty oils or resins—To 10.0 g of Yellow Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.
Residue on ignition  <2.44>  Not more than 0.05% (2 g).
Containers and storage  Containers—Tight containers.

Petroleum Benzin
石油ベンジン

Petroleum Benzin is a mixture of low-boiling point hydrocarbons from petroleum.

Description  Petroleum Benzin occurs as a colorless, clear, volatile liquid. It shows no fluorescence. It has a characteristic odor.
   It is miscible with ethanol (99.5) and with diethyl ether.
   It is practically insoluble in water.
   It is very flammable.
Specific gravity  $d_{20}^0$: 0.65 – 0.71

Purity  (1) Acid—Shake vigorously 10 mL of Petroleum Benzin with 5 mL of water for 2 minutes, and allow to stand: the separated aqueous layer does not change moistened blue litmus paper to red.
   (2) Sulfur compounds and reducing substances—To 10 mL of Petroleum Benzin add 2.5 mL of ammonia-ethanol TS and 2 to 3 drops of silver nitrate TS, and warm the mixture at about 50°C for 5 minutes, protected from light: no brown color develops.
   (3) Fatty oil and sulfur compounds—Drop and evaporate 10 mL of Petroleum Benzin in small portions on odorless filter paper spread on a previously warmed glass plate: no spot or no foreign odor is perceptible.
   (4) Benzene—Warm 5 drops of Petroleum Benzin with 2 mL of sulfuric acid and 0.5 mL of nitric acid for about 10 minutes, allow to stand for 30 minutes, transfer the mixture to a porcelain dish, and dilute with water: no odor of nitrobenzene is perceptible.
   (5) Residue on evaporation—Evaporate 140 mL of Petroleum Benzin on a water bath to dryness, and heat the residue at 105°C to constant mass: the mass is not more than 1 mg.
   (6) Readily carbonizable substances—Shake vigorously 5 mL of Petroleum Benzin with 5 mL of sulfuric acid for readily carbonizable substances for 5 minutes in a Nessler tube, and allow to stand: the sulfuric acid layer has no more color than Matching Fluid A.

Distilling range  <2.57>  50 – 80°C, not less than 90 vol%.
Containers and storage  Containers—Tight containers.
   Storage—Remote from fire, and not exceeding 30°C.

Phenethicillin Potassium
フェネチシリンカリウム

C$_{17}$H$_{19}$KN$_2$O$_5$S: 402.51
Monopotassium (2S,5R,6R)-3,3-dimethyl-7-oxo-6-[(2RS)-2-phenoxypropanoylamino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate
[132-93-4]

Phenethicillin Potassium contains not less than 1400 units and not more than 1480 units per mg, calculated on the dried basis. The potency of Phenethicillin Potassium is expressed as unit based on the amount of phenethicillin potassium (C$_{17}$H$_{19}$KN$_2$O$_5$S). One unit of Phenethicillin Potassium is equivalent to 0.68 μg of phenethicillin potassium (C$_{17}$H$_{19}$KN$_2$O$_5$S).

Description  Phenethicillin Potassium occurs as a white to light yellowish white crystalline powder.
   It is freely soluble in water, and slightly soluble in ethanol (99.5).

Identification  (1) Determine the absorption spectrum of a solution of Phenethicillin Potassium (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
   (2) Determine the infrared absorption spectrum of Phenethicillin Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
   (3) Phenethicillin Potassium responds to Qualitative Tests <1.09> (1) for potassium salt.

Optical rotation  <2.49>  [α]$_D^15$: +217 – +244° (1 g calculated on the dried basis, phosphate TS, 100 mL, 100 mm).

L-α-Phenethicillin potassium  Dissolve about 50 mg of Phenethicillin Potassium in the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_D$ and $A_L$, of p-α-phenethicillin and L-α-phenethicillin by the automatic integration method: $A_D/(A_D + A_L)$ is between 0.50 and 0.70.

Operating conditions—
   Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
   Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadeccysilanized silica gel for liquid chromatography (5 μm in particle diameter).
   Column temperature: A constant temperature of about 30°C.
   Mobile phase: Adjust the pH of a mixture of a solution of
from the standard solution.

Other than D-phenethicillin, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of L-phenethicillin is about 25 minutes.

**System suitability**

- **System performance:** When the procedure is run with 10 µL of the sample solution under the above operating conditions, D-phenethicillin and L-phenethicillin are eluted in this order with the resolution between these peaks being not less than 1.5.
- **System repeatability:** When the test is repeated 6 times with 10 µL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of L-phenethicillin is not more than 2.0%.

**Purity (1)**

- Heavy metals $<1.0%$—Proceed with 1.0 g of Phenethicillin Potassium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).
- **Arsenic (2)**—Prepare the test solution with 1.0 g of Phenethicillin Potassium according to Method 4 and, perform the test (not more than 2 ppm).
- **Related substances**—Dissolve 50 mg of Phenethicillin Potassium in 50 mL of the mobile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and determine each peak area by the automatic integration method: the total of the peak areas other than D-phenethicillin and L-phenethicillin obtained from the sample solution is not larger than 5 times the total of the peak areas of D-phenethicillin and L-phenethicillin from the standard solution.

**Operating conditions—**

- **Detector, column, column temperature, mobile phase, and flow rate:** Proceed as directed in the operating conditions in the L-phenethicillin potassium.
- **Time span of measurement:** About 1.5 times as long as the retention time of L-phenethicillin.

**System suitability**

- Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of L-phenethicillin obtained from 10 µL of this solution is equivalent to 14 to 26% of that from 10 µL of the standard solution.

**Assay**

Weigh accurately an amount of Phenethicillin Potassium equivalent to 0.1 g, in vacuum, at $60^\circ$C, 3 hours.

**Loss on drying** $<2.41>$ Not more than 1.0% (0.1 g, in vacuum, at $60^\circ$C, 3 hours).

**Chloride** $<1.0%$—Dissolve 0.30 g of Phenobarbital in 20 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the sample solution for exactly 15 minutes. Add 0.2 – 0.5 mL of starch TS, and titrate $<2.50>$ with 0.01 mol/L sodium thiosulfate VS until the color of the solution disappears. Separately, to exactly 2 mL each of the sample solution and standard solution add exactly 10 mL of 0.005 mol/L iodine VS, then proceed in the same manner as above allowing to stand for 15 minutes as a blank determination, and make any necessary correction. Determine the volumes, $V_T$ and $V_S$, of 0.005 mol/L iodine VS consumed in the sample solution and standard solution.

\[ \text{Amount (unit) of } C_{12}H_{12}N_2O_5S = M_S \times \frac{V_T}{V_S} \]

$M_S$: Amount (unit) of Phenethicillin Potassium RS

**Containers and storage**

Containers—Well-closed containers.

**Phenobarbital**

フェノバルビタール

![Phenobarbital structure](image)

$C_{12}H_{12}N_2O_5S$: 232.24
5-Ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione [50-06-6]

Phenobarbital, when dried, contains not less than 99.0% and not more than 101.0% of $C_{12}H_{12}N_2O_5S$.

**Description**

Phenobarbital occurs as white crystals or crystalline powder.

It is very soluble in $N,N$-dimethylformamide, freely soluble in ethanol (95) and in acetone, sparingly soluble in acetonitrile, and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

The pH of a saturated solution of Phenobarbital is between 5.0 and 6.0.

**Identification (1)**

Determine the absorption spectrum of a solution of Phenobarbital in boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $<2.24>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phenobarbital as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** $<2.60>$ 175 – 179°C

**Purity (1)**

Clarity and color of solution—Dissolve 0.5 g of Phenobarbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride $<1.0%$—Dissolve 0.30 g of Phenobarbital in 20 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the sample solution.
the test solution. Prepare the control solution as follows: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetic acid and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.035%).

(3) Heavy metals. Proceed with 1.0 g of Phenobarbital according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead solution (not more than 20 ppm).

(4) Phenylbarbituric acid. Boil 1.0 g of Phenobarbital with 5 mL of ethanol (95%) for 3 minutes: the solution is clear.

(5) Related substances. Dissolve 0.10 g of Phenobarbital in 100 mL of acetonitrile, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak other than phenobarbital obtained from the sample solution is not larger than the peak area of phenobarbital from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 45°C.
Mobile phase: A mixture of water and acetonitrile (11:9).
Flow rate: Adjust the flow rate so that the retention time of phenobarbital is about 5 minutes.
Time span of measurement: About 12 times as long as the retention time of phenobarbital, beginning after the solvent peak.
System suitability—
Test for required detectability: Pipet 5 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of phenobarbital obtained with 10 µL of this solution is equivalent to 20 to 30% of that with 10 µL of the standard solution.
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of phenobarbital are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phenobarbital is not more than 3.0%.
Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).
Residue on ignition <2.44> Not more than 0.1% (1 g).
Assay Weigh accurately about 0.5 g of Phenobarbital, previously dried, dissolve in 50 mL of N,N-dimethylformamide, and titrate <2.35d> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution change from yellow to yellow-green (indicator: 1 mL of alizarin yellow G-G-thymolphthalein TS). Perform a blank determination using a mixture of 50 mL of N,N-dimethylformamide and 22 mL of ethanol (95%), and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 23.22 mg of C12H12N2O3.

Containers and storage Containers—Well-closed containers.

**10% Phenobarbital Powder**

**Phenobarbital Powder**

フェノバルビタール散 10%

10% Phenobarbital Powder contains not less than 9.3% and not more than 10.7% of phenobarbital (C12H12N2O3: 232.24).

**Method of preparation**

<table>
<thead>
<tr>
<th>Phenobarbital</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 g Starch, Lactose Hydrate or their mixture</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Granules or Powders, with the above ingredients.

**Identification** (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 238 nm and 242 nm.

(2) To 6 g of 10% Phenobarbital Powder add 150 mL of ethanol, shake well, and filter. Condense the filtrate on a water bath to about 5 mL, add about 50 mL of water, filter to collect the formed crystals, and dry them at 105°C for 2 hours. Determine the infrared absorption spectrum of the crystals as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Dissolution** <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of 10% Phenobarbital Powder is not less than 80%.

Start the test with an accurately weighted about 0.3 g of 10% Phenobarbital Powder, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of phenobarbital for assay, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under
Ultraviolet-visible Spectrophotometry <2.24>, using a mixture of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and water (2:1) as the blank, and determine the absorbances, A1 and A5, at 240 nm.

Dissolution rate (%) with respect to the labeled amount of phenobarbital (C12H12N2O3) = M5/MF × A1/A5 × 1/C × 180

M5: Amount (mg) of phenobarbital for assay
M1: Amount (g) of 10% Phenobarbital Powder
C: Labeled amount (mg) of phenobarbital (C12H12N2O3) in 1 g

Assay Weigh accurately about 0.2 g of 10% Phenobarbital Powder, dissolve in a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL. Pipet 5 mL of this solution, add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of phenobarbital for assay, previously dried at 105°C for 2 hours, and add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL. Pipet 5 mL of this solution, add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 as the blank, and determine the absorbances, A1 and A5, at 240 nm.

Amount (mg) of phenobarbital (C12H12N2O3) = M5 × A1/A5

M5: Amount (mg) of phenobarbital for assay

Containers and storage Containers—Well-closed containers.

Phenol

Phenol / Official Monographs

Phenol for Disinfection

Carbolic Acid for Disinfection

Phenol contains not less than 98.0% of C6H6O.

Description Phenol occurs as colorless to slightly red crystals, crystalline masses, or liquid containing these crystals. It has a characteristic odor. It is very soluble in ethanol (95) and in diethyl ether, and freely soluble in water. Phenol for Disinfection (10 g) is liquefied by addition of 1 mL of water. It cauterizes the skin, turning it white. Congealing point: about 40°C

Identification (1) Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Phenol (1 in 100): a blue-purple color develops.

(2) Add bromine TS dropwise to 5 mL of a solution of Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

Purity (1) Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

(2) Residue on evaporation—Weigh accurately about 5 g of Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the mass of the sample.

Assay Dissolve about 1.5 g of Phenol, accurately weighed, in water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at once stopper the flask, and shake well. Add 1 mL of chloroform, stop the flask, and shake thoroughly. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS = 1.569 mg of C6H6O

Containers and storage Containers—Tight containers. Storage—Light-resistant.
of Phenol for Disinfection, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.10% of the mass of the sample.

**Assay** Dissolve about 1 g of Phenol for Disinfection, accurately weighed, in water to make exactly 1000 mL. Pipet 25 mL of the solution into an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes and allow to stand for 15 minutes. Add 7 mL of potassium iodide TS, stopper immediately, shake well, and titrate \(<2.50\) of the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS = 1.569 mg of C₆H₆O

Containers and storage  Containers—Tight containers.
Storage—Light-resistant.

**Liquefied Phenol**

*Liquefied Carbolic Acid*

液状フェノール

Liquefied Phenol is Phenol maintained in a liquid condition by the presence of 10% of Water, Purified Water or Purified Water in Containers. It contains not less than 88.0% of phenol (C₆H₆O: 94.11)

**Description** Liquefied Phenol is a colorless or slightly reddish liquid. It has a characteristic odor.

It is miscible with ethanol (95), with diethyl ether and with glycerin.

A mixture of equal volumes of Liquefied Phenol and glycerin is miscible with water.

The color changes gradually to dark red on exposure to light or air.

It cauterizes the skin, turning it white.

Specific gravity \(d_{20}^{20}\) about 1.065

**Identification (1)** Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Liquefied Phenol (1 in 100): a blue-purple color develops.

(2) Add bromine TS dropwise to 5 mL of a solution of Liquefied Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

**Boiling point** \(<2.57\) Not more than 182°C.

**Purity (1)** Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Liquefied Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

(2) Residue on evaporation—Weigh accurately about 5 g of Liquefied Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the mass of the sample.

**Assay** Dissolve about 1.7 g of Liquefied Phenol, accurately weighed, in a water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05 mol/L of bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at one stopper the flask tightly, and shake well. Add 1 mL of chloroform, stopper the flask, and shake thoroughly. Titrate \(<2.50\) the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS = 1.569 mg of C₆H₆O

Containers and storage  Containers—Tight containers.
Storage—Light-resistant.

**Dental Phenol with Camphor**

歯科用フェノール・カンフル

**Method of preparation**

<table>
<thead>
<tr>
<th>Phyton</th>
<th>dl-Camphor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>35 g</td>
</tr>
<tr>
<td>dl-Camphor</td>
<td>65 g</td>
</tr>
</tbody>
</table>

To make 100 g

Melt Phenol by warming, add dl-Camphor or dl-Camphor, and mix.

**Description** Dental Phenol with Camphor is a colorless or light red liquid. It has a characteristic odor.

Containers and storage  Containers—Tight containers.
Storage—Light-resistant.

**Phenol and Zinc Oxide Liniment**

フェノール・亜鉛華リニメント

**Method of preparation**

<table>
<thead>
<tr>
<th>Phyton</th>
<th>dl-Camphor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquefied Phenol</td>
<td>22 mL</td>
</tr>
<tr>
<td>Powdered Tragacanth</td>
<td>20 g</td>
</tr>
<tr>
<td>Carmellose Sodium</td>
<td>30 g</td>
</tr>
<tr>
<td>Glycerin</td>
<td>30 mL</td>
</tr>
<tr>
<td>Zinc Oxide</td>
<td>100 g</td>
</tr>
<tr>
<td>Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Mix Liquefied Phenol, Glycerin and Purified Water or Purified Water in Containers, add Powdered Tragacanth in small portions by stirring, and allow the mixture to stand overnight. To the mixture add Carmellose Sodium in small portions by stirring to make a pasty mass, add Zinc Oxide in small portions, and mix. Less than 5 g of Powdered Tragacanth or Carmellose Sodium can be replaced by each other to make 50 g in total.

**Description** Phenol and Zinc Oxide Liniment is a white, pasty mass. It has a slight odor of phenol.

**Identification (1)** Shake well 1 g of Phenol and Zinc Oxide Liniment with 10 mL of diethyl ether, and filter. To the filtrate add 10 mL of dilute sodium hydroxide TS, shake
well, and separate the water layer. To 1 mL of the water layer add 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, shake, and add 3 mL of sodium hydroxide TS: a yellow color develops (phenol).

(2) Place 1 g of Phenol and Zinc Oxide Liniment in a porcelain crucible, heat gradually raising the temperature until the content is charred, and then ignite it strongly: a yellow color develops, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate(II) TS: a white precipitate is produced (zinc oxide).

(3) Shake 0.5 g of Phenol and Zinc Oxide Liniment with 1 mL of water and 5 mL of chloroform, separate the chloroform layer, and use this solution as the sample solution. Separately, dissolve 0.01 g of phenol in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots obtained from the sample solution and the standard solution show the same Rf value.

Containers and storage Containers—Tight containers.

Phenolated Water

フェノール水

Phenolated Water contains not less than 1.8 w/v% and not more than 2.3 w/v% of phenol (C₆H₆O: 94.11).

Method of preparation

Liquefied Phenol 22 mL
Water, Purified Water or Purified Water in Containers a sufficient quantity

To make 1000 mL

Mix the above ingredients.

Description Phenolated Water is a colorless, clear liquid, having the odor of phenol.

Identification (1) Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water: a blue-purple color develops.

(2) To 5 mL of a solution of Phenolated Water (1 in 200) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

Assay Take exactly 2 mL of Phenolated Water into an iodine flask, add 25 mL of water, then add exactly 40 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes, and allow to stand for 15 minutes. Add 7 mL of potassium iodide TS, stopper tightly at once, shake well, and titrate 2.50 mL of the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS = 1.569 mg of C₆H₆O

Containers and storage Containers—Tight containers.

Phenolated Water for Disinfection

消毒用フェノール水

Phenolated Water for Disinfection contains not less than 2.8 w/v% and not more than 3.3 w/v% of phenol (C₆H₆O: 94.11).

Method of preparation

Phenol for Disinfection 31 g
Water, Purified Water or Purified Water in Containers a sufficient quantity

To make 1000 mL

Mix the above ingredients.

Description Phenolated Water for Disinfection is a clear, colorless liquid, having the odor of phenol.

Identification (1) Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water for Disinfection: a blue-purple color develops.

(2) Proceed with 5 mL of a solution of Phenolated Water for Disinfection (1 in 200) as directed in the Identification (2) under Phenol for Disinfection.

Assay Take exactly 5 mL of Phenolated Water for Disinfection, add water to make exactly 100 mL, then pipet 25 mL of the solution into an iodine flask, and proceed as directed in the Assay under Phenol for Disinfection.

Each mL of 0.05 mol/L bromine VS = 1.569 mg of C₆H₆O

Containers and storage Containers—Tight containers.

Phenolsulfonphthalein

フェノールスルホンフタレイン

Phenolsulfonphthalein occurs as a vivid red to dark red, crystalline powder.

It is very slightly soluble in water and in ethanol (95).

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Phenolsulfonphthalein in water, and dilute with chloroform to 10 mL. Add 0.05 mol/L hydrochloric acid VS dropwise to the solution, then add 0.05 mol/L potassium thiocyanate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L hydrochloric acid VS = 0.517 mg of C₁₉H₁₄O₅S

Containers and storage Containers—Tight containers.

Phenolsulfonphthalein, when dried, contains not less than 98.0% of C₁₉H₁₄O₅S.

Description Phenolsulfonphthalein occurs as a vivid red to dark red, crystalline powder.

It is very slightly soluble in water and in ethanol (95).

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Phenolsulfonphthalein in water, and dilute with chloroform to 10 mL. Add 0.05 mol/L hydrochloric acid VS dropwise to the solution, then add 0.05 mol/L potassium thiocyanate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L hydrochloric acid VS = 0.517 mg of C₁₉H₁₄O₅S
Phenolsulfonphthalein Injection

フェノールスルホンフタライン注射液

Phenolsulfonphthalein Injection is an aqueous solution for injection.

It contains not less than 0.54 w/v% and not more than 0.63 w/v% of phenolsulfonphthalein (C₁₉H₁₄O₅S: 354.38).

**Method of preparation**

<table>
<thead>
<tr>
<th>Phenolsulfonphthalein</th>
<th>Sodium Chloride</th>
<th>Sodium Bicarbonate (or Sodium Hydroxide)</th>
<th>Water for Injection or Sterile Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 g</td>
<td>9 g</td>
<td>1.43 g</td>
<td>0.68 g</td>
</tr>
</tbody>
</table>

Prepare as directed under Injections, with the above ingredients.

**Description** Phenolsulfonphthalein Injection is a clear, orange-yellow to red liquid.

**Identification** To 1 mL of Phenolsulfonphthalein Injection add 2 to 3 drops of sodium hydroxide TS, and proceed as directed in the Identification (1) under Phenolsulfonphthalein.

**pH** <2.54> 6.0 – 7.6

**Bacterial endotoxins** <4.01> Less than 7.5 EU/mg.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> Perform the test according to Method 2: it meets the requirement.

**Sensitivity** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Pipet 5 mL of Phenolsulfonphthalein Injection, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of phenolsulfonphthalein for assay, previously dried in a desiccator (silica gel) for 4 hours, and dissolve in a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, $A_f$ and $A_s$, of the sample solution and standard solution at 559 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

**Containers and storage** Containers—Well-closed containers.
1240  1-Phenylalanine / Official Monographs

Amount (mg) of phenolsulfonphthalein (C₁₉H₁₂O₂S)

\[ M_s = \frac{A_s}{A_f} \]

Mₙ: Amount (mg) of phenolsulfonphthalein for assay

Containers and storage  Containers—Hermetic containers.

1-Phenylalanine

L-フェニルアラニン

C₉H₁₁NO₂: 165.19
(2S)-2-Amino-3-phenylpropanoic acid [63-91-2]

L-Phenylalanine, when dried, contains not less than 98.5% of C₉H₁₁NO₂.

Description  L-Phenylalanine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification  Determine the infrared absorption spectrum of L-Phenylalanine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation  \( <2.49> \) [α]₀^D: \( -33.0 - -35.5^\circ \) (after drying, 0.5 g, water, 25 mL, 100 mm).

pH  \( <2.5> \) Dissolve 0.20 g of L-Phenylalanine in 20 mL of water: the pH of this solution is between 5.3 and 6.3.

Purity  (1) Clarity and color of solution—Dissolve 0.5 g of L-Phenylalanine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride  \( <1.03> \) Perform the test with 0.5 g of L-Phenylalanine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate  \( <1.14> \) Perform the test with 0.6 g of L-Phenylalanine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium  \( <1.02> \) Perform the test with 0.25 g of L-Phenylalanine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals  \( <1.07> \) Dissolve 1.0 g of L-Phenylalanine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic  \( <1.17> \) Dissolve 1.0 g of L-Phenylalanine in 5 mL of dilute hydrochloric acid and 15 mL of water, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Phenylalanine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying  \( <2.41> \) Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition  \( <2.44> \) Not more than 0.1% (1 g).

Assay  Weigh accurately about 0.17 g of L-Phenylalanine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate \( <2.50> \) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 16.52 mg of C₉H₁₁NO₂

Containers and storage  Containers—Tight containers.

Phenylbutazone

フェニルプタゾン

C₁₉H₂₃N₂O₂: 308.37
4-Butyl-1,2-diphenylpyrazolidine-3,5-dione [50-33-9]

Phenylbutazone, when dried, contains not less than 99.0% of C₁₉H₂₃N₂O₂.

Description  Phenylbutazone occurs as a white to slightly yellowish white, crystalline powder. It is odorless, and is at first tasteless but leaves a slightly bitter aftertaste.

It is freely soluble in acetone, soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification  (1) To 0.1 g of Phenylbutazone add 1 mL of acetic acid (100) and 1 mL of hydrochloric acid, and heat on a water bath under a reflux condenser for 30 minutes. Add 10 mL of water, and cool with ice water. Filter, and to the filtrate add 3 to 4 drops of sodium nitrite TS. To 1 mL of this solution add 1 mL of 2-naphthol TS and 3 mL of chloroform, and shake: a deep red color develops in the chloroform layer.
(2) Dissolve 1 mg of Phenylbutazone in 10 mL of dilute sodium hydroxide solution, and dilute with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry $<$2.24$>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point** $<$2.60$>$ 104 – 107°C

**Purity** (1) Clarity of solution—Dissolve 1.0 g of Phenylbutazone in 20 mL of sodium hydroxide solution (2 in 25), and allow to stand at 25 ± 1°C for 3 hours: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry $<$2.24$>$: it is not more than 0.05.

(2) Heavy metals $<$1.07$>$—Proceed with 2.0 g of Phenylbutazone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic $<$1.11$>$—Prepare the test solution with 1.0 g of phenylbutazone, according to Method 3, and perform the test (not more than 2 ppm).

(4) Readily carbonizable substances—Dissolve 1.0 g of Phenylbutazone in 20 mL of sulfuric acid, and allow to stand at 25 ± 1°C for exactly 30 minutes: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry $<$2.24$>$: it is not more than 0.10.

**Loss on drying** $<$2.41$>$ Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** $<$2.44$>$ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Phenylbutazone, previously dried, dissolve in 25 mL of acetone, and titrate $<$2.50$>$ with 0.1 mol/L sodium hydroxide VS until the solution shows a blue color which persists for 15 seconds (indicator: 5 drops of bromothymol blue TS). Perform a blank determination with a mixture of 25 mL of acetone and 16 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 30.84 mg of C$_{13}$H$_{13}$NO$_2$.HCl

**Containers and storage** Containers—Tight containers.

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**Phenylephrine Hydrochloride**

フェニレフィリン塩酸塩

C$_9$H$_{13}$NO$_2$.HCl: 203.67

(1R)-1-(3-Hydroxyphenyl)-2-methylaminoethanol monohydrochloride

[67-76-7]

Phenylephrine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of C$_9$H$_{13}$NO$_2$.HCl.

**Description** Phenylephrine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Phenylephrine Hydrochloride (1 in 100) is between 4.5 and 5.5.

**Identification** (1) To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue color is produced. To the solution so obtained add 1 mL of diethyl ether, and shake vigorously: no blue color develops in the diethyl ether layer.

(2) To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of iron (III) chloride TS: a persistent purple color is produced.

(3) Dissolve 0.3 g of Phenylephrine Hydrochloride in 3 mL of water, add 1 mL of ammonia TS, and rub the inner side of the test tube with a glass rod: a precipitate is produced. Collect the precipitate, wash with a few drops of ice-cold water, and dry at 105°C for 2 hours: it melts $<$2.60$>$ between 170°C and 177°C.

(4) A solution of Phenylephrine Hydrochloride (1 in 100) responds to Qualitative Tests $<$1.09$>$ (2) for chloride.

**Optical rotation** $<$2.49$>$ $[\alpha]_D^20$: −42.0 – −47.5° (after drying, 0.5 g, water, 10 mL, 100 mm).

**Melting point** $<$2.60$>$ 140 – 145°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Phenylephrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate $<$1.14$>$—Take 0.5 g of Phenylephrine Hydrochloride, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Ketone—Dissolve 0.20 g of Phenylephrine Hydrochloride in 1 mL of water, and add 2 drops of sodium pentacyanoferrate (III) TS, 1 mL of sodium hydroxide TS and then 0.6 mL of acetic acid (100): the solution has no more color than the following control solution.

Control solution: Prepare as directed above without Phenylephrine Hydrochloride.

**Loss on drying** $<$2.41$>$ Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** $<$2.44$>$ Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.1 g of Phenylephrine Hydrochloride, previously dried, dissolve in 40 mL of water contained in an iodine flask, add exactly measured 50 mL of 0.05 mol/L bromine VS, then add 5 mL of hydrochloric acid, and immediately stopper tightly. Shake the mixture, and allow to stand for 15 minutes. To this solution add 10 mL of potassium iodide TS carefully, stopper tightly immediately, shake thoroughly, allow to stand for 5 minutes, and titrate $<$2.50$>$ with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS = 3.395 mg of C$_{13}$H$_{13}$NO$_2$.HCl

**Containers and storage** Containers—Light-resistant.

Containers—Tight containers.

Storage—Light-resistant.
Phenytoin

Diphenylhydantoin

フェニトイン

Phenytoin, when dried, contains not less than 99.0% of C₁₅H₁₂N₂O₂.

Description Phenytoin occurs as a white, crystalline powder or granules. It is odorless and tasteless. It is sparingly soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water. It dissolves in sodium hydroxide VS.

Melting point: about 296°C (with decomposition).

Identification
1. Dissolve 0.02 g of Phenytoin in 2 mL of ammonia TS, and add 5 mL of silver nitrate TS: a white precipitate is produced.

2. To 25 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 10 mL of ammonia TS: a red, crystalline precipitate is produced.

3. Heat 0.1 g of Phenytoin with 0.2 g of sodium hydroxide, and fuse: the gas evolved turns moistened red litmus paper blue.

4. Add 3 mL of chlorinated lime TS to 0.1 g of Phenytoin, shake for 5 minutes, and dissolve the oil precipitate in 15 mL of hot water. After cooling, add 1 mL of dilute hydrochloric acid dropwise, then add 4 mL of water. Filter the white precipitate thus obtained, wash with water, and press it with dry filter paper to remove the accompanying water. Dissolve the precipitate with 1 mL of chloroform, add 5 mL of diluted ethanol (9 in 10), and rub the inner surface of the flask to produce a white, crystalline precipitate. Collect the precipitate, wash with ethanol (95), and dry: the melting point of the residue is between 165°C and 169°C.

Purity
1. Clarity and color of solution—Dissolve 0.20 g of Phenytoin in 10 mL of 0.2 mol/L sodium hydroxide VS: the solution is clear and colorless. Then heat the solution: no turbidity is produced. Cool, and mix the solution with 5 mL of acetone: the solution is clear and colorless.

2. Acidity or alkalinity—Shake 2.0 g of Phenytoin with 40 mL of water for 1 minute, filter, and perform the following tests using this filtrate as the sample solution.

(i) To 10 mL of the sample solution add 2 drops of thymolphthalein TS: no color develops. Then add 0.15 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(ii) To 10 mL of the sample solution add 0.30 mL of 0.01 mol/L hydrochloric acid VS and 5 drops of methyl red TS: a red to orange color develops.

(3) Chloride <1.0%—Dissolve 0.30 g of Phenytoin in 30 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution from 0.60 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of acetone and 6 mL of dilute nitric acid, and add water to 50 mL (not more than 0.071%).

(4) Heavy metals <1.0%—Proceed with 1.0 g of Phenytoin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.4% Not more than 0.5% (2 g, 105°C, 2 hours).

Residue on ignition <2.4% Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Phenytoin, previously dried, dissolve in 40 mL of ethanol (95) with the aid of gentle heating, add 0.5 mL of thymolphthalein TS immediately, and titrate with 0.1 mol/L sodium hydroxide VS until a light blue color develops. Then add 1 mL of pyridine, 5 drops of phenolphthalein TS and 25 mL of silver nitrate TS, and titrate with 0.1 mol/L sodium hydroxide VS until a light red color, which persists for 1 minute, develops.

Each mL of 0.1 mol/L sodium hydroxide VS = 25.23 mg of C₁₅H₁₂N₂O₂.

Containers and storage Containers—Well-closed containers.

Phenytoin Powder

Diphenylhydantoin Powder

フェニトイン散

Phenytoin Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of phenytoin (C₁₅H₁₂N₂O₂: 252.27).

Method of preparation Prepare as directed under Granules or Powders, with Phenytoin.

Identification Weigh a portion of Phenytoin Powder, equivalent to 0.3 g of Phenytoin according to the labeled amount, stir well with two 100-mL portions of diethyl ether, and extract. Combine the diethyl ether extracts, and filter. Evaporate the filtrate on a water bath to dryness, and proceed with the residue as directed in the Identification under Phenytoin.

Dissolution Being specified separately.

Assay Weigh accurately an amount of Phenytoin Powder, equivalent to about 50 mg of phenytoin (C₁₅H₁₂N₂O₂), add 30 mL of methanol, treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of phenytoin for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the
internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q1 and Q0, of the peak area of phenytoin to that of the internal standard.

\[
M_5: \text{Amount (mg) of phenytoin for assay} \\
= M_S \times \frac{Q_1}{Q_0} \times 2 \\
\]

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 258 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of methanol and 0.02 mol/L phosphate buffer solution, pH 3.5 (11.9).
Flow rate: Adjust the flow rate so that the retention time of phenytoin is about 5 minutes.

System performance—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, phenytoin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of phenytoin to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Well-closed containers.

Phenytoin Tablets

Diphenhydantoin Tablets

フェニトイン錠

Phenytoin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of phenytoin (C15H12N2O2: 252.27).

Method of preparation—Prepare as directed under Tablets, with Phenyltoin.

Identification—Weigh a portion of powdered Phenyltoin Tablets, equivalent to about 0.3 g of Phenyltoin according to the labeled amount, transfer to a separator, and add 1 mL of dilute hydrochloric acid and 10 mL of water. Extract with 100 mL of diethyl ether, then with four 25-mL portions of diethyl ether. Combine the extracts, evaporate the diethyl ether on a water bath, and dry the residue at 105°C for 2 hours. Proceed with the residue as directed in the Identification under Phenyltoin.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Phenyltoin Tablets add 3 V/5 mL of a mixture of water and acetonitrile (1:1), treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly 50 mL so that each mL contains about 1 mg of phenytoin (C15H12N2O2). Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Proceed as directed in the Assay.

\[
M_5: \text{Amount (mg) of phenytoin for assay} \\
= M_S \times \frac{Q_1}{Q_0} \times \frac{V}{25} \\
\]

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25,000).

Dissolution—Being specified separately.

Assay—Weigh accurately the mass of not less than 20 Phenyltoin Tablets, and powder in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 50 mg of phenytoin (C15H12N2O2), add 30 mL of a mixture of water and acetonitrile (1:1), treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of phenytoin for assay, previously dried at 105°C for 2 hours, and dissolve in a mixture of water and acetonitrile (1:1) to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q1 and Q0, of the peak area of phenytoin to that of the internal standard.

\[
M_5: \text{Amount (mg) of phenytoin for assay} \\
= M_S \times \frac{Q_1}{Q_0} \times 2 \\
\]

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 258 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of methanol and 0.02 mol/L phosphate buffer solution, pH 3.5 (11.9).
Flow rate: Adjust the flow rate so that the retention time of phenytoin is about 5 minutes.

System performance—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, phenytoin and the internal standard are eluted in this order with the resolution between these peaks being not

Official Monographs / Phenytoin Tablets 1243
Phenytoin Sodium for Injection

Diphenylhydantoin Sodium for Injection

Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless.

It is soluble in water and in ethanol (95%), and practically insoluble in chloroform and in diethyl ether.

The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12.

It is hygroscopic.

A solution of Phenytoin Sodium for Injection absorbs carbon dioxide gradually when exposed to air, and a crystalline precipitate of phenytoin is produced.

Identification (1) With the residue obtained in the Assay, proceed as directed in the Identification under Phenytoin.

(2) Ignite 0.5 g of Phenytoin Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue, and responds to Qualitative Tests <1.09> (1) for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution is clear and colorless. If any turbidity is produced, boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. 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Loss on drying <2.45> Not more than 2.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately the content of not less than 10 preparations of Phenytoin Sodium for Injection, transfer about 0.3 g of the content, previously dried and accurately weighed, to a separator, dissolve in 50 mL of water, add 10 mL of dilute hydrochloric acid, and extract with 100 mL of diethyl ether, then with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate on a water bath. Dry the residue at 105°C for 2 hours, and weigh it as the mass of phenytoin (C15H11N2O2: 252.27).

Amount (mg) of phenytoin sodium (C15H11N2NaO2) = amount (mg) of phenytoin (C15H11N2NaO2) × 1.087

Containers and storage Containers—Hermetic containers.

Phytonadione

Phytomenadione

Vitamin K1

Phytonadione contains not less than 97.0% and not more than 102.0% of C31H46O2.

Description Phytonadione is a clear yellow to orange-yellow, viscous liquid.

It is miscible with isooctane.

It is soluble in ethanol (99.5), and practically insoluble in water.

It decomposes gradually and changes to a red-brown by light.

Specific gravity d20: about 0.967

Identification (1) Determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phytonadione as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
Refractive index $\leq 2.45$  \( n^\oplus_2 : 1.525 - 1.529 

**Purity (1)** Ratio of absorbances—Determine the absorbances, $A_1$, $A_2$ and $A_3$, of a solution of Phytonadione in isooctane (1 in 100,000) at 284.5 nm, 253.5 nm and 269.5 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry. $2.24 > A_3/A_1 > 0.73$, and the ratio $A_2/A_1$ is between 0.69 and 0.73, and the ratio $A_2/A_3$ is between 0.74 and 0.78. Determine the absorbances, $A_1$, $A_2$, and $A_3$, of a solution of Phytonadione in isooctane (1 in 10,000) at 284.5 nm and 326 nm, respectively: the ratio $A_2/A_1$ is between 0.28 and 0.34.

(2) Heavy metals $< 1.07$—Carbonize 1.0 g of Phytonadione by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, 10 mL of a solution of 3-methyl-1-phenyl-5-pyrazolone in ethanol (95) (1 in 20) and 1 drop of ammonia solution (28), and allow to stand for 2 hours: no blue-purple color develops.

**Isomer ratio** Conduct this procedure rapidly and without exposure to light. Dissolve 30 mg of Phytonadione in 50 mL of the mobile phase. To 4 mL of this solution add the mobile phase to make 25 mL. To 10 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Perform the test with 50 $\mu$L of the sample solution as directed under Liquid Chromatography $2.20$ according to the following conditions, and determine the peak areas of Z-isomer and E-isomer, $A_{TZ}$ and $A_{TE}$: $A_{TZ} / (A_{TZ} + A_{TE})$ is between 0.05 and 0.18.

**Operating conditions—**
Proceed as directed in the operating conditions in the Assay.

**System suitability—**
System performance: When the procedure is run with 50 $\mu$L of the sample solution under the above operating conditions, Z-isomer and E-isomer are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 50 $\mu$L of the sample solution under the above operating conditions, the relative standard deviation of the total area of the peaks of Z-isomer and E-isomer is not more than 2.0%.

**Assay** Conduct this procedure rapidly and without exposure to light. Weigh accurately about 30 mg each of Phytonadione and Phytonadione RS, and dissolve each in the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, and add the mobile phase to make exactly 25 mL. To exactly 10 mL each of these solutions add exactly 7 mL of the internal standard solution and the mobile phase to make 25 mL, and use these as the sample solution and the standard solution, respectively. Perform the test with 50 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography $2.20$ according to the following conditions, and calculate the ratios, $Q_1$ and $Q_5$, of the total area of the peaks of Z-isomer and E-isomer to the peak area of the internal standard.

Amount (mg) of $C_{11}H_{16}O_2\cdot HCl = M_5 \times Q_1/Q_5$

**Internal standard solution**—A solution of cholesterol benzoate in the mobile phase (1 in 400).

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with porous silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of hexane and n-amyl alcohol (4000 : 3).

Flow rate: Adjust the flow rate so that the retention time of the peak of E-isomer of phytonadione is about 25 minutes.

**System suitability—**
System performance: When the procedure is run with 50 $\mu$L of the standard solution under the above operating conditions, the internal standard, Z-isomer and E-isomer are eluted in this order with the resolution between the peaks of Z-isomer and E-isomer being not less than 1.5.

System repeatability: When the test is repeated 6 times with 50 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the total area of the peaks of Z-isomer and E-isomer to the peak area of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, at a cold place or in containers in which air has been displaced by Nitrogen.

### Pilocarpine Hydrochloride

ピロカルピン塩酸塩

C$_{11}$H$_{16}$N$_2$O$_2\cdot$HCl: 244.72

(3S,4R)-3-Ethyl-4-(1-methyl-1H-imidazol-5-ylmethyl)-5,4-dihydrofuran-2(3H)-one monohydrochloride

[54-71-7]

Pilocarpine Hydrochloride, when dried, contains not less than 99.0% of C$_{11}$H$_{16}$N$_2$O$_2\cdot$HCl.

**Description** Pilocarpine Hydrochloride occurs as colorless crystals or white powder. It is odorless, and has a slightly bitter taste.

It is very soluble in acetic acid (100), freely soluble in water, in methanol and in ethanol (95), soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Pilocarpine Hydrochloride (1 in 10) is between 3.5 and 4.5.

It is hygroscopic.

It is affected by light.

**Identification (1)** Dissolve 0.1 g of Pilocarpine Hydrochloride in 5 mL of water, add 1 drop of dilute nitric acid, 1 mL of hydrogen peroxide TS, 1 mL of chloroform and 1 drop of a potassium dichromate solution (1 in 300), and

\[ \text{Amount (mg) of } C_{11}H_{16}N_2O_2 = M_5 \times Q_1/Q_5 \]
shake the mixture vigorously: a violet color develops in the chloroform layer while no color or a light yellow color is produced in the aqueous layer.

(2) To 1 mL of a solution of Pilocarpine Hydrochloride (1 in 20) add 1 mL of dilute nitric acid and 2 to 3 drops of silver nitrate TS: a white precipitate or opalescence is produced.

**Melting point** 200 – 203°C

**Purity** (1) Sulfate—Dissolve 0.5 g of Pilocarpine Hydrochloride in 20 mL of water, and use this solution as the sample solution. To 5.0 mL of the sample solution add 1 mL of dilute hydrochloric acid and 0.5 mL of barium chloride TS: no turbidity is produced.

(2) Nitrate—To 2.0 mL of the sample solution obtained in (1) add 2 mL of iron (II) sulfate TS, and superimpose the mixture upon 4 mL of sulfuric acid: no dark brown color develops at the zone of contact.

(3) Related substances—Dissolve 0.3 g of Pilocarpine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.

Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol (1 in 20), and ammonia TS (85:14:2) to a distance of about 13 cm, and dry the plate at 105°C for 10 minutes. Cool, and spray evenly bismuth potassium iodide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(4) Readily carbonizable substances—Take 0.25 g of Pilocarpine Hydrochloride, and perform the test: the solution has no more color than Matching Fluid B.

**Loss on drying** Not more than 3.0% (1 g, 105°C, 2 hours).

**Residue on ignition** Not more than 0.5% (0.1 g).

**Assay** Weigh accurately about 0.5 g of Pilocarpine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.47 mg of C11H16N2O2.HCl

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Identification** (1) To 3 mg of Pimaricin add 1 mL of hydrochloric acid, and mix: a blue-purple color appears.

(2) Dissolve 5 mg of Pimaricin in a solution of acetic acid (100) in methanol (1 in 100) to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pimaricin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** [α]D25 243° – 259° (0.1 g, acetic acid (100), 25 mL, 100 mm).

**Purity** (1) Heavy metals—Proceed with 1.0 g of Pimaricin according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 20 mg of Pimaricin in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography according to the following conditions, and determine the total area of the peaks other than pimaricin by the automatic integration method: not more than 4.0%.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelengths: 243° – 259°)

**Pimaricin**

**Natamycin**

C33H47NO13: 665.73


[7681-93-8]

Pimaricin is a polyene macrolide substance having antifungal activity produced by the growth of *Streptomyces natalensis*.

It contains not less than 900 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pimaricin is expressed as mass (potency) of pimaricin (C33H47NO13).

**Description** Pimaricin occurs as white to yellowish white crystalline powder.

It is slightly soluble in methanol and in acetic acid (100), and practically insoluble in water and in ethanol (99.5).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelengths: 243° – 259°)
length: 303 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of ammonium acetate in 1000 mL of a mixture of water, methanol and tetrahydrofuran (47:44:2).

Flow rate: Adjust the flow rate so that the retention time of pimaricin is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of pimaricin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add methanol to make exactly 10 mL. Confirm that the peak area of pimaricin obtained from 10 µL of this solution is equivalent to 7 to 13% of that from 10 µL of the solution for system suitability test.

System performance: When the procedure is run with 10 µL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pimaricin are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of pimaricin is not more than 2.0%.

Water

Between 6.0% and 9.0% (0.2 g, volumetric titration, direct titration).

Assay

Weigh accurately an amount of Pimaricin and Pimaricin RS, equivalent to about 25 mg (potency), and dissolve each in methanol to make exactly 100 mL. Pipet 2 mL of each of these solutions, add a solution of acetic acid (100) in methanol (1 in 100) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances at 295.5 nm, A11 and A51, at 303 nm, A12 and A52, and at 311 nm, A13 and A53, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount [µg (potency)] of C28H29F2N3O:

\[
A_{12} = \frac{A_{11} + A_{12}}{2} \times \frac{1000}{M_S} = \frac{A_{51} + A_{52}}{2}
\]

\[
M_S: \text{Amount [mg (potency)] of Pimaricin RS}
\]

Containers and storage

Containers—Tight containers. Storage—Light resistant.

Pimozide

ピモジド

C28H29F2N3O: 461.55
1-[[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl]-1,3-dihydro-2H-benzoimidazol-2-one [2062-78-4]

Pimozide contains not less than 98.5% and not more than 101.0% of C28H29F2N3O.

Description

Pimozide occurs as a white to pale yellowish white powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification

1. Determine the absorption spectrum of a solution of Pimozide in methanol (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Pimozide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point

216 – 220°C

Purity

1. Heavy metals <1.0>—Proceed with 2.0 g of Pimozide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution by using 5 mL of sulfuric acid (not more than 10 ppm).

2. Arsenic <1.11>—Prepare the test solution with 1.0 g of Pimozide according to Method 3, and perform the test (not more than 2 ppm).

3. Related substances—Dissolve 0.10 g of Pimozide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than the peak of pimozide from the sample solution is not larger than the peak area of pimozide from the standard solution, and the total area of the peaks other than the peak of pimozide from the sample solution is not larger than 1.5 times of the peak area of pimozide from the standard solution.

Operating conditions—
Pindolol

ピンドロール

C_{14}H_{20}N_{2}O_{2}: 248.32
(2RS)-1-(1H-インドール-4-ヨキドー)-3-(1-メチルエチル)アミノプロパン-2-オール
[13523-86-9]

Pindolol, when dried, contains not less than 98.5\% of C_{14}H_{20}N_{2}O_{2}.

Description  Pindolol occurs as a white, crystalline powder. It has a slight, characteristic odor.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in dilute sulfuric acid and in acetic acid (100).

Identification (1)  To 1 mL of a solution of Pindolol in methanol (1 in 10,000) add 1 mL of a solution of 1-(4-pyridyl)-pyridinium chloride hydrochloride (1 in 1000) and 1 mL of sodium hydroxide TS, then add 1 mL of hydrochloric acid: a blue to blue-purple color, changing to red-purple, is produced.

(2)  Dissolve 0.05 g of Pindolol in 1 mL of dilute sulfuric acid, and add 1 mL of Reinecke salt TS: a light red precipitate is produced.

(3)  Determine the absorption spectrum of a solution of Pindolol in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4)  Determine the infrared absorption spectrum of Pindolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance 2.24  E_{1\text{cm}}(264 nm): 333 – 350 (10 mg, methanol, 500 mL).

Melting point 2.60  169 – 173°C

Purity (1)  Clarity and color of solution—Dissolve 0.5 g of Pindolol in 10 mL of acetic acid (100), and observe immediately: the solution is clear, and has no more color than the following control solution.

Control solution: Measure accurately 1 mL of a solution of Pindolol in methanol (1 in 10,000) and add 1 mL of 0.02 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction. Each mL of 0.02 mol/L perchloric acid VS = 9.231 mg of C_{14}H_{20}N_{2}O₂.

Containers and storage  Containers—Well-closed containers.

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 2.5 g of ammonium acetate and 8.5 g of tetrabutylammonium hydrogensulfate in water to make 1000 mL.

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>80 → 70</td>
<td>20 → 30</td>
</tr>
<tr>
<td>10 – 15</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

Flow rate: 2.0 mL per minute.

Time span of measurement: 1.5 times as long as the retention time of pimozone.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of pimozone obtained from 10 μL of this solution is equivalent to 8 to 12% of that of pimozone from 10 μL of the standard solution.

System performance: Dissolve 5 mg of Pimozide and 2 mg of mebendazole in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, mebendazole and pimozone are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pimozone is not more than 2.0%.

(4)  Residual solvent—Being specified separately.

Loss on drying 2.41  Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition 2.44  Not more than 0.1% (1 g).

Assay  Weigh accurately about 70 mg of Pimozide, previously dried, dissolve in 25 mL of acetic acid for nonaqueous titration, and titrate 2.50 with 0.02 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS = 9.231 mg of C_{14}H_{20}F_{2}N_{2}O.
tion. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Ultraviolet-spectrophotometry 2.037. Spot 5 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and isopropylamine (5:4:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (3 in 5) and a sodium nitrite solution (1 in 50) on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.4\%\) Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** \(<2.4\%\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Pindolol, previously dried, dissolve in 80 mL of methanol, and titrate with 0.1 mol/L hydrochloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L hydrochloric acid VS = 24.83 mg of \(C_{14}H_{20}N_{2}O_{2}\)

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

### Pioglitazone Hydrochloride

ピオアグリタゾン塩酸塩

\[\text{C}_{19}\text{H}_{20}\text{N}_{2}\text{O}_{3}\text{S.HCl: 392.90}\]

(5RS)-5-[4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl]thiazolidine-2,4-dione monohydrochloride

[112259-15-4]

Pioglitazone Hydrochloride contains not less than 99.0% and not more than 101.0% of \(C_{19}H_{20}N_{2}O_{3}S\). HCl, calculated on the anhydrous basis.

**Description** Pioglitazone Hydrochloride occurs as white crystals or crystalline powder.

It is soluble in \(N,N\)-dimethylformamide and in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Pioglitazone Hydrochloride in \(N,N\)-dimethylformamide (1 in 20) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Pioglitazone Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry 2.247, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pioglitazone Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pioglitazone Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.257, and compare the spectrum with the Reference Spectrum or the spectrum of Pioglitazone Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 50 mg of Pioglitazone Hydrochloride in 1 mL of nitric acid, and add 4 mL of dilute nitric acid: the solution responds to the Qualitative Tests \(<1.0\%\) (2) for chloride.

**Purity (1)** Heavy metals \(<1.0\%\)—Proceed with 1.0 g of Pioglitazone Hydrochloride according to Method 4, and perform the test. After incineration, use 3 mL of hydrobromic acid instead of 3 mL of hydrochloric acid. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Pioglitazone Hydrochloride in 20 mL of methanol, add the mobile phase to make 100 mL, and use this solution as the standard solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 40 \(\mu L\) each of the sample solution and standard solution as directed under Liquid Chromatography 2.017 according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention times of about 0.7, about 1.4 and about 3.0 with respect to pioglitazone from the sample solution, is not larger than 2/5 times the peak area of pioglitazone from the standard solution, and the area of each peak other than the peak of pioglitazone and other than those mentioned above is smaller than 1/5 times the peak area of pioglitazone from the standard solution. Furthermore, the total area of the peaks other than the peak of pioglitazone is not larger than the peak area of pioglitazone from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of pioglitazone, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pioglitazone obtained from 40 \(\mu L\) of this solution is equivalent to 7 to 13% of that of pioglitazone from 40 \(\mu L\) of the standard solution.

System performance: Dissolve 50 mg of Pioglitazone Hydrochloride in 10 mL of a solution of benzophenone in methanol (1 in 750), and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 40 \(\mu L\) of this solution under the above operating conditions, pioglitazone and benzophenone are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 40 \(\mu L\) of the standard solution under the above operat-
ing conditions, the relative standard deviation of the peak area of pioglitazone is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water <2.48> Not more than 0.2% (0.5 g, coulometric titration). For anolyte solution, use anolyte solution for water determination A.

Residue on ignition <2.48> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Pioglitazone Hydrochloride and Pioglitazone Hydrochloride RS (separately, determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), add exactly 10 mL of the internal standard solution and methanol to make 100 mL. Pipet 2 mL each of these solutions, add the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.06> according to the following conditions, and calculate the ratios, Q<sub>T</sub> and Q<sub>S</sub>, of the peak area of pioglitazone to that of the internal standard.

Amount (mg) of pioglitazone hydrochloride (C<sub>19</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>S.HCl)

\[ M_S = M_S \times \frac{Q_T}{Q_S} \]

M<sub>S</sub>: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of benzophenone in methanol (1 in 750).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 269 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of ammonium acetate solution (77 in 10,000), acetonitrile and acetic acid (100) (25:25:1).

Flow rate: Adjust the flow rate so that the retention time of pioglitazone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, pioglitazone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pioglitazone is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

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**Pioglitazone Hydrochloride Tablets**

ピオグリタゾン塩酸塩錠

Pioglitazone Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pioglitazone hydrochloride (C<sub>19</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>S.HCl: 392.90).

Method of preparation Prepare as directed under Tablets, with Pioglitazone Hydrochloride.

Identification To an amount of powdered Pioglitazone Hydrochloride Tablets, equivalent to 2.8 mg of Pioglitazone Hydrochloride according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> using a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly 10 mL of each solution, and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Pioglitazone Hydrochloride RS (separately, determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, add methanol to make exactly 100 mL, and centrifuge. Take exactly V mL of the supernatant liquid, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly V mL so that each mL contains about 26 µg of pioglitazone hydrochloride (C<sub>19</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>S.HCl), and use this solution as the standard solution. Separately, weigh accurately about 33 mg of Pioglitazone Hydrochloride RS, determine the water <2.48> in the same manner as Pioglitazone Hydrochloride, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, and add methanol to make exactly 100 mL. Pipet 4 mL of this solution, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A<sub>T</sub> and A<sub>S</sub>, of the sample solution and standard solution at 269 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) as the blank.

Amount (mg) of pioglitazone hydrochloride (C<sub>19</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>S.HCl)

\[ M_S = M_S \times \frac{A_T}{A_S} \times \frac{V}{V} \times \frac{2/25}{M} \]

M<sub>S</sub>: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution, which is prepared by mixing 50 mL of 0.2 mol/L hydrochloric acid TS and 150 mL of potassium chloride solution (3 in 20), adding water to make 1000 mL and adjusting to pH 2.0 with 5 mol/L hydrochloric acid TS, as the dissolution medium, the dissolution rate in 45 minutes of Pioglitazone Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Pioglitazone Hydrochloride Tablets, withdraw 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the
first 5 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add the dissolution medium to make exactly $V'$ mL so that each mL contains about 18 µg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S\cdotHCl$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Pioglitazone Hydrochloride RS (separately determine the water $<2.4\%$ in the same manner as Pioglitazone Hydrochloride), dissolve in 10 mL of methanol, and add the dissolution medium to make exactly 50 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 269 nm as directed under Ultraviolet-visible Spectrophotometry $<2.2\>$ using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S\cdotHCl$)$\quad M_S = M_5 \times A_T/A_S \times V'/V \times 1/C \times 72$

$M_5$: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

$C$: Labeled amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S\cdotHCl$) in 1 tablet

Assay Accurately weigh the mass of not less than 20 Pioglitazone Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S\cdotHCl$), add 45 mL of methanol and exactly 5 mL of the internal standard solution, agitate with the aid of ultrasonic waves, and centrifuge. To 2 mL of the supernatant liquid add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Pioglitazone Hydrochloride RS (separately, determine the water $<2.4\%$ in the same manner as Pioglitazone Hydrochloride), dissolve in 45 mL of methanol, and add exactly 5 mL of the internal standard solution. Pipet 2 mL of this solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.2\>$ according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of pioglitazone to that of the internal standard.

Amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S\cdotHCl$) $\quad M_S = M_5 \times Q_T/Q_S$

$M_5$: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of benzophenone in methanol (1 in 750).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 269 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of ammonium acetate solution (77 in 10,000), acetonitrile and acetic acid (100) (25:25:1).

Flow rate: Adjust the flow rate so that the retention time of pioglitazone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, pioglitazone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pioglitazone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Pipemidic Acid Hydrate

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C$_{14}$H$_{17}$N$_5$O$_3$·3H$_2$O: 357.36
8-Ethyl-5-oxo-2-(piperazin-1-yl)-5,8-dihydropyrido[2,3-d]pyrimidine-6-carboxylic acid trihydrate [51940-44-4, anhydride]

Pipemidic Acid Hydrate contains not less than 98.5% and not more than 101.0% of pipemidic acid (C$_{14}$H$_{17}$N$_5$O$_3$: 303.32), calculated on the anhydrous basis.

Description Pipemidic Acid Hydrate occurs as a pale yellow, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water and in ethanol (99.5), and practically insoluble in methanol.

It dissolves in sodium hydroxide TS.

It is gradually colored on exposure to light.

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 0.1 g of Pipemidic Acid Hydrate in 20 mL of sodium hydroxide TS, and dilute with water to make 200 mL. To 1 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry $<2.2\>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pipemidic Acid Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25\>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride $<1.0\%$—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, then add 15 mL of dilute nitric acid, shake well, and filter through a glass filter (G3). To 30 mL of the filtrate
add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 13.5 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate <1,14>—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, then add 15 mL of dilute hydrochloric acid, shake well, and filter through a glass filter (G3). To 30 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 5 mL of sodium hydroxide TS, 7.5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1,07>—Proceed with 2.0 g of Pipemidic Acid Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1,11>—Prepare the test solution with 1.0 g of Pipemidic Acid Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Pipemidic Acid Hydrate in 10 mL of diluted acetic acid (100) (1 in 20), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted acetic acid (100) (1 in 20) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2,05>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, formic acid and triethylamine (25:15:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than

Water <2,48> 14.5 – 16.0% (20 mg, coulometric titration).

Residue on ignition <2,44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Pipemidic Acid Hydrate, dissolve in 40 mL of acetic acid (100), and titrate <2,59> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 30.33 mg of C14H17N5O3

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

C23H27N5O7S.H2O: 535.57

1) 1H spectrum of a solution of Piperacillin Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 3) as directed under Nuclear Magnetic Resonance Spectroscopy <2,22>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triple signal A at about δ 1.1 ppm, a single signal B at about δ 4.2 ppm, and a multiple signal C at about δ 7.4 ppm, and the ratio of the integrated intensity of each signal, A:B:C, is about 3:1:5.

Optical rotation <2,49> [c]D20 +162 – +172° (0.2 g, methanol, 20 mL, 100 mm).

Purity (1) Heavy metal <1,07>—Proceed with 2.0 g of Piperacillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances 1—Conduct this procedure rapidly after the preparation of the sample solution and standard solution. Dissolve 20 mg of Piperacillin Hydrate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 2 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and the standard solutions (1) and (2) as directed under Liquid Chromatography <2,01> according to the following conditions, and

**Piperacillin Hydrate**

**ピベラシリン水和物**

Piperacillin Hydrate contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Piperacillin Hydrate is expressed as mass (potency) of piperacillin (C23H27N5O7S: 517.55).

**Description** Piperacillin Hydrate occurs as a white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5) and in dimethylsulfoxide, and very slightly soluble in water.

**Identification (1)** Determine the infrared absorption spectrum of Piperacillin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,25>, and compare the spectrum with the Reference Spectrum or the spectrum of Piperacillin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(2)** Determine the 1H spectrum of a solution of Piperacillin Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 3) as directed under Nuclear Magnetic Resonance Spectroscopy <2,22>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triple signal A at about δ 1.1 ppm, a single signal B at about δ 4.2 ppm, and a multiple signal C at about δ 7.4 ppm, and the ratio of the integrated intensity of each signal, A:B:C, is about 3:1:5.

**Optical rotation <2,49>** [c]D20 +162 – +172° (0.2 g, methanol, 20 mL, 100 mm).

**Purity (1) Heavy metal <1,07>—Proceed with 2.0 g of Piperacillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).**
determine each peak area by the automatic integration method: the total area of the peaks, having the relative retention time of about 0.38 and about 0.50 with respect to piperacillin, obtained from the sample solution is not larger than 2 times the peak area of piperacillin from the standard solution (2), the total area of the peaks, having the relative retention time of about 0.82 and about 0.86 with respect to piperacillin, obtained from the sample solution is not larger than the peak area of piperacillin from the standard solution (2), and the area of the peak other than piperacillin and other than the peaks having the relative retention time of about 0.38, about 0.50, about 0.82 and about 0.86 with respect to piperacillin, obtained from the sample solution, is not larger than the peak area of piperacillin from the standard solution (2). Furthermore, the total area of the peaks other than piperacillin obtained from the sample solution is not larger than the peak area of piperacillin from the standard solution (1).

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of piperacillin, beginning after the solvent peak.

**System suitability**—

Test for required detectability: Confirm that the peak area of piperacillin obtained from 20 μL of the standard solution (2) is equivalent to 15 to 25% of that from 20 μL of the standard solution (1).

System performance: When the procedure is run with 20 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 3.0%.

(3) Related substances 2—Dissolve 20 mg of Piperacillin Hydrate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 2 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution, and the standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 6.6 with respect to piperacillin, obtained from the sample solution is not larger than 3 times the peak area of piperacillin from the standard solution (2), and the area of the peaks other than the peak of piperacillin and the peak having the relative retention time of about 6.6 with respect to piperacillin from the sample solution are not larger than 1.4 times the peak area of piperacillin from the standard solution (2). Furthermore, the total area of the peaks other than the peak of piperacillin from the sample solution is not larger than the area of the peak of piperacillin from the standard solution (1). For these calculations, use the area of the peak, having the relative retention time of about 6.6 with respect to piperacillin, after multiplying by the relative response factor, 2.0.

**Operating conditions**—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Take 60.1 g of acetic acid (100) and 101.0 g of triethylamine, add water to make 1000 mL. To 25 mL of this solution add 300 mL of acetonitrile and 25 mL of dilute acetic acid, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 1.2 minutes.

Time span of measurement: About 8 times as long as the retention time of piperacillin, beginning after the piperacillin peak.

**System suitability**—

Test for required detectability: Confirm that the peak area of piperacillin obtained from 20 μL of the standard solution (2) is equivalent to 15 to 25% of that from 20 μL of the standard solution (1).

System performance: When the procedure is run with 20 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 4.0%.

(4) Residual solvents—Transfer exactly 10 mg of Piperacillin Hydrate to an about 3-mL vial, add exactly 1 mL of saturated sodium hydrogen carbonate solution to dissolve and stop the vial tightly. After heating this at 90°C for 10 minutes, use the gas inside the container as the sample gas. Separately, measure exactly 1 mL of ethyl acetate, dissolve in water to make exactly 200 mL. Pipet 10 mL of this solution, add water to make exactly 20 mL. Pipet 2 μL of this solution in an about 3-mL vial containing exactly 1 mL of saturated sodium hydrogen carbonate solution, and stop the vial tightly. Run the procedure similarly to the sample, and use the gas as the standard gas. Perform the test with exactly 0.5 mL each of the sample gas and standard gas as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area of ethyl acetate by the automatic integration method: the peak area of ethyl acetate obtained from the sample gas is not larger than that from the standard gas.

**Operating conditions**—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with porous styene-divinyl benzene copolymer for gas chromatography (average pore diameter of 0.0085 μm, 300 – 400 m2/g) with the particle size of 125 to 150 μm.

Column temperature: A constant temperature of about 145°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of ethyl acetate is about 4 minutes.

**System suitability**—

System performance: Take 1 mL of saturated sodium hydrogen carbonate solution in an about 3-mL vial, add 2 μL each of ethyl acetate solution (1 in 400) and acetone solution (1 in 400), and stop the vial tightly. When the procedure...
is run under the above operating conditions, acetone and ethyl acetate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: Take 1 mL of saturated sodium hydrogen carbonate solution in an about 3 mL-vial, add 2 \( \mu \)L of ethyl acetate solution (1 in 400), stop the vial tightly, and perform the test under the above operating conditions.

When the procedure is repeated 6 times, the relative standard deviation of the peak area of ethyl acetate is not more than 10%.

Water \(<2.48\) Not less than 3.2% and not more than 3.8% (0.5 g, volumetric titration, direct titration).

Residue on ignition \(<2.46\) Not more than 0.1% (1 g).

Bacterial endotoxins \(<4.01\) Less than 0.07 EU/mg (potency).

Assay Weigh accurately an amount of Piperacillin Hydrate and Piperacillin RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\> according to the following conditions, and calculate the ratios, \( H_1 \) and \( H_2 \), of the peak height of piperacillin to that of the internal standard.

Amount [\( \mu \)g (potency)] of piperacillin (C\(_{23}\)H\(_{27}\)N\(_{5}\)O\(_{7}\)S) = \( M_5 \times H_1 / H_2 \times 1000 \)

\( M_5 \): Amount [mg (potency)] of Piperacillin RS

Internal standard solution—A solution of acetonilide in the mobile phase (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Take 60.1 g of acetic acid (100) and 101.0 g of triethylamine, add water to make 1000 mL. To 25 mL of this solution add 210 mL of acetonitrile and 25 mL of dilute acetic acid, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 \( \mu \)L of the standard solution under the above operating conditions, the internal standard and piperacillin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of piperacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

### Piperacillin Sodium

![Piperacillin Sodium](image)

C\(_{23}\)H\(_{26}\)N\(_2\)NaO\(_3\): 539.54

Monosodium (25,5R,6R)-6-[(2R)-2-[(4-ethyl-3-dioxopiperazine-1-carboxy)amino]-2-phenylacetylamino]-3,3-dimethyl-7-oxy-4-thiazil-1-azabicyclo[3.2.0]heptane-2-carboxylate

\([59703-84-3]\)

Piperacillin Sodium contains not less than 863 \( \mu \)g (potency) per mg, calculated on the anhydrous basis. The potency of Piperacillin Sodium is expressed as mass (potency) of piperacillin (C\(_{23}\)H\(_{27}\)N\(_{2}\)O\(_{7}\)S: 517.55).

Description Piperacillin Sodium occurs as a white powder or mass.

It is very soluble in water, freely soluble in methanol and in ethanol (95), and practically insoluble in acetonitrile.

Identification

(1) Determine the infrared absorption spectrum of Piperacillin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\>\> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Piperacillin Sodium responds to Qualitative Tests \(<1.09\> (1) for sodium salt.

Optical rotation \(<2.49\> [\alpha]_D^{20} +175 – +190° (0.8 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH \(<2.54\> Dissolve 1.0 g of Piperacillin Sodium in 4 mL of water: the pH of the solution is between 5.0 and 7.0.

Purity

(1) Clarity and color of solution—Dissolve 1.0 g of Piperacillin Sodium in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals \(<1.07\>—Proceed with 2.0 g of Piperacillin Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic \(<1.11\>—Prepare the test solution with 2.0 g of Piperacillin Sodium according to Method 4, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.1 g of Piperacillin Sodium in 50 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\>\> according to the following conditions, and determine the areas of each peak by the automatic integration method: the area of the peak of ampicillin appeared at the retention time of about 7 minutes from the sample solution is not larger than 1/2 times that of piperacillin from the standard solu-
tion, the total area of related compounds 1 appeared at the retention times of about 17 minutes and about 21 minutes is not larger than 2 times of the peak area of piperacillin from the standard solution, the peak area of related compound 2 appeared at the retention time of about 56 minutes is not larger than that of piperacillin from the standard solution, and the total area of the peaks other than piperacillin is not larger than 5 times of the peak area of piperacillin from the standard solution. The peak areas of ampicillin, related compounds 1 and related compound 2 are used after multiplying by their relative response factors, 1.39, 1.32 and 1.11, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, acetonitrile and 0.2 mol/L potassium dihydrogenphosphate (45:4:1).

Mobile phase B: A mixture of acetonitrile, water and 0.2 mol/L potassium dihydrogenphosphate (25:24:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 7</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7 – 13</td>
<td>100 ( \rightarrow ) 83</td>
<td>0 ( \rightarrow ) 17</td>
</tr>
<tr>
<td>13 – 41</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>41 – 56</td>
<td>83 ( \rightarrow ) 20</td>
<td>17 ( \rightarrow ) 80</td>
</tr>
<tr>
<td>56 – 60</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute. The retention time of piperacillin is about 33 minutes.

Time span of measurement: About 1.8 times as long as the retention time of piperacillin beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 3 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of piperacillin is not more than 2.0%.

Water <2.48> Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Piperacillin Sodium, equivalent to about 0.1 g (potency), and dissolve in water to make exactly 100 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Piperacillin RS, equivalent to about 0.1 g (potency), and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 5 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak height of piperacillin to that of the internal standard.

Amount [\( \mu g \) (potency)] of piperacillin (C\(_{23}\)H\(_{27}\)N\(_5\)O\(_7\)S) = \( M_S \times Q_1/Q_2 \times 1000 \)

\( M_S \): Amount [mg (potency)] of Piperacillin RS

Internal standard solution—A solution of acetaldehyde in the mobile phase (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 60.1 g of acetic acid (100) and 101.0 g of triethylamine add water to make exactly 1000 mL. To 25 mL of this solution add 25 mL of dilute acetic acid and 210 mL of acetonitrile, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 \( \mu \)L of the standard solution under the above operating conditions, the internal standard and piperacillin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of piperacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Piperacillin Sodium for Injection

注射用ピペラシンリンナトリウム

Piperacillin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of piperacillin (C\(_{23}\)H\(_{27}\)N\(_5\)O\(_7\)S: 517.55).

Method of preparation Prepare as directed under Injections, with Piperacillin Sodium.

Description Piperacillin Sodium for Injection is a white powder or masses.

Identification Piperacillin Sodium for Injection is a preparation for injection which is dissolved before use.

\( pH <2.54> \) The \( pH \) of a solution prepared by dissolving an amount of Piperacillin Sodium for Injection, equivalent to 1.0 g (potency) of Piperacillin Sodium according to the la-
Sterility

(1) Clarity and color of solution—Dissolve an amount of Piperacillin Sodium for Injection, equivalent to 4.0 g (potency) of Piperacillin Sodium according to the labeled amount, in 17 mL of water: the solution is clear and colorless.

(2) Related substances—Proceed as directed in the Purity (4) under Piperacillin Sodium.

Water <2.48> Not more than 1.0% (3 g, volumetric titration, direct titration).

Bacterial endotoxins <4.01> Less than 0.04 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Piperacillin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 20 mg (potency) of Piperacillin Sodium, dissolve in water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Piperacillin RS, and dissolve in the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Proceed as directed in the Assay under Piperacillin Sodium.

Amount [mg (potency)] of piperacillin (C₂₃H₂₇N₅O₇S) = Mₛ × Qₙ/Qₛ

Mₛ: Amount [mg (potency)] of Piperacillin RS

Internal standard solution—A solution of acetonilide in the mobile phase (1 in 5000).

Containers and storage Containers—Well-closed containers. Plastic containers for aqueous injections may be used.

Piperazine Adipate

ピペラジンアジピン酸塩

C₅H₁₀N₂·C₆H₁₀O₄: 232.28

Piperazine hexanedioate

[142-88-1]

Piperazine Adipate, when dried, contains not less than 98.5% of C₅H₁₀N₂·C₆H₁₀O₄.

Description Piperazine Adipate occurs as a white, crystal-pine powder. It is odorless, and has a slightly acid taste.

It is soluble in water and in acetic acid (100), and practically insoluble in ethanol (95), in acetone and in diethyl ether.

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 0.5 g of Piperazine Adipate in 10 mL of water, add 1 mL of hydrochloric acid, and extract with two 20-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness on a water bath, and dry the residue at 105°C for 1 hour: the melting point <2.60> is between 152°C and 155°C.

(2) To 3 mL of a solution of Piperazine Adipate (1 in 100) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the infrared absorption spectrum of Piperazine Adipate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> The pH of a solution of Piperazine Adipate (1 in 20) is between 5.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Piperazine Adipate in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Piperazine Adipate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Piperazine Adipate, previously dried, dissolve in a mixture of 20 mL of acetic acid for nonaqueous titration and 40 mL of acetone for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the red-purple color of the solution changes to blue-purple (indicator: 6 drops of bromoresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 11.61 mg of C₅H₁₀N₂·C₆H₁₀O₄

Containers and storage Containers—Well-closed containers.

Piperazine Phosphate Hydrate

ピペラジリン酸塩水和物

C₅H₁₀N₂·H₃PO₄·H₂O: 202.15

Piperazine monophosphate monohydrate

[1853-4-18-4]

Piperazine Phosphate Hydrate contains not less
than 98.5% of piperazine phosphate (C₄H₁₀N₂.H₃PO₄: 184.13), calculated on the anhydrous basis.

**Description** Piperazine Phosphate Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste.

It is soluble in formic acid, sparingly soluble in water, very slightly soluble in acetic acid (100), and practically insoluble in methanol, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid.

Melting point: about 222°C (with decomposition).

**Identification** (1) To 3 mL of a solution of Piperazine Phosphate Hydrate (1 in 100) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Piperazine Phosphate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Piperazine Phosphate Hydrate (1 in 100) responds to Qualitative Tests <1.09> (1) and (3) for phosphate.

**pH** <2.5> Dissolve 1.0 g of Piperazine Phosphate Hydrate in 100 mL of water: the pH of the solution is between 6.0 and 6.5.

**Purity** (1) Chloride <1.07>—To 0.5 g of Piperazine Phosphate Hydrate add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(2) Heavy metals <1.11>—To 2.0 g of Piperazine Phosphate Hydrate add 5 mL of dilute hydrochloric acid, 30 mL of water and 2 mL of dilute acetic acid, and dissolve. Add sodium hydroxide TS, adjust the pH of the solution to 3.3, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.17>—Dissolve 2.0 g of Piperazine Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and use this solution as the test solution. Perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 50 mg of Piperazine Phosphate Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28), acetone and ethanol (99:5) (8:3:3:2) to a distance of about 13 cm, and air-dry the plate. Spray evenly 4-dimethylaminocinnamaldehyde TS, and allow to stand for 15 minutes: the spots other than the principal spot and the spot on the starting line from the sample solution are not more intense than the spot from the standard solution.

**Water** <2.48> 8.0 - 9.5% (0.3 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 0.15 g of Piperazine Phosphate Hydrate, dissolve in 10 mL of formic acid, add 60 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 9.207 mg of C₄H₁₀N₂.H₃PO₄

**Containers and storage** Containers—Well-closed containers.

### Piperazine Phosphate Tablets

**ピペラジンリン酸塩錠**

Piperazine Phosphate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of piperazine phosphate hydrate (C₄H₁₀N₂.H₃PO₄.H₂O: 202.15).

**Method of preparation** Prepare as directed under Tablets, with Piperazine Phosphate Hydrate.

**Identification** Take a quantity of Piperazine Phosphate Tablets equivalent to 0.1 g of Piperazine Phosphate Hydrate according to the labeled amount, previously powdered, add 10 mL of water, shake while warming for 10 minutes, allow to cool, and filter. To 3 mL of the filtrate add 3 drops of Reinecke salt TS: a light red precipitate is formed.

**Disintegration** <6.09> It meets the requirement. The time limit of the test is 10 minutes.

**Assay** Weigh accurately not less than 20 Piperazine Phosphate Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 0.15 g of piperazine phosphate hydrate (C₄H₁₀N₂.H₃PO₄.H₂O). Add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. To the residue add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. Repeat twice the same procedure with 5 mL each of acetic acid (100), combine all the supernatant liquids, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 10.11 mg of C₄H₁₀N₂.H₃PO₄.H₂O

**Containers and storage** Containers—Tight containers.
Pirarubicin
ピラルビシン

\[
\text{C}_{32}\text{H}_{37}\text{NO}_{12}: 627.64 \\
(25,45)-4\{3\text{-Amino}-2,3,6\text{-trideoxy}-4\text{-O\{[(2R)-3,4,5,6-} \\
\text{tetrahydro-2H-pyran-2-yl]\text{-6-L-lyxo-hexopyranosyloxy}}} \\
\text{2,5,12-trihydroxy-2-hydroxyacetyl}-7\text{-methoxy-1,2,3,4-} \\
tetrahydrotetracene-6,11\text{-dione}
\]

Pirarubicin is a derivative of daunorubicin.
It contains not less than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pirarubicin is expressed as mass (potency) of pirarubicin (C_{32}H_{37}NO_{12}).

**Description** Pirarubicin occurs as a red-orange crystalline powder.
It is soluble in chloroform, very slightly soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** (1) Dissolve 10 mg of Pirarubicin in 80 mL of methanol and 6 mL of diluted hydrochloric acid (1 in 5000), and add water to make 100 mL. To 10 mL of this solution add diluted methanol (4 in 5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pirarubicin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Pirarubicin and Pirarubicin RS in 5 mL of chloroform, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (5:1) to a distance of about 10 cm, and air-dry the plate. Examine the spots with the necked eye: the principal spot obtained from the sample solution and the spot from the standard solution show a red-orange color and the same Rf value.

**Optical rotation** <2.49> \([\alpha]_{D}^{20}: +195 – +215^\circ\) (10 mg, chloroform, 10 mL, 100 mm).

**Purity** (1) Clarity and color of solution—Dissolve 10 mg of Pirarubicin in 10 mL of 0.01 mol/L hydrochloric acid TS: the solution is clear and red.
(2) Heavy metals <1.07>—Proceed with 1.0 g of Pirarubicin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Pirarubicin in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of doxorubicin, having the relative retention time of about 0.45 with respect to pirarubicin, and the area of the peak, having the relative retention time of about 1.2 with respect to pirarubicin, obtained from the sample solution are not larger than the peak area of pirarubicin from the standard solution, respectively, and the sum of the areas of the peaks, having the relative retention times of about 1.9 and about 2.0 with respect to pirarubicin, from the sample solution is not larger than 5 times the peak area of pirarubicin from the standard solution. For these calculations, use the peak area for doxorubicin after multiplying by the relative response factor 0.94 and the area for the two peaks, having the relative retention times of about 1.9 and about 2.0, after multiplying by their relative response factors, 1.09, respectively.

**Operating conditions**—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 4 times as long as the retention time of pirarubicin.

**System suitability**—
Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pirarubicin obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.
System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

**Water** <2.48> Not more than 2.0% (0.1 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Pirarubicin and Pirarubicin RS, equivalent to about 10 mg (potency), and dissolve in the mobile phase to make exactly 10 mL. Pipet 5 mL of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{S} and Q_{S}, of the peak area of pirarubicin to that of the internal standard.

\[\text{Amount} \ [\mu \text{g (potency)}] \text{of } C_{32}H_{37}NO_{12} \times 1000 = M \times Q_S / Q_S \times \text{Amount} \ [\mu \text{g (potency)}] \text{of Pirarubicin RS}\]

\[M_S: \text{Amount} \ [\mu \text{g (potency)}] \text{of Pirarubicin RS}\]

**Internal standard solution**—A solution of 2-naphthol in the mobile phase (1 in 1000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Pirenoxine

Description Pirenoxine occurs as a yellow-brown powder. It is odorless, and has a slightly bitter taste.

It is very slightly soluble in dimethylsulfoxide, and practically insoluble in water, in acetonitrile, in ethanol (95%), in carboxylic acid of phosphate buffer solution, pH 6.5, add 5 mL of a solution of phosphoric acid. To 700 mL of this solution add 200 mL of acetonitrile and 30 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of pirarubicin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, pirarubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pirarubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Purity (1) Heavy metals \( <1.0 \%) — Proceed with 1.0 g of Pirenoxine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 10 mg of Pirenoxine in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.0 \) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than pirenoxine is not larger than the peak area of pirenoxine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.39 g of tetra n-butylammonium chloride and 4.5 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, and adjust the pH to 6.5 with phosphoric acid. To 700 mL of this solution add 200 mL of acetonitrile and 30 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of pirenoxine is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of pirenoxine.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 30 mL. Confirm that the peak area of pirenoxine obtained from 5 \( \mu \)L of this solution is equivalent to 5 to 8% of that of pirenoxine obtained from 5 \( \mu \)L of the standard solution.

System performance: Dissolve 3 mg of Pirenoxine and 16 mg of methyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 5 \( \mu \)L of this solution under the above operating conditions, pirenoxine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pirenoxine is not more than 1.0%.

Loss on drying \( <2.4 \%) — Not more than 1.5% (0.5 g, in vacuum, 80°C, 3 hours).

Residue on ignition \( <2.4 \%) — Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Pirenoxine, previously dried, dissolve in 140 mL of dimethylsulfoxide by heating on a water bath. After cooling, add 30 mL of water, and titrate \( <2.5 \) immediately with 0.02 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium hydroxide VS 7 = 6.165 mg of C\(_{16}\)H\(_8\)N\(_2\)O\(_5\).

Containers and storage Containers—Tight containers.
Pirenzepine Hydrochloride Hydrate

ピレンゼピン塩酸塩水和物

\[ \text{Pirenzepine Hydrochloride Hydrate} \]

C\textsubscript{19}H\textsubscript{21}N\textsubscript{5}O\textsubscript{2}.2HCl·H\textsubscript{2}O: 442.34

11-[(4-Methylpiperazin-1-yl)acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one dihydrochloride monohydrate

[29868-97-1, anhydride]

Pirenzepine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of pirenzepine hydrochloride (C\textsubscript{19}H\textsubscript{21}N\textsubscript{5}O\textsubscript{2}.2HCl: 424.32), calculated on the anhydrous basis.

**Description**

Pirenzepine Hydrochloride Hydrate occurs as a white to pale yellow crystalline powder.

It is freely soluble in water and in formic acid, slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

The pH of a solution by dissolving 1 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water is between 1.0 and 2.0.

Melting point: about 245°C (with decomposition).

It is gradually colored by light.

**Identification (1)**

Determine the absorption spectrum of a solution of Pirenzepine Hydrochloride Hydrate (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pirenzepine Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pirenzepine Hydrochloride Hydrate (1 in 50) responds to Qualitative Tests <1.09> for chloride.

**Purity (1)**

Clarity and color of solution—A solution obtained by dissolving 1.0 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water is clear and not more color than that of the following control solution.

Control solution: To 1.2 mL of Matching fluid for color F add 8.8 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Pirenzepine Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.3 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water. To 1 mL of this solution add 5 mL of methanol and the mobile phase A to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add 5 mL of methanol and the mobile phase A to make exactly 10 mL. Pipet 1 mL of this solution, add 5 mL of methanol and the mobile phase A to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than pirenzepine is not larger than 3/10 times the peak area of pirenzepine from the standard solution, and the total area of the peaks other than pirenzepine is not larger than 3/5 times the peak area of pirenzepine from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 2 g of sodium lauryl sulfate in 900 mL of water, adjust the pH to 3.2 with acetic acid (100), and add water to make 1000 mL.

Mobile phase B: Methanol.

Mobile phase C: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
<th>Mobile phase C (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 15</td>
<td>55 → 25</td>
<td>30</td>
<td>15 → 45</td>
</tr>
<tr>
<td>15 –</td>
<td>25</td>
<td>30</td>
<td>45</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of pirenzepine is about 8 minutes.

Time span of measurement: About 2 times as long as the retention time of pirenzepine beginning after the solvent peak.

**System suitability**—

Test for required detectability: Pipet 1 mL of the standard solution, and add 5 mL of methanol and the mobile phase A to make exactly 10 mL. Confirm that the peak area of pirenzepine obtained from 10 µL of this solution is equivalent to 7 to 13% of that from 10 µL of the standard solution.

System performance: Dissolve 0.1 g of phenylpiperazine hydrochloride in 10 mL of methanol. Mix 1 mL of this solution and 1 mL of the sample solution, and add 5 mL of methanol and the mobile phase A to make 10 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, pirenzepine and phenylpiperazine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pirenzepine is not more than 2.0%.

**Water <2.48>**

Not less than 3.5% and not more than 5.0%
***Piroxicam***

![Structure of Piroxicam](image)

C₁₅H₁₃N₃O₄S: 331.35

4-Hydroxy-2-methyl-N-(pyridin-2-yl)-2H-1,1-
benzothiazine-3-carboxamide 1,1-dioxide

[36322-90-4]

Piroxicam contains not less than 98.5% and not more than 101.0% of C₁₅H₁₃N₃O₄S, calculated on the dried basis.

**Description** Piroxicam occurs as a white to pale yellow crystalline powder.

It is sparingly soluble in acetic anhydride, slightly soluble in acetonitrile, in methanol and in ethanol (99.5), very slightly soluble in acetic acid (100), and practically insoluble in water.

Melting point: about 200°C (with decomposition).

**Identification**

1. Dissolve 0.1 g of Piroxicam in a mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 200 mL. To 1 mL of this solution add the mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 100 mL. Determine the absorption spectrum of this solution as directed under UV-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Piroxicam as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample with dichloromethane, evaporate the solvent, dry the residue on a water bath, and perform the test.

**Purity**

1. Heavy metals (1.07)—Proceed with 1.0 g of Piroxicam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

2. Related substances—Dissolve 75 mg of Piroxicam in 50 mL of acetonitrile for liquid chromatography, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile for liquid chromatography to make exactly 10 mL. Pipet 1 mL of this solution, add acetonitrile for liquid chromatography to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01), according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than piroxicam obtained with the sample solution is not larger than the peak area of piroxicam with the standard solution, and the total area of the peaks other than piroxicam is not larger than 2 times the peak area of piroxicam with the standard solution.

**Operating conditions**

- Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and acetonitrile for liquid chromatography (3:2).
- Flow rate: Adjust the flow rate so that the retention time of piroxicam is about 10 minutes.
- Time span of measurement: About 5 times as long as the retention time of piroxicam beginning after the solvent peak.

**System suitability**

- Test for required detectability: To exactly 5 mL of the standard solution add acetonitrile for liquid chromatography to make exactly 20 mL. Confirm that the peak area of piroxicam obtained with 20 μL of this solution is equivalent to 17.5 to 32.5% of that with 20 μL of the standard solution.
- System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piroxicam are not less than 6000 and not more than 1.5, respectively.
- System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of piroxicam is not more than 2.0%.

**Loss on drying** (2.4) Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** (2.44) Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.25 g of Piroxicam, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100): (1:1), and titrate (2.50) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.14 mg of C₁₅H₁₃N₃O₄S

**Containers and storage** Containers—Tight containers.
Pivmecillinam Hydrochloride

ピブメシリンアム塩酸塩

\[
\text{C}_{21}\text{H}_{33}\text{N}_{3}\text{O}_{5}\text{S.HCl}: 476.03
\]

2,2-Dimethylpropanoyloxymethyl (25S,5R,6R)-6-[(azepan-1-ylmethylene)amin]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [32887-03-9]

Pivmecillinam Hydrochloride contains not less than 630 μg (potency) and not more than 710 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pivmecillinam Hydrochloride is expressed as mass (potency) of mecillinam (C\text{15}H\text{23}N\text{3}O\text{3}S: 325.43).

Description Pivmecillinam Hydrochloride occurs as a white to yellowish white crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in water and in ethanol (99.5), and soluble in acetonitrile.

Identification (1) Determine the infrared absorption spectrum of Pivmecillinam Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Pivmecillinam Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.5 g of Pivmecillinam Hydrochloride in 10 mL of water, and add 1 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation <2.49> \([\alpha]_D^20 + 200 – +220^\circ\) (1 g calculated on the anhydrous basis, water, 100 mm).

Purity (1) Heavy metals <1.07>—To 1.0 g of Pivmecillinam Hydrochloride in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 3 mL of hydrochloric acid to the residue, dissolve by warming on a water bath, and heat to dryness. To the residue add 10 mL of water, and dissolve by warming on a water bath. After cooling, adjust the pH to 3 to 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, and wash the crucible and the filter with 10 mL of water. Put the filtrate and the washings to a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution in the same manner as the test solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pivmecillinam Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Pivmecillinam Hydrochloride in 4.0 mL of a mixture of acetonitrile and acetic acid (100:97:3), and use this solution as the sample solution. Separately, dissolve 2.0 mg of Pivmecillinam Hydrochloride RS in 4.0 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography, allow to stand for 30 minutes, then spot 2 μL of the sample solution on the plate. Immediately, develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 10 minutes in iodine vapor: the spot from the sample solution appeared at the position corresponding to the spot obtained from the standard solution is not larger and not more intense than the spot from the standard solution, and any spot other than the principal spot and the above spot is not observable.

Water <2.49> Not more than 1.0% (0.25 g, coulometric titration).

Assay Weigh accurately an amount of Pivmecillinam Hydrochloride and Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, \(Q_1\) and \(Q_2\), of the peak area of pivmecillinam to that of the internal standard.

\[
\text{Amount} [\mu g \text{ (potency)}] \text{ of mecillinam} (\text{C}_{15}\text{H}_{23}\text{N}_{3}\text{O}_{3}\text{S}) = M_5 \times Q_1 / Q_2 \times 1000
\]

\(M_5\): Amount [mg (potency)] of Pivmecillinam Hydrochloride RS

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12,500).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 3.5 with acetic acid (100), and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of pivmecillinam is about 6.5 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pivmecillinam and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pivmecillinam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Pivmecillinam Hydrochloride Tablets

ビブメシリナム塩酸塩錠

Pivmecillinam Hydrochloride Tablets contains not less than 93.0% and not more than 107.0% of the labeled potency of mecillinam (C\textsubscript{15}H\textsubscript{23}N\textsubscript{3}O\textsubscript{3}S: 325.43).

**Method of preparation** Prepare as directed under Tablets, with Pivmecillinam Hydrochloride.

**Identification** Powder Pivmecillinam Hydrochloride Tablets, dissolve a portion of the powder, equivalent to 35 mg (potency) of Pivmecillinam Hydrochloride according to the labeled amount, in 4 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \text{m} \). Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately dissolve 25 mg of Pivmecillinam Hydrochloride RS in 2 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 2 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and immediately develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 10 minutes: the principal spot obtained from the sample solution has the same \( R_f \) value as the spot from the standard solution. Then, proceed as directed in the Assay under Pivmecillinam Hydrochloride.

**Internal standard solution**—A solution of diphenyl in the mobile phase (1 in 12,500).

**Water** Not more than 3.0% (1 g of powdered Pivmecillinam Hydrochloride Tablets, volumetric titration, direct titration).

**Uniformity of dosage units** Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Pivmecillinam Hydrochloride Tablets add 40 mL of the mobile phase, shake vigorously for 10 minutes, and add the mobile phase to make exactly 50 mL. Pipet 40 mL of the mobile phase, shake vigorously for 10 minutes, and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \text{m} \), discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \text{m} \), discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Pivmecillinam Hydrochloride.

\[
M_5 = \frac{M_S \times Q_1}{Q_2} \times \frac{V}{5} \times 5
\]

**Containers and storage** Containers—Tight containers.

**Live Oral Poliomyelitis Vaccine**

経口生ポリオワクチン

Live Oral Poliomyelitis Vaccine contains live attenuated poliovirus of type I, II and III. Monovalent or bivalent product may be prepared, if necessary. Live Oral Poliomyelitis Vaccine conforms to the requirements of Live Oral Poliomyelitis Vaccine in the Minimum Requirements for Biological Products.

**Description** Live Oral Poliomyelitis Vaccine is a light yellow-red to light red, clear liquid.

**Polymixin B Sulfate**

ポリミキシン B 硫酸塩

Polymixin B Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of Bacillus polymyxa.

It contains not less than 6500 units per mg, calculated on the dried basis. The potency of Polymixin B
Sulfate is expressed as mass unit of polymixin B (C_{35.56}H_{86.98}N_{16}O_{13}). One unit of Polymixin B Sulfate is equivalent to 0.129 μg of polymixin B sulfate (C_{35.56}H_{86.98}N_{16}O_{13}.1\cdot2H_2SO_4).

**Description** Polymixin B Sulfate occurs as a white to yellow-brown powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

**Identification (1)** To 5 mL of a solution of Polymixin B Sulfate (1 in 10) add 5 mL of a solution of sodium hydroxide (1 in 10), 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) while shaking: a purple color develops.

(2) Transfer 5 mg each of Polymixin B Sulfate and Polymixin B Sulfate RS separately into two glass-stoppered test tubes, add 1 mL of diluted hydrochloric acid (1 in 2), stopper the tube, heat at 135°C for 5 hours, then heat to dryness on a water bath, and keep the heating until no more hydrochloric acid odor is evolved. Dissolve the residue in 0.5 mL of water, and use these solutions as the sample solution and standard solution (1). Separately, dissolve 20 mg each of l-leucine, l-threonine, phenylalanine and l-serine separately in 10 mL of water, and use these solutions as the standard solutions (2), (3), (4) and (5), respectively. Perform the test in 10 mL of water, and use these solutions as the standard stock solution and the low concentration standard solution and the high concentration standard solution.

(iv) Sample solutions—Weigh accurately an amount of Polymixin B Sulfate, equivalent to about 200,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iii) Standard solutions—Weigh accurately an amount of Polymixin B Sulfate RS, equivalent to about 200,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Polyoxyl 40 Stearate**

Polyoxyl 40 Stearate is the monostearate of condensation polymers of ethylene oxide represented by the formula H(OCH2CH2)_nOCOC17H35, in which n is approximately 40.

**Description** Polyoxyl 40 Stearate occurs as a white to light yellow, waxy solid or powder. It is odorless or has a faint fat-like odor.

It is soluble in water, in ethanol (95) and in diethyl ether.

**Consealing point** 39.0 – 44.0°C

**Consealing point of the fatty acid** Not below 53°C.

**Acid value** Not more than 1.

**Saponification value** 25 – 35
### Polysorbate 80

ポリソルベート 80

Polysorbate 80 is a polyoxyethylene ether of anhydrous sorbitol, partially esterified with oleic acid.

**Description** Polysorbate 80 is a colorless or orange-yellow, viscous liquid, having a faint, characteristic odor and a warm, slightly bitter taste.

It is miscible with methanol, with ethanol (95), with warm ethanol (95), with pyridine and with chloroform.

It is freely soluble in water and slightly soluble in diethyl ether.

The pH of a solution of Polysorbate 80 (1 in 20) is between 5.5 and 7.5.

**Identification** (1) To 5 mL of a solution of Polysorbate 80 (1 in 20) add 5 mL of sodium hydroxide TS, boil for 5 minutes, cool, and acidify with dilute hydrochloric acid: the solution is opalescent.

(2) To 5 mL of a solution of polysorbate 80 (1 in 20) add 2 to 3 drops of bromine TS: the color of the test solution is discharged.

(3) Mix 6 mL of Polysorbate 80 with 4 mL of water at an ordinary, or lower than ordinary, temperature: a jelly-like mass is produced.

(4) To 10 mL of a solution of Polysorbate 80 (1 in 20) add 5 mL of ammonium thiocyanate-cobalt (II) nitrate TS, shake well, add 5 mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer.

**Viscosity** <2.53> 345 – 445 mm²/s (Method 1, 25°C).

**Specific gravity** <1.13> d²⁰⁰: 1.065 – 1.095

**Acid value** <1.13> Not more than 2.0.

**Saponification value** <1.13> 45 – 55

**Iodine value** <1.13> 19 – 24 Use chloroform instead of cyclohexane, and titrate <2.50> without using an indicator, until the yellow color of iodine disappears.

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Polysorbate 80 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Polysorbate 80 according to Method 3, and perform the test (not more than 2 ppm).

### Potassium Bromide

カリウムの亜化物

Potassium Bromide contains not less than 40.0% as fatty acids.

**Method of preparation**

<table>
<thead>
<tr>
<th>Fixed oil</th>
<th>470 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Hydroxide</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td>Water, Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td>To make</td>
<td>1000 g</td>
</tr>
</tbody>
</table>

Dissolve Potassium Hydroxide, in required quantity for saponification, in Water, Purified Water or Purified Water in Containers, add this solution to fixed oil, previously warmed, add a sufficient quantity of Ethanol if necessary, stir thoroughly, heat in a water bath, and continue the saponification. After complete saponification, add Water, Purified Water or Purified Water in Containers to make 1000 g.

**Description** Potash Soap occurs as a yellow-brown, transparent, unctuous, soft mass, having a characteristic odor.

It is freely soluble in water and in ethanol (95).

**Purity** Silicic acid and alkalinity—Dissolve 10 g of Potash Soap in 30 mL of ethanol (95), and add 0.50 mL of 1 mol/L hydrochloric acid VS: no turbidity is produced. Add 1 drop of phenolphthalein TS to this solution: no red color develops.

**Assay** Weigh accurately about 5 g of Potash Soap, dissolve in 100 mL of hot water, and transfer to a separator. Acidify the mixture with dilute sulfuric acid, and cool. Extract the solution with 50-mL, 40-mL, and 30-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 10-mL portions of water until the washing contains no acid. Transfer the diethyl ether solution to a tared flask, evaporate diethyl ether on a water bath at a temperature as low as possible. Dry the residue at 80°C to constant mass, and weigh as fatty acids.

**Containers and storage** Containers—Tight containers.

### Potassium Bromide

カリウムの亜化物

Potassium Bromide, when dried, contains not less than 99.0% of KBr.

**Description** Potassium Bromide occurs as colorless or
white crystals, granules or crystalline powder. It is odorless.

It is freely soluble in water and in glycerin, soluble in hot ethanol (95), and slightly soluble in ethanol (95).

**Identification** A solution of Potassium Bromide (1 in 10) responds to Qualitative Tests 1.09 for potassium salt and for bromide.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Bromide in 3 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Potassium Bromide in 10 mL of water, add 0.10 mL of 0.05 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boiling, and cool: no color develops.

(3) Chloride—Make a calculation from the result obtained in the Assay: not more than 84.5 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Potassium Bromide.

(4) Sulfate 1.14—Proceed with 2.0 g of Potassium Bromide, and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Iodide—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform, and shake: no red-purple to purple color develops in the chloroform layer.

(6) Bromate—Dissolve 1.0 g of Potassium Bromide in 10 mL of freshly boiled and cooled water, and add 0.1 mL of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently, and allow to stand for 5 minutes: no blue color develops.

(7) Heavy metals 1.07—Proceed with 2.0 g of Potassium Bromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(8) Barium—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

(9) Arsenic 1.11—Prepare the test solution with 1.0 g of Potassium Bromide according to Method 1, and perform the test (not more than 2 ppm).

**Loss on drying** 2.41 Not more than 1.0% (1 g, 110°C, 4 hours).

**Assay** Weigh accurately about 0.4 g of Potassium Bromide, previously dried, and dissolve in 50 mL of water. Add 10 mL of dilute nitric acid and exactly measured 50 mL of 0.1 mol/L silver nitrate VS, and titrate 2.50 the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS = 11.90 mg of KBr

**Containers and storage** Containers—Tight containers.

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**Potassium Canrenoate**

*Potassium Canrenoate* occurs as a pale yellowish white to pale yellow-brown, crystalline powder.

It is freely soluble in water, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in chloroform and in diethyl ether.

**Identification** (1) Dissolve 2 mg of Potassium Canrenoate in 2 drops of sulfuric acid: an orange color develops. Observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence. Add 1 drop of acetic anhydride to this solution: the color of the solution changes to red.

(2) Determine the absorption spectrum of a solution of Potassium Canrenoate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Potassium Canrenoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) The solution of Potassium Canrenoate (1 in 10) responds to Qualitative Tests 1.09 (1) for potassium salt.

**Optical rotation** 2.49 [α]D 0.71 -76° (after drying, 0.2 g, methanol, 20 mL, 100 mm).

**pH** 2.54 Dissolve 1.0 g of Potassium Canrenoate in 20 mL of water: the pH of this solution is between 8.4 and 9.4.

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Potassium Canrenoate in 5 mL of water: the solution is clear, and shows a pale yellow to light yellow color.

(2) Heavy metals 1.07—Proceed with 2.0 g of Potassium Canrenoate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic 1.11—Prepare the test solution with 1.0 g of Potassium Canrenoate according to Method 3, and perform the test (not more than 2 ppm).

(4) Canrenone—Place 0.40 g of Potassium Canrenoate in a glass-stoppered centrifuge tube, cool in ice-water to a
temperature not higher than 5°C, add 6 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0. being cooled to a temperature not higher than 5°C to dissolve, and add 8 mL of water being cooled to a temperature not higher than 5°C. Add exactly 10 mL of chloroform, allow to stand for 3 minutes at a temperature not higher than 5°C, shake vigorously for 2 minutes, and centrifuge. Drain off the water layer, collect 5 mL of the chloroform layer, transfer to a glass-stoppered centrifuge tube containing 3 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, cooled to a temperature not higher than 5°C, and 4 mL of water cooled to a temperature not higher than 5°C, shake for 1 minute, and centrifuge. Drain off the water layer, pipet 2 mL of the chloroform layer, and add chloroform to make exactly 10 mL. Determine the absorbance of this solution at 283 nm as directed under Ultraviolet-visible Spectrophotometry \( \leq 0.67 \).

**Loss on drying** \( < 2.4 \) Not more than 0.5% (1 g, 105°C, 4 hours).

**Assay** Weigh accurately about 0.2 g of Potassium Car-renoate, previously dried, dissolve in 75 mL of acetic acid (100), and titrate \( < 2.5 \) with 0.1 mol/L perchloric acid VS (potentiometric titration). Use a solution of saturated potassium chloride-acetic acid (100) as the internal liquid.). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
\[ = \frac{39.66 \text{ mg of } C_2H_2KO_4}{1000 \text{ mL}} \]

**Containers and storage** Containers—Tight containers.

### Potassium Carbonate

炭酸カリウム

\( K_2CO_3 \): 138.21

**Potassium Carbonate**, when dried, contains not less than 99.0% of \( K_2CO_3 \).

**Description** Potassium Carbonate occurs as white granules or powder. It is odorless.

It is very soluble in water, and practically insoluble in ethanol (95).

A solution of Potassium Carbonate (1 in 10) is alkaline.

It is hygroscopic.

**Identification** A solution of Potassium Carbonate (1 in 10) responds to Qualitative Tests \( < 1.09 \) for potassium salt and for carbonate.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Carbonate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals \( < 1.07 \)—Dissolve 1.0 g of Potassium Carbonate in 2 mL of water and 6 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 15 mL of water and 2 mL of dilute acetic acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 6 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution to dryness, and dilute with water to 50 mL (not more than 20 ppm).

(3) Sodium—Dissolve 1.0 g of Potassium Carbonate in 20 mL of water, and perform the test as directed under Flame Coloration Test \( < 1.04 \) (1): no persisting yellow color is produced.

(4) Arsenic \( \leq 1.11 \)—Prepare the test solution with 0.5 g of Potassium Carbonate, according to Method 1, and perform the test (not more than 4 ppm).

**Loss on drying** \( < 2.4 \) Not more than 1.0% (3 g, 180°C, 4 hours).

**Assay** Dissolve about 1.5 g of Potassium Carbonate, previously dried and accurately weighed, in 25 mL of water, titrate with 0.5 mol/L sulfuric acid VS until the blue color of the solution changes to yellow-green, boil cautiously, then cool, and titrate \( < 2.5 \) until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS
\[ = \frac{69.11 \text{ mg of } K_2CO_3}{1000 \text{ mL}} \]

**Containers and storage** Containers—Tight containers.

### Potassium Chloride

塩化カリウム

KCl: 74.55

Potassium Chloride, when dried, contains not less than 99% of KCl.

**Description** Potassium Chloride occurs as colorless or white crystals or crystalline powder. It is odorless, and has a saline taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Potassium Chloride (1 in 10) is neutral.

**Identification** A solution of Potassium Chloride (1 in 50) responds to Qualitative Tests \( < 1.09 \) for potassium salt and for chloride.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Chloride in 5 mL of water: the solution is clear and colorless.

(2) Acidity and alkalinity—Dissolve 5.0 g of Potassium Chloride in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS: no red color develops. Then add 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Bromide—Dissolve 1.0 g of Potassium Chloride in water to make 100 mL. To 5 mL of the solution add 3 drops of dilute hydrochloric acid and 1 mL of chloroform, and add 3 drops of sodium toluenesulfonchloramide TS dropwise while shaking: no yellow to yellow-red color develops in the chloroform layer.

(4) Iodide—Dissolve 0.5 g of Potassium Chloride in 10 mL of water, add 3 drops of iron (III) chloride TS and 1 mL of chloroform, shake, allow to stand for 30 minutes, and shake again: no red-purple to purple color develops in the chloroform layer.
Potassium Clavulanate / Official Monographs

Potassium Clavulanate

\[
\text{C}_8\text{H}_8\text{KNO}_5: 237.25
\]

Monopotassium (2R,5R)-3-[[1Z]-2-hydroxyethylidene]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptan-2-carboxylate

[6177-45-5]

Potassium Clavulanate is the potassium salt of a substance having \(\beta\)-lactamase inhibiting activity produced by the growth of \(\text{Streptomyces clavuligerus}\). It contains not less than 810 µg (potency) and not more than 860 µg (potency) per mg, calculated on the anhydrous basis. The potency of Potassium Clavulanate is expressed as mass (potency) of clavulanic acid (\(\text{C}_8\text{H}_8\text{KNO}_5\)): 199.16.

**Description** Potassium Clavulanate occurs as a white to light yellowish white, crystalline powder.

It is very soluble in water, soluble in methanol, and slightly soluble in ethanol (95).

It is hygroscopic.

**Identification** (1) To 1 mL of a solution of Potassium Clavulanate (1 in 50,000) add 5 mL of imidazole TS, and warm in a water bath at 30°C for 12 minutes. After cooling, determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Potassium Clavulanate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Potassium Clavulanate responds to Qualitative Tests <1.09> (1) for potassium salt.

**Optical rotation** <2.49> \([\alpha]_D^20 = +53 - +63^\circ\) (0.5 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Clavulanate according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Potassium Chloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Potassium Clavulanate in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak other than clavulanic acid from the sample solution is not larger than the peak area of clavulanic acid from the standard solution, and the total area of the peaks other than clavulanic acid from the sample solution is not larger than 2 times the peak area of clavulanic acid from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Adjust the pH 0.05 mol/L sodium dihydrogen phosphate TS to 4.0 with phosphoric acid.

Mobile phase B: A mixture of the mobile phase A and methanol (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 4</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4 – 15</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>15 – 25</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute.

Time span of measurement: About 6 times as long as the retention time of clavulanic acid.

**System suitability**—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase A to make exactly 10
mL. Confirm that the peak area of clavulanic acid obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: Dissolve 10 mg each of Potassium Clavulanate and Amoxycillin in 100 mL of the mobile phase A. When the procedure is run with 20 μL of this solution under the above operating conditions, clavulanic acid and amoxycillin are eluted in this order with the resolution between these peaks being not less than 8 and the number of theoretical plates of the peak of clavulanic acid is not less than 2500.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clavulanic acid is not more than 2.0%.

Water <2.48> Not more than 1.5% (5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Potassium Clavulanate and Lithium Clavulanate RS, equivalent to about 12.5 mg (potency), dissolve each in 30 mL of water, add exactly 5 mL of the internal standard solution and water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.04> according to the following conditions, and calculate the ratios, Qt and Qs, of the peak area of clavulanic acid to that of the internal standard.

\[
M_s = \frac{M_s \times Q_t}{Q_s} \times 1000
\]

\[
M_s: \text{Amount [mg (potency)] of Lithium Clavulanate RS}
\]

Internal standard solution—Dissolve 0.3 g of sulfanilamide in 30 mL of methanol, and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.36 g of sodium acetate trihydrate in 900 mL of water, adjust to pH 4.5 with diluted acetic acid (31) (2 in 5), and add 30 mL of methanol and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of clavulanic acid is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, clavulanic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clavulanic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

---

Potassium Guaiacolsulfonate

グアヤコールスルホン酸カリウム

\[
\text{C}_7\text{H}_7\text{KO}_5\text{S}: 242.29
\]

Monopotassium 4-hydroxy-3-methoxybenzenesulfonate [1321-14-8]

Potassium Guaiacolsulfonate contains not less than 98.5% of C7H7KO5S, calculated on the anhydrous basis.

Description Potassium Guaiacolsulfonate occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor and a slightly bitter taste.

It is freely soluble in water and in formic acid, soluble in methanol, and practically insoluble in ethanol (95), in acetic anhydride and in diethyl ether.

Identification (1) To 10 mL of a solution of Potassium Guaiacolsulfonate (1 in 100) add 2 drops of iron (III) chloride TS: a blue-purple color develops.

(2) Dissolve 0.25 g of Potassium Guaiacolsulfonate in water to make 500 mL, and to 10 mL of this solution add phosphate buffer solution, pH 7.0, to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.4> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Potassium Guaiacolsulfonate (1 in 10) responds to Qualitative Tests <1.06> for potassium salt.

pH <2.54> Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the pH of the solution is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <1.42>—Perform the test with 0.8 g of Potassium Guaiacolsulfonate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Potassium Guaiacolsulfonate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Potassium Guaiacolsulfonate according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Potassium Guaiacolsulfonate in 200 mL of mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.04> according to the following conditions. Determine each peak
area obtained from these solutions by the automatic integration method: the total area of peaks other than the peak of potassium guaiacolsulfonate from the sample solution is not larger than the peak area of potassium guaiacolsulfonate from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 279 nm).
Column: A stainless steel column 4 mm in inside diameter and 20 to 25 cm in length, packed with dimethylamino-propylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: A mixture of 0.05 mol/L potassium dihydrogenphosphate TS and methanol (20:1).
Flow rate: Adjust the flow rate so that the retention time of potassium guaiacolsulfonate is about 10 minutes.
Selection of column: Weigh 50 mg each of potassium guaiacolsulfonate and guaiacol, and dissolve in 50 mL of the mobile phase. Proceed with 5 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of guaiacol and potassium guaiacolsulfonate in order with the resolution of these peaks being not less than 4.
Detection sensitivity: Adjust the sensitivity so that the peak height of potassium guaiacolsulfonate from 5 μL of the standard solution is not less than 10 mm.
Time span of measurement: About twice as long as the retention time of potassium guaiacolsulfonate.

**Water** <2.48> 3.0 – 4.5% (0.3 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 0.3 g of Potassium Guaiacolsulfonate, dissolve in 2.0 mL of formic acid, add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.23 mg of C7H7KO5S

**Containers and storage** Containers—Well-closed containers.
Storage—Light-resistant.

**Potassium Hydroxide**

水酸化カリウム

KOH: 56.11

Potassium Hydroxide contains not less than 85.0% of KOH.

**Description** Potassium Hydroxide occurs as white fused masses, in small pellets, in flakes, in sticks and in other forms. It is hard and brittle, and shows a crystalline fracture.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It rapidly absorbs carbon dioxide in air.

It deliquesces in the presence of moisture.

**Identification (1)** A solution of Potassium Hydroxide (1 in 500) is alkaline.

(2) A solution of Potassium Hydroxide (1 in 25) responds to Qualitative Tests <1.06> for potassium salt.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Potassium Hydroxide in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 2.0 g of Potassium Hydroxide in water, and add water to make 100 mL. To 25 mL of the solution add 8 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.7 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.050%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Potassium Hydroxide in 5 mL of water, add 7 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid and 1 drop of ammonia TS, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 7 mL of dilute hydrochloric acid on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and add water to make 50 mL (not more than 30 ppm).

(4) Sodium—Dissolve 0.10 g of Potassium Hydroxide in 10 mL of dilute hydrochloric acid, and perform the test as directed under Flame Coloration Test <1.04> (1): no persistent yellow color develops.

(5) Potassium carbonate—The amount of potassium carbonate (K₂CO₃: 138.21) is not more than 2.0% when calculated by the following equation using B (mL) obtained in the Assay.

\[ \text{Amount of potassium carbonate (mg)} = 138.21 \times B \]

**Assay** Weigh accurately about 1.5 g of Potassium Hydroxide, and dissolve in 40 mL of freshly boiled and cooled water. Cool the solution to 15°C, add 2 drops of phenolphthalein TS, and titrate <2.50> with 0.5 mol/L sulfuric acid VS until the red color of the solution disappears. Record the amount A (mL) of 0.5 mol/L sulfuric acid VS consumed, then add 2 drops of methyl orange TS, and titrate <2.50> again with 0.5 mol/L sulfuric acid VS until the solution changes to a persistent light red color. Record the amount B (mL) of 0.5 mol/L sulfuric acid VS consumed.

Calculate the amount KOH from the amount, A (mL) = B (mL).

Each mL of 0.5 mol/L sulfuric acid VS = 56.11 mg of KOH

**Containers and storage** Containers—Tight containers.
crystals, or a white crystalline powder.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

It is slightly deliquescent in moist air.

**Identification** A solution of Potassium Iodide (1 in 20) responds to Qualitative Tests \(<1.09\) for potassium salt and for iodide.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Iodide in 2 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 0.50 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS: no color develops.

(3) Chloride, bromide and thiosulfate—Dissolve 0.20 g of Potassium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for 2 to 3 minutes, and filter. To 10 mL of the filtrate, add 15 mL of dilute nitric acid: no brown color develops. The solution has no more turbidity than that of the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ammonia TS, and 7.5 mL of 0.1 mol/L silver nitrate VS and 15 mL of dilute nitric acid.

(4) Nitrate, nitrite and ammonium—Place 1.0 g of Potassium Iodide in a 40-mL test tube, and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert the absorbent cotton in the mouth of the test tube, and place a piece of moistened red litmus paper on it. Heat the test tube carefully on a water bath for 15 minutes: the gas evolved does not turn red litmus paper to blue.

(5) Cyanide—Dissolve 0.5 g of Potassium Iodide in 10 mL of water. To 5 mL of this solution add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm, then add 4 mL of hydrochloric acid: no green color develops.

(6) Iodate—Dissolve 0.5 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color develops immediately.

(7) Heavy metals \(<1.07\)—Proceed with 2.0 g of Potassium Iodide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(8) Barium—Dissolve 0.5 g of Potassium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 5 minutes: no turbidity is produced.

(9) Sodium—Dissolve 1.0 g of Potassium Iodide in 10 mL of water, and perform the Flame Coloration Test (1) \(<1.04\): a yellow color develops, but does not persist.

(10) Arsenic \(<1.11\)—Prepare the test solution with 0.40 g of Potassium Permanganate for the preparation of the test solution. Evaporate the solution on a sand bath nearly to dryness. Dissolve the residue in 5 mL of water, and perform the test with this solution as the test solution: the color produced is not more intense than the following standard color.

Standard color: To 10 mL of water add 1 mL of sulfuric acid and the same volume of hydroperoxide (30) as used for the preparation of the test solution. Evaporate the solution on a sand bath nearly to dryness, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and carry out the test with this solution in the same manner as the test solution (not more than 5 ppm).

**Loss on drying** \(<2.4\>) Not more than 1.0% (2 g, 105°C, 4 hours).

**Assay** Weigh accurately about 0.6 g of Potassium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform, and titrate \(<2.5\) with 0.05 mol/L potassium iodate VS with shaking until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS = 16.60 mg of KMnO₄

**Containers and storage** Containers—Tight containers.

**Potassium Permanganate**

過マンガ酸カリウム

\[ \text{KMnO}_4 : 158.03 \]

Potassium Permanganate, when dried, contains not less than 99.0% of KMnO₄.

**Description** Potassium Permanganate occurs as dark purple crystals and has a metallic luster.

It is soluble in water.

A solution of Potassium Permanganate (1 in 1000) has a slightly sweet, astringent taste.

**Identification** A solution of Potassium Permanganate (1 in 100) responds to Qualitative Tests \(<1.09\) for permanganate.

**Purity** (1) Water-insoluble substances—Dissolve 2.0 g of Potassium Permanganate, previously powdered, in 200 mL of water. Filter the insoluble substances through a tared glass filter (G4), wash with water until the last washing shows no color, and dry at 105°C for 2 hours: the mass of the residue is not more than 4 mg.

(2) Arsenic \(<1.11\)>—Dissolve 0.40 g of Potassium Permanganate in 10 mL of water, add 1 mL of sulfuric acid, add hydrogen peroxide (30) dropwise until the solution remains colorless, and evaporate on a sand bath nearly to dryness. Dissolve the residue in 5 mL of water, and perform the test with this solution as the test solution: the color produced is not more intense than the following standard color.

Standard color: To 10 mL of water add 1 mL of sulfuric acid and the same volume of hydroperoxide (30) as used for the preparation of the test solution. Evaporate the solution on a sand bath nearly to dryness, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and carry out the test with this solution in the same manner as the test solution (not more than 5 ppm).

**Loss on drying** \(<2.4\>) Not more than 0.5% (1 g, silica gel, 18 hours).

**Assay** Weigh accurately about 0.6 g of Potassium Permanganate, previously dried, in 200 mL, and use this solution as the sample solution. Pipet 25 mL of 0.05 mol/L oxalic acid VS into a 500-mL conical flask, add 200 mL of diluted sulfuric acid (1 in 20), and keep at a temperature between 30°C and 35°C. Transfer the sample solution to a buret. Add quickly 23 mL of the sample solution from the buret to the flask while shaking gently, and then allow the flask to stand until the red color disappears. Warm the mixture to a temperature between 55°C and 60°C, and continue the titration \(<2.5\) slowly until the red color persists for 30 seconds.

Each mL of 0.05 mol/L oxalic acid VS = 3.161 mg of KMnO₄

**Containers and storage** Containers—Tight containers.
Potassium Sulfate

Potassium Sulfate, when dried, contains not less than 99.0% of K₂SO₄.

**Description**  Potassium Sulfate occurs as colorless crystals or a white, crystalline powder. It has a slightly saline, somewhat bitter taste.

It is soluble in water and practically insoluble in ethanol (95).

**Identification**  A solution of Potassium Sulfate (1 in 20) responds to Qualitative Tests <1.09> for potassium salt and for sulfate.

**Purity**  (1) Clarity and color of solution, and acid or alkali—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water: the solution is clear, colorless and neutral.

(2) Chloride <1.07>—Perform the test with 0.5 g of Potassium Sulfate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Sodium—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water, and perform the test as directed under Flame Coloration Test <1.04> (1): no persistent yellow color develops.

(5) Arsenic <1.10>—Prepare the test solution with 0.40 g of Potassium Sulfate according to Method 1, and perform the test (not more than 5 ppm).

**Loss on drying**  <2.41>  Not more than 1.0% (1 g, 110°C, 4 hours).

**Assay**  Weigh accurately about 0.5 g of Potassium Sulfate, previously dried, boil with 200 mL of water and 1.0 mL of hydrochloric acid, and add gradually 8 mL of boiling barium chloride TS. Heat the mixture on a water bath for 1 hour, collect the precipitate, and wash the precipitate with water until the last washing shows no opalescence on the addition of silver nitrate TS. Dry, heat strongly to constant mass until the last washing shows no opalescence on the addition of water: the solution is clear, colorless and neutral.

Amount (mg) of K₂SO₄ = amount (mg) of barium sulfate (BaSO₄) × 0.747

**Containers and storage**  Containers—Well-closed containers.

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Potato Starch

Amylum Solani

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (•  •).

Potato Starch consists of starch granules derived from the tuber of Solanum tuberosum Linné (Solanaceae).

**Description**  Potato Starch occurs as a white powder. It is practically insoluble in water and in ethanol (99.5).

**Identification**  (1) Examined under a microscope <5.01> using a mixture of water and glycerin (1:1), Potato Starch presents granules, either irregularly shaped, ovoid or pear-shaped, usually 30–100 μm in size but occasionally exceeding 100 μm, or rounded, 10–35 μm in size. There are occasional compound granules having two to four components. The ovoid and pear-shaped granules have an eccentric hilum and the rounded granules acentric or slightly eccentric hilum. All granules show clearly visible concentric striations. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Potato Starch add 50 mL of water, boil for 1 minute, and allow to cool: a subtle white-turbid, pasty liquid is formed.

(3) To 1 mL of the pasty liquid obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): an orange-red to deep blue color is formed, and the color disappears by heating.

**pH**  <2.54>  Put 5.0 g of Potato Starch in a non-metal vessel, add 25.0 mL of freshly boiled and cooled water, mix gently for 1 minute, and allow to stand for 15 minutes: the pH of the solution is between 5.0 and 8.0.

**Purity**  (1) Iron—To 1.5 g of Potato Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use as the control solution. Put 10 mL each of the test solution and the control solution into test tubes, add 2 mL of a solution of citric acid (1 in 5) and 0.1 mL of mercapto acetic acid, and mix. Alkalize with ammonia solution (28) to litmus paper, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not darker than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Potato Starch add 50.0 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate <2.50> with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm,
Sulfur dioxide—

(i) Apparatus Use as shown in the figure.

A: Boiling flask (500 mL)
B: Funnel (100 mL)
C: Condenser
D: Test-tube
E: Tap

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Potato Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

\[
\text{Amount (ppm) of sulfur dioxide} = \frac{V}{M} \times 1000 \times 3.203
\]

\[
M: \text{Amount (g) of the sample}
\]

\[
V: \text{Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed}
\]

(4) Foreign matter—Under a microscope, Potato Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant.

Loss on drying <2.4> Not more than 20.0% (1 g, 130°C, 90 minutes).

Residue on ignition <2.44> Not more than 0.6% (1 g).

*Containers and storage Containers—Well-closed containers.

Povidone

Polyvidone

Polyvinylpyrrolidone

ポビドン

\[
(C_{6}H_{9}NO)_{n}
\]

Poly[(2-oxopyrrolidin-1-yl)ethylene]

[9003-39-8]

Povidone is a chain polymer of 1-vinyl-2-pyrrolidone.

It contains not less than 11.5% and not more than 12.8% of nitrogen (N: 14.01), calculated on the anhydrous basis.

It has a nominal K-value of not less than 25 and not more than 90.

The nominal K-value is shown on the label.

Description Povidone occurs as a white to slightly yellowish fine powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water, in methanol and in ethanol (95), slightly soluble in acetone, and practically insoluble in diethyl ether.

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Povidone, previously dried at 105°C for 6 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Povidone RS previously dried at 105°C for 6 hours: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 1.0 g of Povidone in 20 mL of water: the pH of this solution is between 3.0 and 5.0 for Povidone having the nominal K-value of 30 or less, and between 4.0 and 7.0 for Povidone having the nominal K-value exceeding 30.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Povidone in 20 mL of water: the solution is clear and colorless to pale yellow, or pale red.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Povidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Aldehydes—Weigh accurately about 1.0 g of Povi-
done and dissolve in 0.05 mol/L pyrophosphate buffer solution, pH 9.0 to make exactly 100 mL. Stopper, heat at 60°C for 60 minutes, allow to cool to room temperature, and use this solution as the standard solution. Separately, dissolve 0.100 g of freshly distilled acetaldehyde in water previously cooled to 4°C to make exactly 100 mL. Allow to stand at 4°C for about 20 hours, pipet 1 mL of this solution, add 0.05 mol/L pyrophosphate buffer solution, pH 9.0 to make exactly 100 mL, and use this solution as the standard solution. Measure 0.5 mL each of the sample solution, standard solution and water (for blank test), transfer to separate cells, add 2.5 mL of 0.05 mol/L pyrophosphate buffer solution, pH 9.0, and 0.2 mL of β-nicotinamide adenine dinucleotide TS to each of these cells, mix and stopper tightly. Allow to stand for 2 to 3 minutes at 22 ± 2°C, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the control solution. Determine the absorbances, \( A_{T1}, A_{S1} \) and \( A_{B1} \) of the subsequent solutions of the sample solution, the standard solution and water at 340 nm. Add 0.05 mL of aldehyde dehydrogenase solution to each of the cells, mix and stopper tightly. Allow to stand for 5 minutes at 22 ± 2°C. Determine the absorbances, \( A_{T2}, A_{S2} \) and \( A_{B2} \) of these solutions in the same manner as above: the content of aldehydes is not more than 500 ppm (expressed as acetaldehyde).

\[
\text{Content (ppm) of aldehydes expressed as acetaldehyde} = \frac{1000 \times (A_{T2} - A_{T1}) - (A_{B2} - A_{B1})}{M} \\
\text{M: Amount (g) of Povidone, calculated on the anhydrous basis}
\]

(4) 1-Vinyl-2-pyrrolidone—Weigh accurately about 0.25 g of Povidone, dissolve in diluted methanol (1 in 5) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 1-vinyl-2-pyrrolidone in methanol to make exactly 100 mL. Pipet 1 mL of this solution and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 µL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of 1-vinyl-2-pyrrolidone in each solution: the content of 1-vinyl-2-pyrrolidone is not more than 10 ppm.  

\[
\text{Content (ppm) of 1-vinyl-2-pyrrolidone} = \frac{2.5 \times M \times A_T}{A_S}
\]

\( M \): Amount (g) of Povidone, calculated on the anhydrous basis

**Operating conditions—**  
Detector: An ultraviolet spectrophotometer (detection wavelength: 254 nm).

Column: Stainless steel columns about 4 mm in inside diameter and about 25 mm in length, and about 4 mm in inside diameter and about 250 mm in length, packed with octylsilsanized silica gel for liquid chromatography (5 µm in particle diameter), and use them as a guard column and a separation column, respectively.

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of 1-vinyl-2-pyrrolidone is about 10 minutes.

Selection of column: Dissolve 0.01 g of 1-vinyl-2-pyrrolidone and 0.5 g of vinyl acetate in 100 mL of methanol. To 1 mL of this solution add diluted methanol (1 in 5) to make 100 mL. Proceed with 50 µL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of 1-vinyl-2-pyrrolidone and vinyl acetate in this order with the resolution between these peaks being not less than 2.0.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 1-vinyl-2-pyrrolidone obtained from 50 µL of the standard solution is between 10 mm and 15 mm.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of obtained peak areas of 1-vinyl-2-pyrrolidone is not more than 2%.

Washing of the guard column: After each test with the sample solution, wash away the polymeric material of Povidone from the guard column by passing the mobile phase through the column backwards for about 30 minutes at the same flow rate as applied in the test.

(5) Peroxides—Weigh exactly an amount of Povidone, equivalent to 4.0 g calculated on the anhydrous basis, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. To 25 mL of the sample solution add 2 mL of titanium (III) chloride-sulfuric acid TS, and mix. Allow to stand for 30 minutes, and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by adding 2 mL of 13% sulfuric acid to 25 mL of the sample solution as a blank: the absorbance of the subsequent solution of the sample solution at 405 nm is not more than 0.35 (not more than 400 ppm, expressed as hydrogen peroxide).

(6) Hydrazine—Transfer 2.5 g of Povidone to a 50-mL centrifuge tube, add 25 mL of water, and stir to dissolve. Add 500 µL of a solution of salicylaldehyde in methanol (1 in 20), stir and warm at 60°C for 15 minutes in a water bath. Allow to cool, add 2.0 mL of toluene, stopper tightly, shake vigorously for 2 minutes, centrifuge, and use the upper layer of the mixture as the sample solution. Separately, dissolve 0.09 g of salicylaldehyde in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 µL each of the sample solution and standard solution on a plate coated with a 0.25-mm layer of dimethylsilinezsilica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and water (2:1) to a distance of about three-fourths of the length of the plate, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the RF value of the fluorescent spot from the standard solution is about 0.3, and the fluorescence of the spot from the sample solution corresponding to the spot from the standard solution is not more intense than that of the spot from the standard solution (not more than 1 ppm).

**Water <2.48>** Not more than 5.0% (0.5 g, volumetric titration, direct titration).

**Residue on ignition <2.44>** Not more than 0.1% (1 g).
**K-value**
Weigh accurately an amount of Povidone, equivalent to 1.00 g calculated on the anhydrous basis, and dissolve in water to make exactly 100 mL, allow to stand for 60 minutes, and use this solution as the sample solution. Perform the test with the sample solution and with water at 25°C as directed in Method 1 under Viscosity Determination ＜2.53＞, and calculate the K-value by the following formula.

\[
K = \frac{1.5 \log \eta_{ag} - 1}{0.15 + 0.003 c} + \frac{\sqrt{200 c \log \eta_{ag} + (c + 1.5 c \log \eta_{ag})^2}}{0.15 c + 0.003 c^2}
\]

c: Mass (g) of Povidone in 100 mL of the solution, calculated on the anhydrous basis
\(\eta_{ag}\): Kinematic viscosity of the sample solution relative to that of water

The K-value of Povidone is not less than 90% and not more than 108% of the nominal K-value.

**Assay**
Weigh accurately about 0.1 g of Povidone, and place in a Kjeldahl flask. Add 5 g of a powdered mixture of 33 g of potassium sulfate, 1 g of copper (II) sulfate pentahydrate and 1 g of titanium (IV) oxide, and wash down any adhering sample from the neck of the flask with a small amount of water. Add 7 mL of sulfuric acid allowing to flow down the inside wall of the flask. Heat the flask gradually over a free flame until the solution has a clear, yellow-green color and the inside wall of the flask is free from a car-bonaceous material, and then heat for further 45 minutes. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 mL of a solution of boric acid (1 in 25), 5 drops of bromocresol green-methyl red TS and sufficient distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small amount of water, and titrate with 0.025 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.025 mol/L sulfuric acid VS = 0.700 mg of N

**Containers and storage**
Containers—Tight containers.

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**Povidone-Iodine**

ポビドンヨード

(P_6H_9NO)_n\cdot xI
Poly[(2-oxopyrrolidin-1-yl)ethylene] iodine
[25655-41-8]

Povidone-Iodine is a complex of iodine with 1-vinyl-2-pyrrolidone polymer.

It contains not less than 9.0% and not more than 12.0% of available iodine (I: 126.90), and not less than 9.5% and not more than 11.5% of nitrogen (N: 14.01), calculated on the dried basis.

**Identification**
(1) To 10 mL of diluted starch TS (1 in 10) add 1 drop of a solution of Povidone-Iodine (1 in 10): a deep blue color develops.

(2) To 1 mL of a solution of Povidone-Iodine (1 in 100) add 1 mL of sodium thiosulfate TS, and add 1 mL of ammonium thiocyanate-cobalt (II) nitrate TS and 2 drops of 1 mol/L hydrochloric acid TS: a blue color develops, and a blue precipitate is gradually formed.

**Purity**
(1) Clarity and color of solution—Dissolve 0.30 g of Povidone-Iodine in 100 mL of water: the solution is clear and brown.

(2) Heavy metals ＜1.0＞—Proceed with 1.0 g of Povidone-Iodine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ＜1.1＞—Prepare the test solution with 1.0 g of Povidone-Iodine according to Method 4, and perform the test (not more than 2 ppm).

(4) Iodide ion—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 100 mL of water, and add sodium hydrosulfite TS until the color of iodine completely disappears. To this solution add exactly 25 mL of 0.1 mol/L silver nitrate VS, shake well with 10 mL of nitric acid, titrate with 0.025 mol/L ammonium thiocyanate VS until the solution develops a red-brown color, and calculate the total amount of iodine (indicator: 1 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L ammonium thiocyanate VS = 12.69 mg of I

Obtain the amount of iodide ion, calculated on the dried basis, by deducting the amount (%) of available iodine from the total amount (%) of iodine: it is not more than 6.6%.

**Loss on drying** ＜2.41＞ Not more than 8.0% (1 g, 100°C, 3 hours).
Pranoprofen

プラノプロフェン

Pranoprofen, when dried, contains not less than 98.5% of C_{15}H_{13}NO_3.

**Description** Pranoprofen occurs as a white to pale yellowish-white crystalline powder.

It is freely soluble in N,N-dimethylformamide, soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in acetonitrile, in ethanol (95) and in acetic anhydride, very slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Pranoprofen in N,N-dimethylformamide (1 in 30) shows no optical rotation.

**Identification** (1) Dissolve 0.2 g of Pranoprofen in 1 mol/L hydrochloric acid TS to make 100 mL, and dilute 10 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pranoprofen as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** \(<2.60\) 186 - 190°C

**Purity** (1) Chloride \(<1.03\)—Dissolve 0.5 g of Pranoprofen in 40 mL of methanol, and 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.3 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Heavy metals \(<1.07\)—Proceed with 2.0 g of Pranoprofen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of the Standard Lead Solution (not more than 10 ppm).

(3) Related Substances—Dissolve 50 mg of Pranoprofen in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.0\) according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the each area of the peaks other than the peak of pranoprofen from the sample solution is not larger than the peak area of pranoprofen from the standard solution, and the total peak area of them is not larger than 2 times the peak area of pranoprofen from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.02 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust the pH to 2.5 with perchloric acid. To 2 volumes of this solution add 1 volume of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pranoprofen is about 10 minutes.

Selection of column: Dissolve 4 mg each of Pranoprofen and ethyl parahydroxybenzoate in 200 mL of the mobile phase. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pranoprofen and ethyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 2.1.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of pranoprofen from 10 μL of the standard solution is between 10 mm and 20 mm.

Time span of measurement: About three times as long as the retention time of pranoprofen.

**Loss on drying** \(<2.44\) Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition** \(<2.44\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Pranoprofen, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate \(<2.50\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 25.53 mg of C_{15}H_{13}NO_3

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.
Pravastatin Sodium

プラバスタチンナトリウム

C₂₃H₃₅NaO₇·4·6·51
Monosodium (3R,5R)-3,5-dihydroxy-7-[(1S,2S,6S,8S,8aR)-6-hydroxy-2-methyl-8-[(2S)-2-methylbutanoyloxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoate [81131-70-6]

Pravastatin Sodium contains not less than 98.5% and not more than 101.0% of C₂₃H₃₅NaO₇, calculated on the anhydrous basis and corrected on the amount of the residual solvent.

Description

Pravastatin Sodium occurs as a white to yellowish white, powder or crystalline powder.

It is freely soluble in water and in methanol, and soluble in ethanol (99.5).

It is hygroscopic.

Identification

(1) Determine the absorption spectrum of a solution of Pravastatin Sodium (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pravastatin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2970 cm⁻¹, 2880 cm⁻¹, 1727 cm⁻¹ and 1578 cm⁻¹.

(3) Dissolve 50 mg of Pravastatin Sodium in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 24 mg of Pravastatin 1,1,3,3-Tetramethylbutyrammonium RS in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

(4) A solution of Pravastatin Sodium (1 in 10) responds to Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49>: +153° to +159° (0.1 g calculated on the anhydrous basis and corrected on the amount of residual solvent, water, 20 mL, 100 mm).

pH <2.54>: The pH of a solution obtained by dissolving 1.0 g of Pravastatin Sodium in 20 mL of freshly boiled and cooled water is between 7.2 and 8.2.

Purity

(1) Heavy metals <1.07>—Proceed with 1.0 g of Pravastatin Sodium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Pravastatin Sodium in 100 mL of a mixture of water and methanol (11:9), and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 5 mL of this solution, add the mixture of water and methanol (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than pravastatin is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin is not larger than the peak area of pravastatin from the standard solution. Keep the sample solution and standard solution at not over than 15°C.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of pravastatin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of water and methanol (11:9) to make exactly 50 mL. Confirm that the peak area of pravastatin obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: Dissolve 5 mg of pravastatin sodium in 50 mL of the mixture of water and methanol (11:9). When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water <2.48>—Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Assay

Weigh accurately about 0.1 g of Pravastatin Sodium, and dissolve in a mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (11:9) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Pravastatin 1,1,3,3-Tetramethylbutyrammonium RS (previously determine the water <2.48> with 0.5 g by direct titration in volumetric titration) dissolve in the mixture of water and methanol (11:9) to make exactly 25 mL. Proceed with exactly 10 mL of this solution in the same manner for the preparation of the sample solution, and use the solution so obtained as the standard solution. Per-
form the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of pravastatin to that of the internal standard.

\[
M_S = \frac{M_S \times Q_1}{Q_2 \times 4 \times 1.052}
\]

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mixture of water and methanol (11:9) (3 in 4000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 238 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of water, methanol, acetic acid (100) and triethylamine (550:450:1:1).
Flow rate: Adjust the flow rate so that the retention time of pravastatin is about 21 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Pravastatin Sodium Fine Granules
プラバスタチンナトリウム細粒

Pravastatin Sodium Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium (C_{23}H_{35}NaO_{7}: 446.51).

Method of preparation Prepare fine particles as directed under Granules, with Pravastatin Sodium.

Identification To an amount of Pravastatin Sodium Fine Granules, equivalent to 10 mg of Pravastatin Sodium according to the labeled amount, add 20 mL of water, agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and add water to 1 mL of the subsequent filtrate to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 237 nm and 241 nm.

Purity Related substances—The sample solution and the standard solution are stored at not exceeding 5°C after preparation. To an amount of Pravastatin Sodium Fine Granules, equivalent to 25 mg of Pravastatin Sodium according to the labeled amount, add 25 mL of a mixture of water and methanol (1:1), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention time of about 0.36 and about 1.9 to pravastatin, obtained from the sample solution is not larger than 1/2 times and 3 times the peak area of pravastatin from the standard solution, respectively, the area of the peak other than pravastatin and the peaks mentioned above obtained from the sample solution is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin obtained from the sample solution is not larger than 4.5 times the peak area of pravastatin from the standard solution. For this calculation, use the area of peaks, obtained by automatic integration method of related substances having the relative retention time of about 0.36, about 0.28 and about 0.88 to pravastatin, after multiplying by their relative response factors, 0.58, 0.86 and 0.82, respectively.

Operating conditions—
Detector: An ultraviolet spectrophotometer (wavelength: 238 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase A: A mixture of water, methanol, acetic acid (100) and triethylamine (50:450:1:1).
Mobile phase B: A mixture of methanol, water, acetic acid (100) and triethylamine (650:350:1:1).
Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>50 – 75</td>
<td>50 → 0</td>
<td>50 → 100</td>
</tr>
</tbody>
</table>

Flow rate: 1.3 mL per minute.
Time span of measurement: For 75 minutes after injection, beginning after the solvent peak.

System suitability—
Test for required detectability: To exactly 1 mL of the standard solution add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.
System performance: When the procedure is run with 20 μL of the standard solution under the above operating con-
ditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 1.5%.

**Uniformity of dosage units <6.02>** Perform the test according to the following method: the Pravastatin Sodium Fine Granules in single-unit container meets the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Pravastatin Sodium Fine Granules add exactly V mL of the internal standard solution so that each mL contains 0.25 mg of pravastatin sodium (C_{23}H_{35}NaO_{7}), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate add a mixture of water and methanol (1:1) to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pravastatin sodium (C_{23}H_{35}NaO_{7})

\[ M_s = M_s \times \frac{Q_1/Q_s \times V}{100 \times 1.052} \]

\( M_s \): Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

**Dissolution** <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Pravastatin Sodium Fine Granules is not less than 80%.

Start the test with an accurately weighed amount of Pravastatin Sodium Fine Granules, equivalent to about 5 mg of pravastatin sodium (C_{23}H_{35}NaO_{7}) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 23 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water <2.48>) in the same manner as Pravastatin Sodium, and dissolve in water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_{T1} \) and \( A_{S1} \), at 238 nm and \( A_{T2} \) and \( A_{S2} \) at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of pravastatin sodium (C_{23}H_{35}NaO_{7})

\[ \frac{M_s}{M_t} \times \left( \frac{A_{T1} - A_{T2}}{A_{S1} - A_{S2}} \right) \times \frac{1}{C} \times \frac{27}{0.806} \]

\( M_s \): Amount (mg) of pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

\( C \): Labeled amount (mg) of pravastatin sodium (C_{23}H_{35}NaO_{7}) in 1 g

**Particle size <6.03>** It meets the requirements of Fine granules.

**Assay** Weigh accurately an amount of Pravastatin Sodium Fine Granules, equivalent to about 5 mg of pravastatin sodium (C_{23}H_{35}NaO_{7}), add exactly 20 mL of the internal standard solution, agitate for 15 minute with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, to 2 mL of the subsequent filtrate add a mixture of water and methanol (1:1) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 32 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water <2.48>) in the same manner as Pravastatin Sodium), and dissolve in a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_s \), of the peak area of pravastatin to that of the internal standard.

Amount (mg) of pravastatin sodium (C_{23}H_{35}NaO_{7})

\[ M_s = M_s \times \frac{Q_1/Q_s \times 1/5 \times 1.052}{} \]

\( M_s \): Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

**Operating conditions**—
Proceed as directed in the operating conditions in the Assay under Pravastatin Sodium.

**System suitability**—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

**Containers and storage** Container—Well-closed containers.

**Pravastatin Sodium Solution**

プラバスタチンナトリウム液

Pravastatin Sodium Solution contains not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium (C_{23}H_{35}NaO_{7}; 446.51).

**Method of preparation** Prepare as directed under Liquids
and Solutions for Oral Administration, with Pravastatin Sodium.

**Identification** Pass a volume of Pravastatin Sodium Solution, equivalent to 1 mg of Pravastatin Sodium according to the labeled amount, through a column [5.5 mm in inside diameter, packed with 30 mg of divinylbenzene-\(N\)-vinyl pyrrolidone copolymer for column chromatography (30 \(\mu m\) in particle size), and washed with 1 mL of methanol and 1 mL of water]. Then wash with 1 mL of water, and elute with 1 mL of methanol. To 0.1 mL of the eluate add water to make 10 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.45\>) it exhibits a maximum between 237 nm and 241 nm.

**pH** Being specified separately.

**Purity** Related substances—The sample solution and the standard solution are stored at not exceeding 15°C after preparation. To a volume of Pravastatin Sodium Solution, equivalent to 2 mg of Pravastatin Sodium according to the labeled amount, add a mixture of methanol and water (5:3) to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu L\) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\>) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention time about 0.24 and 0.85 to pravastatin, obtained from the sample solution is not larger than 3/10 times the peak area of pravastatin and the peaks mentioned above obtained from the sample solution is not larger than 3/10 times the peak area of pravastatin from the standard solution.

**Operating conditions**—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of pravastatin, beginning after the solvent peak.

**System suitability**—
Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin obtained with 10 \(\mu L\) of this solution is equivalent to 15 to 25% of that with 10 \(\mu L\) of the standard solution.

System performance: When the procedure is run with 10 \(\mu L\) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3400 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 10 \(\mu L\) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 2.5%.

**Uniformity of dosage units** \(<4.02\>) The solution in single-unit container meets the requirement of the Mass variation test.

**Microbial limit** \(<4.05\>) The acceptance criteria of TAMC and TYMC are 10^2 CFU/mL and 10^3 CFU/mL, respectively. *Escherichia coli* is not observed.

**Assay** To a volume of Pravastatin Sodium Solution, equivalent to 2 mg of pravastatin sodium (C_{23}H_{35}NaO_{7}), add exactly 5 mL of the internal standard solution, add water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water \(<2.45\>) in the same manner as Pravastatin Sodium), and dissolve in a solution of disodium hydrogen phosphate dodecahydrate (1 in 200) to make exactly 50 mL. Pipet 6 mL of this solution, add exactly 5 mL of the internal standard solution, add water to make 100 mL, and use this solution as the standard solution. Perform the test with 10 \(\mu L\) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\>) according to the following conditions. Calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of pravastatin to that of the internal standard.

Amount (mg) of pravastatin sodium

\[
M_S = M_S \times \frac{Q_T}{Q_S} \times \frac{3}{25} \times 1.052
\]

\(M_S\): Amount (mg) of pravastatin in Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis.

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in methanol (3 in 10,000).

**Operating conditions**—
Detector: An ultraviolet spectrophotometer (wavelength: 238 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (10 \(\mu m\) in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, methanol, acetic acid (100) and triethylamine (500:500:1:1).

Flow rate: Adjust the flow rate so that the retention time of pravastatin is about 20 minutes.

**System suitability**—
System performance: When the procedure is run with 10 \(\mu L\) of the standard solution under the above operating conditions, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 \(\mu L\) of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

**Containers and storage** Container—Tight containers.
Pravastatin Sodium Tablets

プラバスタチンナトリウム錠

Pravastatin Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium (C23H35NaO7: 446.51).

Method of preparation  Prepare as directed under Tablets, with Pravastatin Sodium.

Identification  To a quantity of powdered Pravastatin Sodium Tablets, equivalent to 10 mg of Pravastatin Sodium according to the labeled amount, add 20 mL of water, agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and add water to 1 mL of the subsequent filtrate to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 237 nm and 241 nm.

Purity  Related substances—The sample solution and the standard solution are stored at not exceeding 15°C after preparation. To a quantity of powdered Pravastatin Sodium Tablets, equivalent to 50 mg of Pravastatin Sodium according to the labeled amount, add 40 mL of a mixture of water and methanol (1:1), agitate with the aid of ultrasonic waves, then add a mixture of water and methanol (1:1) to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL of the standard solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention time about 0.36 and about 1.9 to pravastatin obtained from the sample solution is not larger than 3/10 times and 2 times the peak area of pravastatin from the standard solution, respectively, the area of the peak other than pravastatin and the peaks mentioned above obtained from the sample solution is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin obtained from the sample solution is not larger than 3 times the peak area of pravastatin from the standard solution. For this calculation, use the area of the peaks, having the relative retention time about 0.36, about 0.28 and about 0.88 to pravastatin, after multiplying by their respective response factors, 0.58, 0.86 and 0.82, respectively. Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, methanol, acetic acid (100) and triethylamine (750:250:1:1).

Mobile phase B: A mixture of methanol, water, acetic acid (100) and triethylamine (650:350:1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>50 – 75</td>
<td>50 → 0</td>
<td>50 → 100</td>
</tr>
</tbody>
</table>

Flow rate: 1.3 mL per minute.

Time span of measurement: For 75 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 1.5%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Pravastatin Sodium Tablets add exactly V mL of the internal standard solution so that each mL contains 0.25 mg of pravastatin sodium (C23H35NaO7), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. To 2 mL of the supernatant liquid add a mixture of water and methanol (1:1) to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pravastatin sodium (C23H35NaO7) = Mₛ × Qₛ / Qₛ × V / 100 × 1.052

Mₛ: Amount (mg) of pravastatin in Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Pravastatin Sodium Tablets is not less than 85%.

Start the test with 1 tablet of Pravastatin Sodium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 3.5 μg of pravastatin (C23H36O7) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Pravastatin
1,1,3,3-Tetramethylbutylammonium RS (separately determine the water 2.48) in the same manner as Pravastatin Sodium), and dissolve in water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, $A_{11}$ and $A_{31}$, at 238 nm and $A_{12}$ and $A_{32}$ at 256 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24.

Dissolution rate (%) with respect to the labeled amount of pravastatin sodium ($C_{23}H_{35}NaO_7$)

$$M_S = \frac{M_S}{C \times V} = \frac{A_{11} - A_{12}}{A_{31} - A_{32}} \times \frac{1}{C} \times \frac{V}{L}$$

$M_S$: Amount (mg) of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

$C$: Labeled amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Pravastatin Sodium Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), add exactly 40 mL of the internal standard solution, stir for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, to 2 mL of the subsequent filtrate add a mixture of water and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 32 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water 2.48 in the same manner as Pravastatin Sodium), and dissolve in a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions. Calculate the ratios, $Q_T$ and $Q_S$, of the peak area of pravastatin to that of the internal standard.

Amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$)

$$M_S = \frac{M_S}{C \times V} = \frac{A_{11} - A_{12}}{A_{31} - A_{32}} \times \frac{1}{C} \times \frac{V}{L}$$

$M_S$: Amount (mg) of pravastatin in Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) in 10,000.

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Pravastatin Sodium.

System suitability—

System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage Container—Well-closed containers.

Prazepam

プラゼパム

C$_{19}$H$_{17}$ClN$_2$O: 324.80
7-Chloro-1-(cyclopropylmethyl)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one [2955-38-6]

Prazepam, when dried, contains not less than 98.5% of C$_{19}$H$_{17}$ClN$_2$O.

Description Prazepam occurs as white to light yellow crystals or crystalline powder. It is odorless. It is freely soluble in acetone, soluble in acetic anhydride, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 0.01 g of Prazepam in 3 mL of sulfuric acid, and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a grayish blue fluorescence.

(2) Dissolve 0.01 g of Prazepam in 1000 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Prazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the Flame Coloration Tests 2.04 (2) with Prazepam: a green color appears.

Melting point 2.60 145 – 148°C

Purity (1) Chloride 1.05—To 1.0 g of Prazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate 1.14—To 20 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals 1.07—Proceed with 2.0 g of Praze-
Prazepam Tablets

プラゼパム錠

Prazepam Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of prazepam (C₁₉H₁₇ClN₂O: 324.80).

Method of preparation  Prepare as directed under Tablets, with Prazepam.

Identification (1)  To a quantity of powdered Prazepam Tablets, equivalent to 0.05 g of Prazepam according to the labeled amount, add 25 mL of acetone, shake well, and filter. Take 5 mL of the filtrate, evaporate on a water bath to dryness, and dissolve the residue in 3 mL of sulfuric acid. With this solution, proceed as directed in the Identification (1) under Prazepam.

(2)  To a quantity of powdered Prazepam Tablets, equivalent to 0.02 g of Prazepam according to the labeled amount, add 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000), shake well, and filter. To 5 mL of the filtrate add a solution of sulfuric acid in ethanol (99.5) (5 in 1000) to make 50 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 241 nm and 245 nm, between 283 nm and 287 nm and between 363 nm and 367 nm, and minima between 263 nm and 267 nm and between 334 nm and 338 nm.

Dissolution  When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of 0.1 mol/L hydrochloric acid TS as the dissolution medium, the dissolution rate in 30 minutes of Prazepam Tablets is not less than 80%.

Start the test with 1 tablet of Prazepam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, measure exactly the subsequent V mL of the filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 5 μg of prazepam (C₁₉H₁₇ClN₂O) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of prazepam for assay, previously dried at 105°C for 2 hours, add 200 mL of the dissolution medium and dissolve with shaking, or by ultrasonication if necessary, add the dissolution medium to make exactly 1000 mL, and use this solution as the standard solution. Determine the absorbances, A₇ and A₆, of the sample solution and the standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of prazepam (C₁₉H₁₇ClN₂O)

\[
M_s = \frac{A_7}{A_6} \times \frac{V}{V} \times \frac{1}{C} \times 90
\]

C: Labeled amount (mg) of prazepam (C₁₉H₁₇ClN₂O) in 1 tablet

Assay  Weigh accurately not less than 20 Prazepam Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 50 mg of prazepam (C₁₉H₁₇ClN₂O), add 30 mL of acetone, shake well, centrifuge, and separate the supernatant liquid. Repeat the same procedure twice with 30 mL each of acetone, combine all the supernatants liquid, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.02 mol/L perchloric acid VS (potiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS = 6.496 mg of C₁₉H₁₇ClN₂O

Containers and storage  Containers—Tight containers.
Prazosin Hydrochloride

プラゾシン塩酸塩

\[
\text{C}_{19}\text{H}_{21}\text{N}_{5}\text{O}_{4}\cdot\text{HCl}: 419.86
\]
1-(4-Amino-6,7-dimethoxy-quinazolin-2-yl)-4-(2-furoyl)piperazine monohydrochloride
[19237-84-4]

Prazosin Hydrochloride, when dried, contains not less than 97.0% and not more than 103.0% of \( \text{C}_{19}\text{H}_{21}\text{N}_{5}\text{O}_{4}\cdot\text{HCl} \).

**Description**

Prazosin Hydrochloride occurs as a white crystalline powder.

- It is slightly soluble in methanol, very slightly soluble in ethanol (99.5) and practically insoluble in water.
- It gradually turns pale yellowish white on exposure to light.

- Melting point: about 270°C (with decomposition).

**Identification (1)**

Determine the absorption spectrum of a solution of Prazosin Hydrochloride in 0.01 mol/L hydrochloric acid-methanol TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.24 \), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Prazosin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Identification (2)**

Determine the infrared absorption spectrum of Prazosin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry \( \leq 2.25 \), and compare the spectrum with the Reference Spectrum or the spectrum of Prazosin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Identification (3)**

To 0.1 g of Prazosin Hydrochloride add 5 mL of water and 1 mL of ammonia TS, shake, allow to stand for 5 minutes, and filter. Render the filtrate acid with acetic acid (100): the solution responds to the Qualitative Tests \( \leq 1.09 \) for chlorides.

**Purity (1)**

Heavy metals \( \leq 1.07 \)—Proceed with 1.0 g of Prazosin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

**Operating conditions**

- **Detector:** An ultraviolet absorption photometer (wavelength: 254 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).
- **Temperature:** A constant temperature of about 25°C.

**Mobile phase:** Dissolve 3.484 g of sodium 1-pentane sulfonate and 18 mL of tetramethylammonium hydroxide in 900 mL of water, adjust the pH to 5.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 1000 mL of methanol.

- **Flow rate:** Adjust the flow rate so that the retention time of prazosin is about 9 minutes.
- **Time span of measurement:** About 6 times as long as the retention time of prazosin.

**System suitability**

- **Test for required detectability:** Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of prazosin obtained from 20 \( \mu \)L of this solution is equivalent to 35 to 65% of that of prazosin from 20 \( \mu \)L of the standard solution.

**System performance:** When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of prazosin are not less than 4000 and not more than 2.0, respectively.

**System reconstitution:** When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of prazosin is not more than 2.0%.

**Residual solvent**—Being specified separately.

**Loss on drying** \( \leq 2.47 \) Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition** \( \leq 2.48 \) Not more than 0.2% (1 g).

**Assay**

Weigh accurately about 25 mg each of Prazosin Hydrochloride and Prazosin Hydrochloride RS, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 3 mL each of these solutions, and add a mixture of methanol and water (7:3) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( \leq 2.01 \) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of prazosin in each solution.

\[
\text{Amount (mg) of prazosin hydrochloride (C}_{19}\text{H}_{21}\text{N}_{5}\text{O}_{4}\cdot\text{HCl)} = M_S \times A_T/A_S
\]

\( M_S \): Amount (mg) of Prazosin Hydrochloride RS
ter and 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol, water, acetic acid (100) and diethylamine (3500:1500:50:1).

Flow rate: Adjust the flow rate so that the retention time of prazosin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of prazosin are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of prazosin is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Prednisolone

プレドニゾロン

\[
\text{C}_{21}\text{H}_{28}\text{O}_{5} : 360.44 \\
\text{11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione [50-24-8]}
\]

Prednisolone, when dried, contains not less than 97.0% and not more than 102.0% of \(\text{C}_{21}\text{H}_{28}\text{O}_{5}\).

Description Prednisolone occurs as a white, crystalline powder.

It is soluble in methanol and in ethanol (95), slightly soluble in ethyl acetate and in chloroform, and very slightly soluble in water.

Melting point: about 235°C (with decomposition).

Identification (1) To 2 mg of Prednisolone add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Prednisolone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(\text{<2.25>}\), and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Prednisolone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Prednisolone and Prednisolone RS in ethyl acetate, respectively, then evaporate the ethyl acetate to dryness, and repeat the test on the residues.

Optical rotation \(\text{<2.49>}\) \([\alpha]_{B}^{20^\circ} = +113 \text{ to } +119^\circ\) (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

Purity (1) Selenium—To 0.10 g of Prednisolone add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 2 mL of nitric acid, and heat on a water bath until no more brown gas evolves and the solution becomes to be a light yellow clear solution. After cooling, add 4 mL of nitric acid to this solution, then add water to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 3 mL of Standard Selenium Solution, add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 6 mL of nitric acid, then add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry \(\text{<2.26>}\) according to the following conditions, and determine constant absorbances, \(A_{T}\) and \(A_{S}\), obtained on a recorder after rapid increasing of the absorption: \(A_{T}\) is smaller than \(A_{S}\) (not more than 30 ppm).

Perform the test by using a hydride generating system and a thermal absorption cell.

Lamp: A selenium hollow cathode lamp.

Wavelength: 196.0 nm.

Temperature of sample atomizer: When an electric furnace is used, about 1000°C.

Carrier gas: Nitrogen or argon.

(2) Related substances—Dissolve 20 mg of Prednisolone in exactly 2 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Separately, dissolve 20 mg of hydrocortisone and 10 mg of prednisolone acetate each in a mixture of methanol and chloroform (1:1) to make exactly 100 mL, and use these solutions as the standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography \(\text{<2.07>}\). Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, toluene and diethylamine (55:45:2) to a distance of about 15 cm, and air-dry the plate (do not dip the filter paper in the developing vessel). Spray evenly alkaline blue tetrazolium TS on the plate: the spots from the sample solution corresponding to those from the standard solutions (1) and (2) are not more intense than the spots from the standard solutions (1) and (2), and no spots other than the principal spot, hydrocortisone and prednisolone acetate appear from the sample solution.

Loss on drying \(\text{<2.41>}\) Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition \(\text{<2.44>}\) Not more than 0.1% (0.5 g).

Assay Dissolve about 25 mg each of Prednisolone and Prednisolone RS, previously dried, and accurately weighed, in 50 mL of methanol, add exactly 25 mL of the internal standard solution to each, and add methanol to make 100 mL. To 1 mL each of these solutions add the mobile phase to make 10 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography \(\text{<2.07>}\) according to the following conditions, and calculate the ratios, \(Q_{T}\) and \(Q_{S}\), of the peak area of prednisolone to that of the internal standard.

Amount (mg) of \(\text{C}_{21}\text{H}_{28}\text{O}_{5} = M_{S} \times Q_{T}/Q_{S}\)
Prednisolone Tablets

Prednisolone Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of prednisolone (C_{21}H_{28}O_{5}; 360.44).

**Method of preparation** Prepare as directed under Tablets, with Prednisolone.

**Identification (1)** Weigh a quantity of powdered Prednisolone Tablets equivalent to 0.05 g of Prednisolone according to the labeled amount, add 10 mL of chloroform, shake for 15 minutes, and filter. Evaporate the filtrate on a water bath to dryness. Dry the residue at 105°C for 1 hour, and proceed as directed in the Identification (1) under Prednisolone.

(2) Determine the infrared absorption spectra of the residue obtained in (1) and Prednisolone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: the spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the RS in ethyl acetate, evaporate to dryness, and repeat the test on the residues.

**Uniformity of dosage units (<6.02)** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Prednisolone Tablets to a volumetric flask, and shake with 10 mL of water until the tablet is disintegrated. Add 50 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, and add methanol to make exactly V mL to provide a solution that contains about 10 μg of prednisolone (C_{21}H_{28}O_{5}) per mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Prednisolone RS, previously dried at 105°C for 3 hours, dissolve in 10 mL of water and 50 mL of methanol, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry.

\[
\text{Amount (mg) of prednisolone (C}_{21}\text{H}_{28}\text{O}_{5}) = M_S \times A_T/A_S \times V/V \times 1/10
\]

**Dissolution (<6.10)** When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Prednisolone Tablets is not less than 70%.

Start the test with 1 tablet of Prednisolone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of Prednisolone RS, previously dried at 105°C for 3 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at the maximum wavelength at about 242 nm as directed under Ultraviolet-visible Spectrophotometry, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of prednisolone (C_{21}H_{28}O_{5})

\[
\text{M}_S = \frac{V}{C} \times \frac{A_T}{A_S} \times \frac{1}{T} \times 45
\]

**Assay** Weigh accurately and powder not less than 20 Prednisolone Tablets using an agate mortar. Weigh accurately a portion of the powder, equivalent to about 5 mg of prednisolone (C_{21}H_{28}O_{5}) add 1 mL of water, and shake gently. Add exactly 5 mL of the internal standard solution and 15 mL of methanol, and shake vigorously for 20 minutes. To 1 mL of this solution add the mobile phase to make 10 mL, and filter through a membrane filter with a pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone RS, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, add exactly 25 mL of the internal standard solution, and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 10 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Prednisolone with these solutions.
Amount (mg) of prednisolone (C₂₃H₃₀O₆)  
\[ M_S = M_S \times Q_I / Q_S \times 1/5 \]

\[ M_S : \text{Amount (mg) of Prednisolone RS} \]

*Internal standard solution*—A solution of methyl parahydroxybenzoate in methanol (1 in 2000).

**Containers and storage** Containers—Tight containers.

**Prednisolone Acetate**

プレドニゾロン酸酸エステル

![Prednisolone Acetate](image)

C₂₃H₃₀O₆: 402.48  
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione  
21-acetate [52-21-1]

Prednisolone Acetate, when dried, contains not less than 96.0% and not more than 102.0% of C₂₃H₃₀O₆.

**Description** Prednisolone Acetate occurs as a white, crystalline powder.

It is slightly soluble in methanol, in ethanol (95), in chloroform, and practically insoluble in water.

Melting point: about 235°C (with decomposition).

**Identification (1)** To 2 mg of Prednisolone Acetate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a deep red color, without fluorescence, develops. To this solution add 10 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a gray, flocculent precipitate is formed.

2.25 mL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of prednisolone acetate to that of the internal standard is not more than 10.

**Residue on ignition**<sup>2.44</sup> Not more than 0.1% (0.5 g, 105°C, 3 hours).

**Assay** Dissolve about 10 mg each of Prednisolone Acetate and Prednisolone Acetate RS, previously dried and accurately weighed, in 60 mL each of methanol, add exactly 2 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography<sup>2.01</sup> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak height of prednisolone acetate to that of the internal standard.

\[ M_S : \text{Amount (mg) of C₂₃H₃₀O₆} = M_S \times Q_I / Q_S \]

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (3 in 1000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of prednisolone acetate is about 10 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, prednisolone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of prednisolone acetate to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
Prednisolone Sodium Phosphate

Prednisolone Sodium Phosphate contains not less than 97.0% and not more than 103.0% of C₂₁H₂₇Na₂O₈P, calculated on the anhydrous basis.

Description Prednisolone Sodium Phosphate occurs as a white to pale yellow powder.

It is freely soluble in water, soluble in methanol, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Moisten 1.0 g of Prednisolone Sodium Phosphate with a small amount of sulfuric acid, and gradually heat to incinerate. After cooling, dissolve the residue in 10 mL of dilute nitric acid, and heat in a water bath for 30 minutes. After cooling, filter if necessary. This solution responds to the Qualitative Tests \( <1.0 \) for phosphate.

(2) Dissolve 2 mg of Prednisolone Sodium Phosphate in 2 mL of sulfuric acid, and allow to stand for 2 minutes: a deep red color, without fluorescence, develops.

(3) Determine the absorption spectrum of a solution of Prednisolone Sodium Phosphate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Prednisolone Sodium Phosphate as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.22> \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) The solution obtained in (1) responds to the Qualitative Tests \( <1.09> \) for sodium salt.

Optical rotation \( <2.49> \) \( \langle \alpha \rangle _{D}^{10} = +96 - +103^\circ \) (1 g, calculated on the anhydrous basis, phosphate buffer solution, pH 7.0, 100 mL, 100 mm).

pH \( <2.54> \) Dissolve 1.0 g of Prednisolone Sodium Phosphate in 100 mL of water: the pH of the solution is between 7.5 and 9.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Prednisolone Sodium Phosphate in 10 mL of water: the solution is clear and not more colored than the following control solution.

Control solution: To a mixture of 3.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.4 mL of Copper (II) Sulfate CS add diluted hydrochloric acid (1 in 40) to make 10 mL. To 2.5 mL of this solution add diluted hydrochloric acid (1 in 40) to make 100 mL.

(2) Heavy metals \( <1.07> \)—Proceed with 0.5 g of Prednisolone Sodium Phosphate according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 40 ppm).

(3) Free phosphoric acid—Weigh accurately about 0.25 g of Prednisolone Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Phosphoric Acid Standard Solution, add 2.5 mL of hexammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-napthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20 ± 1°C for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), using a solution prepared with 5 mL of water in the same manner as the blank. Determine the absorbance, \( A_t \) and \( A_s \), of each solution from the sample solution and standard solution at 740 nm: the content of free phosphoric acid is not more than 1.0%.

Content (\%) of free phosphoric acid (H₃PO₄)

\[
M = \frac{A_t}{A_s} \times 258.0
\]

where \( M \) is the amount (mg) of Prednisolone Sodium Phosphate, calculated on the anhydrous basis.

(4) Related substances—Dissolve 10 mg of Prednisolone Sodium Phosphate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.07> \) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of prednisolone phosphate from the sample solution is not larger than 1.5 times the peak area of prednisolone phosphate from the standard solution, and the total area of the peaks other than the peak of prednisolone phosphate from the sample solution is not larger than 2.5 times the peak area of prednisolone phosphate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (3 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 2.5 with phosphoric acid. To 1000 mL of this solution add 250 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of prednisolone phosphate is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of prednisolone phosphate.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of prednisolone phosphate ob-
tained from 20 μL of this solution is equivalent to 7 to 13% of that of prednisolone phosphate from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of prednisolone phosphate are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of prednisolone phosphate is not more than 2.0%.

(5) Residual solvent—Being specified separately.

Water 2.49 Not more than 8.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Prednisolone Sodium Phosphate, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add 1 mL of alkaline phosphatase TS, and allow to stand for 2 hours with occasional shaking. To this solution add exactly 20 mL of 1-octanol, and shake vigorously. Centrifuge this solution, pipet 10 mL of the 1-octanol layer, add 1-octanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone RS, previously dried at 105°C for 3 hours, and dissolve in 1-octanol to make exactly 100 mL. Pipet 6 mL of this solution, add a solution prepared by adding 1 mL of alkaline phosphatase TS to 2 mL water and being allowed to stand for 2 hours with occasional gentle shaking, add exactly 14 mL of 1-octanol, and shake vigorously. Proceed in the same manner as the sample solution to make the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24, using 1-octanol as the blank, and determine the absorbances, A_r and A_s, at 245 nm.

\[
\text{Amount (mg) of prednisolone sodium phosphate} = M_r \times \frac{A_t}{A_s} \times 3 \times 1.344
\]

\[
M_r: \text{Amount (mg) of Prednisolone RS}
\]

Containers and storage Containers—Tight containers.

Prednisolone Succinate

プレドニゾロンコハク酸エステル

\[
\text{C}_{25}\text{H}_{32}\text{O}_{8}: 460.52
\]

11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione
21-(hydrogen succinate)
[2920-86-7]

Prednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of C_{25}\text{H}_{32}\text{O}_{8}.

Description Prednisolone Succinate occurs as a white, fine, crystalline powder. It is odorless.

It is freely soluble in methanol, soluble in ethanol (95), and very slightly soluble in water and in diethyl ether.

Melting point: about 205°C (with decomposition).

Identification (1) To 2 mg of Prednisolone Succinate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Prednisolone Succinate as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum or the spectrum of Prednisolone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation 2.49 [α]_D^22: +114 – +120° (after drying, 67 mg, methanol, 10 mL, 100 mm).

Purity Related substances—Dissolve 0.10 g of Prednisolone Succinate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 30 mg of prednisolone in methanol to make exactly 10 mL. Pipet 1 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 5 μL of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ethanol (95) (2:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying 2.41 Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 6 hours).

Residue on ignition 2.44 Not more than 0.1% (1 g).

Assay Weigh accurately about 10 mg each of Prednisolone Succinate and Prednisolone Succinate RS, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, A_r and A_s, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry 2.24.

\[
\text{Amount (mg) of C}_{25}\text{H}_{32}\text{O}_{8} = M_s \times \frac{A_t}{A_s}
\]

\[
M_s: \text{Amount (mg) of Prednisolone Succinate RS}
\]

Containers and storage Containers—Tight containers.
Prednisolone Sodium Succinate for Injection

注射用プレドニゾロンコハク酸エステルナトリウム

C₂₅H₃₁NaO₈: 482.50
Monosodium 11β,17,21-trihydroxypregna-1,4-diene-3,20-dione 21-succinate [1715-33-9]

Prednisolone Sodium Succinate for Injection is a preparation for injection which is dissolved before use.

It contains not less than 72.4% and not more than 83.2% of prednisolone sodium succinate (C₂₅H₃₁NaO₈), and the equivalent of not less than 90.0% and not more than 110.0% of the labeled amount of prednisolone (C₂₁H₂₈O₅: 360.44).

The amount should be stated as the amount of prednisolone (C₂₁H₂₈O₅).

Method of preparation Prepare as directed under Injections, with Prednisolone Succinate and Dried Sodium Carbonate or Sodium Hydroxide.

It contains a suitable buffer agent.

Description Prednisolone Sodium Succinate for Injection occurs as a white powder or porous, friable mass.

It is freely soluble in water.

It is hygroscopic.

Identification (1) To 2 mg of Prednisolone Sodium Succinate for Injection add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Dissolve 0.01 g of Prednisolone Sodium Succinate for Injection in 1 mL of methanol, add 1 mL of Fehling’s TS, and heat: an orange to red precipitate is formed.

(3) Dissolve 0.1 g of Prednisolone Sodium Succinate for Injection in 2 mL of sodium hydroxide TS, allow to stand for 10 minutes, and filter. Add 1 mL of dilute hydrochloric acid to the filtrate, shake, and filter if necessary. Adjust the solution with diluted ammonia TS (1 in 10) to a pH of about 6, and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

(4) Prednisolone Sodium Succinate for Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> Dissolve 1.0 g of Prednisolone Sodium Succinate for Injection in 40 mL of water: the pH of the solution is between 6.5 and 7.2.

Purity Clarity and color of solution—Dissolve 0.25 g of Prednisolone Sodium Succinate for Injection in 10 mL of water: the solution is clear and colorless.

Loss on drying <2.41> Not more than 2.0% (0.15 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 2.4 EU/mg of prednisolone (C₂₁H₂₈O₅).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take a quantity of sealed containers of Prednisolone Sodium Succinate for Injection, equivalent to about 0.1 g of prednisolone (C₂₁H₂₈O₅), and dissolve the contents in a suitable amount of diluted methanol (1 in 2), and transfer to a 100-mL volumetric flask. Wash each container with diluted methanol (1 in 2), collect the washings in the volumetric flask, and add diluted methanol (1 in 2) to make volume. Pipet 4 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone Succinate RS, previously dried in a desiccator for 6 hours (in vacuum, phosphorus (V) oxide, 60°C), dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography according <2.01> to the following conditions, and calculate the ratios, \( Q_s \) and \( Q_t \), of the peak area of prednisolone succinate to that of the internal standard.

\[
\text{Amount (mg) of prednisolone sodium succinate (C₂₅H₃₁NaO₈)} = M_s \times Q_s / Q_t \times 5 \times 1.048
\]

\[
\text{Amount (mg) of prednisolone (C₂₁H₂₈O₅)} = M_s \times Q_s / Q_t \times 5 \times 0.783
\]

\( M_s \): Amount (mg) of Prednisolone Succinate RS

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 25,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.32 g of tetra n-butyrammonium bromide, 3.22 g of disodium hydrogen phosphate dodecahydrate and 6.94 g of potassium dihydrogen phosphate in 1000 mL of water. To 840 mL of this solution add 1160 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of prednisolone succinate is about 15 minutes.

System suitability—

System performance: When the procedure is run with 10 \( \mu \text{L} \) of the standard solution under the above operating conditions, prednisolone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of prednisolone succinate to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Hermetic containers.

**Primidone**

プリミドン

![Chemical Structure](image)

C\( _{12} \)H\( _{14} \)N\( _{2} \)O\( _{2} \): 218.25

5-Ethyl-5-phenyl-2,3-dihydropyrimidine-4,6(1\( H \),5\( H \))-dione

[125-33-7]

Primidone, when dried, contains not less than 98.5% of C\( _{12} \)H\( _{14} \)N\( _{2} \)O\( _{2} \).

**Description**  Primidone occurs as a white, crystalline powder or granules. It is odorless and has a slightly bitter taste. It is soluble in \( N,N \)-dimethylformamide, sparingly soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

**Identification** (1)  Heat 0.5 g of Primidone with 5 mL of diluted sulfuric acid (1 in 2): the odor of formaldehyde is perceptible.

(2)  Mix 0.2 g of Primidone with 0.2 g of anhydrous sodium carbonate, and heat: the gas evolved changes moistened red litmus paper to blue.

**Melting point** 279 – 284°C

**Purity** (1)  Clarity and color of solution—Dissolve 0.10 g of Primidone in 10 mL of \( N,N \)-dimethylformamide: the solution is clear and colorless.

(2)  Heavy metals 1.07 —Proceed with 2.0 g of Primidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3)  2-Ethyl-2-phenylmalonediamide—Dissolve 0.10 g of Primidone in 2 mL of pyridine, add exactly 2 mL of the internal standard solution, then add 1 mL of bis-trimethyl silyl acetamide, shake well, and heat at 100°C for 5 minutes. Cool, add pyridine to make 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 2-ethyl-2-phenylmalonediamide in pyridine to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, proceed in the same manner as Primidone, and use this solution as the standard solution. Perform the test with 2 \( \mu \)L of the sample solution and standard solution as directed under Gas Chromatography 2.02 according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard: \( Q_1 \) is not more than 2.60.

*Internal standard solution*A solution of stearylalcohol in pyridine (1 in 2000).

**Operating conditions**—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 150 cm in length, packed with siliconeous earth for gas chromatography (125 to 150 \( \mu \text{m} \) in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 195°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of stearylalcohol is about 10 minutes.

**System suitability**—

System performance: When the procedure is run with 2 \( \mu \)L of the standard solution under the above operating conditions, 2-ethyl-2-phenylmalonediamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 2 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard is not more than 1.5%.

**Loss on drying**  Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition**  Not more than 0.2% (1 g).

**Assay**  Weigh accurately about 20 mg each of Primidone and Primidone RS, previously dried, dissolve each in 20 mL of ethanol (95) by warming, and after cooling, add ethanol (95) to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbance, \( A_1 \), of the sample solution and standard solution at the wavelength of maximum absorption at about 267 nm, and the absorbances, \( A_2 \) and \( A_3 \), at the wavelength of minimum absorption at about 254 nm and at about 261 nm, as directed under Ultraviolet-visible Spectrophotometry 2.24, using ethanol (95) as the blank.

\[
\text{Amount (mg)} = M_5 \times \left( \frac{(2A_1 - A_2 - A_3)/(2A_1 - A_2 - A_3)_s}{(2A_1 - A_2 - A_3)_t} \right)
\]

where, \((2A_1 - A_2 - A_3)_t\) is the value from the sample solution, and \((2A_1 - A_2 - A_3)_s\) is from the standard solution.

**Containers and storage**  Containers—Tight containers.
**Probencid**

**Description** Probencid occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste, followed by unpleasant bitter.

It is sparingly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in sodium hydroxide TS and in ammonia TS.

Melting point: 198 – 200°C

**Identification (1)** Heat Probencid strongly: the odor of sulfur dioxide is perceptible.

(2) Determine the absorption spectrum of a solution of Probencid in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probencid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)** Acidity—To 2.0 g of Probencid add 100 mL of water, heat on a water bath with occasional shaking for 30 minutes, cool, and filter. To the filtrate add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). After 30 minutes, cool, and filter. To the filtrate add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS = 28.54 mg of C₁₃H₁₉NO₄S

**Containers and storage** Containers—Well-closed containers.

**Probencid Tablets**

Probencid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of probencid (C₁₃H₁₉NO₄S: 285.36).

**Method of preparation** Prepare as directed under Tablets, with Probencid.

**Identification (1)** Weigh a quantity of powdered Probencid Tablets, equivalent to 0.5 g of Probencid according to the labeled amount, add 50 mL of ethanol (95) and 1 mL of 1 mol/L hydrochloric acid TS, shake, and filter. Evaporate the filtrate on a water bath to about 20 mL. After cooling, collect produced crystals, recrystallize with 50 mL of dilute ethanol, and dry at 105°C for 4 hours: it melts <2.60> between 196°C and 200°C. With the crystals so obtained, proceed as directed in the Identification (1) under Probencid.

(2) Determine the absorption spectrum of a solution of the dried crystals obtained in (1) in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probencid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Uniformity of dosage units** <2.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Probencid Tablets add 30 mL of water and 2 mL of 1 mol/L hydrochloric acid TS, treat with ultrasonic waves with occasional shaking to disintegrate the tablet completely, and add ethanol (99.5) to make exactly 100 mL. Centrifuge this solution, pipet 3 mL of the supernatant liquid, and add 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, and add ethanol (99.5) to make exactly 20 mL so that each mL contains about 15 μg of probencid (C₁₃H₁₉NO₄S), and use this solution as the standard solution. Separately, weigh accurately about 0.125 g of Probencid RS, previously dried at 105°C for 4 hours, dissolve in 15 mL of water, 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, and add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared by adding
ethanol (99.5) to 1 mL of 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, as the blank, and determine the absorbances, \( A_1 \) and \( A_5 \), at 248 nm.

\[
\text{Amount (mg) of probenecid (C}_{13}\text{H}_{19}\text{NO}_{4}\text{S}) = M_5 \times A_1 / A_S \times V / 25
\]

\( M_5 \): Amount (mg) of Probucol RS

**Dissolution 6.10**  When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Probucol Tablets is not less than 80%.

Start the test with 1 tablet of Probucol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \( \mu \)m. Discard the first 10 mL of the filtrate, pipet \( V \) mL of the subsequent filtrate, add the dissolution medium to make exactly \( V \) mL so that each mL contains about 14 \( \mu \)g of probenecid (C\(_{13}\)H\(_{19}\)NO\(_4\)S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of Probucol RS, previously dried at 105°C for 4 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, \( A_1 \) and \( A_5 \), at 244 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24.5.

\[
\text{Dissolution rate (% with respect to the labeled amount of probenecid (C}_{13}\text{H}_{19}\text{NO}_{4}\text{S}) = M_5 \times A_1 / A_S \times V / V \times 1 / C \times 18
\]

\( M_5 \): Amount (mg) of Probucol RS

\( C \): Labeled amount (mg) of probenecid (C\(_{13}\)H\(_{19}\)NO\(_4\)S) in 1 tablet

**Assay** Weigh accurately, and powder not less than 20 Probucol Tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of probenecid (C\(_{13}\)H\(_{19}\)NO\(_4\)S), add 30 mL of water and 2 mL of 1 mol/L hydrochloric acid TS, shake, add 30 mL of ethanol (99.5), disperse the particles with the aid of ultrasonic waves, and add ethanol (99.5) to make exactly 100 mL. Centrifuge the solution, pipet 3 mL of the supernatant liquid, add 1 mL of 1 mol/L hydrochloric acid TS, and add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of the solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of Probucol RS, previously dried at 105°C for 4 hours, add 15 mL of water and 1 mL of 1 mol/L hydrochloric acid TS, then add ethanol (99.5) to make exactly 50 mL. Pipet 3 mL of the solution, add 1 mL of 1 mol/L hydrochloric acid TS, and add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of the solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, \( A_1 \) and \( A_5 \), of the sample solution and standard solution at 248 nm as directed under Ultraviolet-visible Spectrophotometry 2.24.5, using a solution, prepared by mixing 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient ethanol (99.5) to make exactly 50 mL, as the blank.

\[
\text{Amount (mg) of probenecid (C}_{13}\text{H}_{19}\text{NO}_{4}\text{S}) = M_5 \times A_1 / A_S \times V / 25
\]

\( M_5 \): Amount (mg) of Probucol RS

**Containers and storage**  Containers—Well-closed containers.

**Probucol**

![Probucol structure](image)

C\(_{31}\)H\(_{48}\)O\(_2\)S\(_2\): 516.84

4,4’-[Propan-2,2-diylbis(sulfandiyl)]bis[2,6-bis(1,1-dimethylethyl)phenol]

[23289-49-5]

Probucol, when dried, contains not less than 98.5% and not more than 101.0% of C\(_{31}\)H\(_{48}\)O\(_2\)S\(_2\).

**Description**  Probucol occurs as a white crystalline powder.

It is very soluble in tetrahydrofuran, freely soluble in ethanol (99.5), soluble in methanol, and practically insoluble in water.

It gradually turns light yellow on exposure to light.

**Identification (1)**  Determine the absorption spectrum of a solution of Probucol in methanol (in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24.5, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probucol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Probucol as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25.5, and compare the spectrum with the Reference Spectrum or the spectrum of Probucol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point**  2.60° 125 – 128°C

**Purity (1)**  Heavy metals 1.07g—Proceed with 2.0 g of Probucol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.40 g of Probucol in 5 mL of ethanol (99.5), add the mobile phase to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.9 with respect to probucol from the sample solution is not larger than the peak area of probucol from the standard solution; the area of peak hav-
Perform the test with 10 μL of the standard solution under the above operating conditions, the internal standard and probucol are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

Containers and storage—Tight containers.
Storage—Light-resistant.

**Probucol Fine Granules**

Probucol Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of probucol (C$_{31}$H$_{48}$O$_2$S$_2$; 516.84).

**Method of preparation** Prepare as directed under Granules, with Probucol.

**Identification** To an amount of pulverized Probucol Fine Granules, equivalent to 50 mg of Probucol according to the labeled amount, add 100 mL of methanol, shake, and filter. To 2 mL of the filtrate add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits a maximum between 240 nm and 244 nm.

**Uniformity of dosage units** (2.02) Perform the test according to the following method: the granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Probucol Fine Granules add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet 20 mL of the supernatant liquid, equivalent to about 5 mg of probucol (C$_{31}$H$_{48}$O$_2$S$_2$), add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of probucol (C$_{31}$H$_{48}$O$_2$S$_2$) = $M_5 \times Q_1/Q_5$

$M_5$: Amount (mg) of Probucol RS

Internal standard solution—Dissolve 0.2 g of bis(cis-3,3,5-trimethylcyclohexyl) phthalate in 1 mL of tetrahydrofuran, and add the mobile phase to make 50 mL.

**Operating conditions**
- Detector: An ultraviolet absorption photometer (wavelength: 242 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: A mixture of acetonitrile and water (93:7).
- Flow rate: Adjust the flow rate so that the retention time of probucol is about 13 minutes.

**System suitability**
- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and probucol are eluted in this order with the resolution between these peaks being not less than 6.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

Containers—Tight containers.
Storage—Light-resistant.
**Particle size**  6.02  It meets the requirements of Fine granules.

**Assay** Weigh accurately an amount of pulverized Probucol Fine Granules, equivalent to about 0.25 g of probucol (C₃₁H₄₈O₂S₂), add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Probucol RS, previously dried under reduced pressure at 80°C for 1 hour, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Qₜ and Qₛ, of the peak area of probucol to that of the internal standard.

\[
\text{Amount (mg) of probucol (C₃₁H₄₈O₂S₂)} = Mₚ × Qₜ/Qₛ × 5
\]

\[
Mₚ: \text{Amount (mg) of Probucol RS}
\]

Internal standard solution—A solution of bis(cis-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).

Operating conditions—

Detector, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Probucol.

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and probucol are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Well-closed containers.

**Probucol Tablets**

**プロブコール錠**

Probucol Tablets contain not less than 95.0% and not more than 105.0% of probucol (C₃₁H₄₈O₂S₂: 516.84).

**Method of preparation** Prepare as directed under Tablets, with Probucol.

**Identification** To an amount of pulverized Probucol Tablets, equivalent to 50 mg of Probucol according to the labeled amount, add 100 mL of methanol, shake, and filter. To 2 mL of the filtrate add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits a maximum between 240 nm and 244 nm.

**Uniformity of dosage units**  6.02  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Shake 1 tablet of Probucol Tablets with a suitable amount of methanol until the tablet is disintegrated, and add methanol to make exactly V mL so that each mL of the solution contains about 2.5 mg of probucol (C₃₁H₄₈O₂S₂). Centrifuge the solution, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of probucol (C₃₁H₄₈O₂S₂)} = Mₚ × Qₜ/Qₛ × V/20
\]

\[
Mₚ: \text{Amount (mg) of Probucol RS}
\]

Internal standard solution—A solution of bis(cis-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).

**Disintegration**  6.09  It meets the requirement.

**Assay** Weigh accurately the mass of 20 Probucol Tablets, and powder the tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of probucol (C₃₁H₄₈O₂S₂), add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Probucol RS, previously dried under reduced pressure at 80°C for 1 hour, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Qₜ and Qₛ, of the peak area of probucol to that of the internal standard.

\[
\text{Amount (mg) of probucol (C₃₁H₄₈O₂S₂)} = Mₚ × Qₜ/Qₛ × 5
\]

\[
Mₚ: \text{Amount (mg) of Probucol RS}
\]

Internal standard solution—A solution of bis(cis-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).
ing conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

### Procainamide Hydrochloride

Procainamide Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of C₁₃H₂₁N₃O.HCl.

**Description** Procainamide Hydrochloride occurs as a white to light yellow crystalline powder. It is very soluble in water and soluble in ethanol (99.5). It is hygroscopic.

**Identification** (1) Determine the infrared absorption spectrum of Procainamide Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Procainamide Hydrochloride (1 in 20) responds to the Qualitative Tests <1.092> for chloride.

**pH** <2.54> Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.5.

**Melting point** <2.60> 165 – 169°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Procainamide Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.15>—Prepare the test solution with 1.0 g of Procainamide Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Procainamide Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.012> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than procainamide from the sample solution is not larger than the peak area of procainamide from the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and methanol (9:1).

Flow rate: Adjust the flow rate so that the retention time of procainamide is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of procainamide.

**System suitability**—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of procainamide obtained from 10 μL of this solution is equivalent to 40 to 60% of that of procainamide from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of procainamide are not less than 10,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of procainamide is not more than 2.0%.

**Loss on drying** <2.4> Not more than 0.3% (2 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (2 g).

**Assay** Weigh accurately about 0.5 g of Procainamide Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 27.18 mg of C₁₃H₂₁N₃O.HCl

**Containers and storage** Containers—Tight containers.

### Procainamide Hydrochloride Injection

Procainamide Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of procainamide hydrochloride (C₁₃H₂₁N₃O.HCl: 271.79).

**Method of preparation** Prepare as directed under Injections, with Procainamide Hydrochloride.
**Description**
Procaainamide Hydrochloride Injection is a clear, colorless or light yellow liquid. pH: 4.0–6.0

**Identification**
1. To a volume of Procaainamide Hydrochloride Injection, equivalent to 10 mg of Procaainamide Hydrochloride according to the labeled amount, add 1 mL of dilute hydrochloric acid and water to make 5 mL: the solution responds to the Qualitative Tests \(<1.09\) (1) for primary aromatic amines.

2. To a volume of Procaainamide Hydrochloride Injection, equivalent to 0.1 g of Procaainamide Hydrochloride according to the labeled amount, add water to make 100 mL. To 1 mL of this solution add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(2.24\): it exhibits a maximum between 277 nm and 281 nm.

3. Procaainamide Hydrochloride Injection responds to the Qualitative Tests \(<1.09\) (2) for chloride.

**Bacterial endotoxins** \(<4.01\)
Less than 0.30 EU/mg.

**Extractable volume** \(<6.05\)
It meets the requirement.

**Foreign insoluble matter** \(<6.06\)
Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** \(<6.07\)
It meets the requirement.

**Sterility** \(<4.06\)
Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay**
Dilate an accurately measured volume of Procaainamide Hydrochloride Injection, equivalent to about 0.5 g of procaainamide hydrochloride \((C_{13}H_{21}N_{3}O.HCl)\), with 5 mL of hydrochloric acid and water to 50 mL, add 10 mL of potassium bromide solution \((3 \to 10)\), cool to 15°C or lower, and titrate \(<2.50\) with 0.1 mol/L sodium nitrite VS (potentiometric titration method or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS \(= 27.18 \text{ mg of } C_{13}H_{21}N_{3}O.HCl\)

**Containers and storage**
Containers—Hermetic containers.

**Procaainamide Hydrochloride Tablets**
プロカインアミド塩酸塩錠

Procaainamide Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of procaainamide hydrochloride \((C_{13}H_{21}N_{3}O.HCl): 271.79\).

**Method of preparation**
Prepare as directed under Tablets, with Procaainamide Hydrochloride.

**Identification**
To a quantity of powdered Procaainamide Hydrochloride Tablets, equivalent to 1.5 g of Procaainamide Hydrochloride according to the labeled amount, add 30 mL of water, shake well, filter, and use the filtrate as the sample solution. To 0.2 mL of the sample solution add 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests \(<1.09\) for primary aromatic amines.

**Uniformity of dosage units** \(<6.02\)
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Procaainamide Hydrochloride Tablets add \(3V/5\) mL of 0.02 mol/L phosphate buffer solution, pH 3.0, treat with ultrasonic waves to disintegrate the tablet completely, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly \(V\) mL so that each mL contains about 2.5 mg of procaainamide hydrochloride \((C_{13}H_{21}N_{3}O.HCl)\), and shake for 5 minutes. Centrifuge this solution, pipet 1 mL of the supernatant liquid, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 250 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of procaainamide hydrochloride \((C_{13}H_{21}N_{3}O.HCl)\)
\[ M_S = M_S \times \frac{A_T}{A_S} \times \frac{V}{V/20} \]

\(M_S\): Amount (mg) of procaainamide hydrochloride for assay

**Dissolution** \(<6.10\)
When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Procaainamide Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Procaainamide Hydrochloride Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly \(V\) mL so that each mL contains about 7 μg of procaainamide hydrochloride \((C_{13}H_{21}N_{3}O.HCl)\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of procaainamide hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 1000 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), and determine the absorbances, \(A_T\) and \(A_S\), at 278 nm.

Dissolution rate (%) with respect to the labeled amount of procaainamide hydrochloride \((C_{13}H_{21}N_{3}O.HCl)\)
\[ M_S = M_S \times \frac{A_T}{A_S} \times \frac{V/V}{V \times 1/C \times 9/2} \]

\(M_S\): Amount (mg) of procaainamide hydrochloride for assay

C: Labeled amount (mg) of procaainamide hydrochloride \((C_{13}H_{21}N_{3}O.HCl)\) in 1 tablet

**Assay**
To 10 Procaainamide Hydrochloride Tablets add about 300 mL of 0.02 mol/L phosphate buffer solution, pH 3.0, and treat with ultrasonic waves to disintegrate the tablets completely. To this solution add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 500 mL, and stir for 5 minutes. Centrifuge this solution, pipet \(V\) mL of the supernatant liquid, and add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly \(V\) mL so that each mL contains about 10 μg of procaainamide hydrochloride \((C_{13}H_{21}N_{3}O.HCl)\). Filter this solution through a membrane
filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of procainamide hydrochloride for assay, previously dried at 105°C for 4 hours, dissolve in 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 100 mL. Pipet 2 mL of this solution, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the peak areas, A₁ and A₅, of procainamide in each solution.

\[
M₅ = \frac{M₅ \times A₁ \times V/V \times 1/10}{A₅}
\]

Amount (mg) of procainamide hydrochloride
(C₁₃H₂₀N₂O₂.HCl) = 272.77

## Operating conditions—

**Detector:** An ultraviolet absorption photometer (wavelength: 270 nm).

**Column:** A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** A mixture of 0.02 mol/L phosphate buffer solution, pH 3.0, and methanol (9:1).

**Flow rate:** Adjust the flow rate so that the retention time of procainamide is about 9 minutes.

## System suitability—

**System performance:** When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of procainamide are not less than 10,000 and not more than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of procainamide is not more than 1.0%.

**Containers and storage**

Containers—Tight containers.

### Proca in Hydrochloride

プロカイン塩酸塩

C₁₃H₂₃N₂O₂.HCl: 272.77

2-(Diethylamino)ethyl 4-aminobenzoate monohydrochloride [31-05-8]

Proca in Hydrochloride, when dried, contains not less than 99.0% of C₁₃H₂₃N₂O₂.HCl.

**Description**

Proca in Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

**Identification**

1. Determine the absorption spectrum of a solution of Proca in Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Proca in Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

3. A solution of Proca in Hydrochloride (1 in 10) responds to the Qualitative Tests 1.09 for chloride.

**pH**

The pH of a solution prepared by dissolving 1.0 g of Proca in Hydrochloride in 20 mL of water is between 5.0 and 6.0.

**Melting point**

155 – 158°C

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Proca in Hydrochloride in 10 mL of water: the solution is clear and colorless.

2. Heavy metals—Proceed with 1.0 g of Proca in Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

3. Related substances—To 1.0 g of Proca in Hydrochloride add 5 mL of ethanol (95), dissolve by mixing well, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 4-aminobenzoic acid in ethanol (95) to make exactly 20 mL, then pipet 1 mL of this solution, add 4 mL of ethanol (95) and water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.02. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dibutyl ether, n-hexane and acetic acid (100) (20:4:1) to a distance of about 10 cm, and air-dry the plate. After drying the plate more at 105°C for 10 minutes, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. The principal spot from the sample solution stays at the origin.

**Loss on drying**

Not more than 0.5% (1 g, silica gel, 4 hours).

**Residue on ignition**

Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.4 g of Proca in Hydrochloride, previously dried, dissolve in 5 mL of hydrochloric acid and 60 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool to below 15°C, and titrate 2.50 with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS = 27.28 mg of C₁₃H₂₃N₂O₂.HCl

**Containers and storage**

Containers—Well-closed contain-
Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 3.0 with phosphoric acid, and add an amount of sodium 1-pentane sulfonate to make a solution so that containing 0.1%. To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of procaine is about 10 minutes.

System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, procaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of procaine to that of the internal standard is not more than 1.0%.

Containers and storage
Containers—Hermetic containers.

Procaine Hydrochloride Injection

プロカイン塩酸塩注射液

Procaine Hydrochloride Injection is an aqueous solution for injection.
It contains not less than 95.0% and not more than 105.0% of the labeled amount of procaine hydrochloride (C₁₃H₂₀N₂O₂.HCl: 272.77).

Method of preparation
Prepare as directed under Injections, with Procaine Hydrochloride.

Description
Procaine Hydrochloride Injection is a clear, colorless liquid.

Identification (1)
To a volume of Procaine Hydrochloride Injection, equivalent to 0.01 g of Procaine Hydrochloride according to the labeled amount, add water to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits maxima between 219 nm and 223 nm, and between 289 nm and 293 nm.

(2) Procaine Hydrochloride Injection responds to the Qualitative Tests 1.09 (2) for chloride.

pH 2.5–3.0

Bacterial endotoxins 4.01
Less than 0.02 EU/unit. Apply to the preparations intended for intraspinal administration.

Extractable volume 6.03
Perform the test according to Method 1: it meets the requirement.

Foreign insoluble matter 6.06
Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter 6.07
It meets the requirement.

Sterility
4.06
Perform the test according to the Membrane filtration method: it meets the requirement.

Assay
To an exactly measured volume of Procaine Hydrochloride Injection, equivalent to about 20 mg of procaine hydrochloride (C₁₃H₂₀N₂O₂.HCl), add the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of procaine hydrochloride for assay, previously dried in a desiccator (silica gel) for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q₁ and Q₀, of the peak area of procaine hydrochloride to that of the internal standard.

\[
\text{Amount (mg) of procaine hydrochloride} = M_s \times \frac{Q_1}{Q_0} \times 2/5
\]

Mₕ: Amount (mg) of procaine hydrochloride for assay

Procaine Hydrochloride

Procarbazine Hydrochloride

プロカルバジン塩酸塩

C₁₂H₁₉N₃O.HCl: 257.76
N-\{(1-Methylethyl)-\-
4-[\(\text{2-methylhydrazino}\)methyl]benzamid monohydrochloride

C₁₂H₁₈N₄O.HCl: 257.76

Description
Procarbazine Hydrochloride occurs as white to light yellowish white crystals or crystalline powder.
It is freely soluble in water, and slightly soluble in ethanol (99.5).
It dissolves in dilute hydrochloric acid.
Melting point: about 223°C (with decomposition).

Identification (1)
Dissolve 0.01 g of Procarbazine Hydrochloride in 1 mL of diluted copper (II) sulfate TS (1 in 10), and add 4 drops of sodium hydroxide TS: a green precipitate is formed immediately, and the color changes from green through yellow to orange.
(2) Determine the absorption spectrum of a solution of Procarbazine Hydrochloride in 0.1 mol/L hydrochloric acid (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Procarbazine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Procarbazine Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

**pH** <2.54> Dissolve 0.10 g of Procarbazine Hydrochloride in 10 mL of water: the pH of this solution is between 3.0 and 5.0.

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Procarbazine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Procarbazine Hydrochloride in 5.0 mL of a solution of L-cysteine hydrochloride monohydrate in diluted methanol (7 in 10) (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of L-cysteine hydrochloride monohydrate in diluted methanol (7 in 10) (1 in 200) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Immers slowly, by inclining, a plate of silica gel with fluorescent indicator for thin-layer chromatography in a solution of Procarbazine Hydrochloride (1 in 2) shows no optical rotation.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.15 g of Procarbazine Hydrochloride, previously dried, place in a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, and cool to room temperature. To this solution add 5 mL of chloroform, and titrate <2.50>, while shaking, with 0.05 mol/L potassium iodate VS until the purple color of the chloroform layer disappears. The end point is reached when the red-purple color of the chloroform layer no more appears within 5 minutes after the purple color disappeared.

Each mL of 0.05 mol/L potassium iodate VS = 8.592 mg of C16H22N2O3.HCl

**Containers and storage** Containers—Tight containers.

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**Procaterol Hydrochloride Hydrate**

プロカテロール塩酸塩水和物

C₁₆H₂₂N₂O₃.HCl: 335.83
8-Hydroxy-5-[(1RS,2SR)-1-hydroxy-2-[(1-methylethyl)amino]butyl]quinolin-2(1H)-one monohydrochloride hemihydrate

[62929-97-3, anhydride]

Procaterol Hydrochloride Hydrate contains not less than 98.5% of procaterol hydrochloride (C₁₆H₂₂N₂O₃.HCl: 326.82), calculated on the anhydrous basis.

**Description** Procaterol Hydrochloride Hydrate occurs as white to pale yellowish white crystals or crystalline powder. It is soluble in water, in formic acid and in methanol, slightly soluble in ethanol (95%), and practically insoluble in diethyl ether.

The pH of a solution of Procaterol Hydrochloride Hydrate (1 in 100) is between 4.0 and 5.0. It is gradually colored by light.

The solution of Procaterol Hydrochloride Hydrate (1 in 20) shows no optical rotation.

Melting point: about 195°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Procaterol Hydrochloride Hydrate (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Procaterol Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Procaterol Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Procaterol Hydrochloride Hydrate in 30 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3.0 mL of Iron (III) Chloride CS add water to make 50 mL.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Procaterol Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Procaterol Hydrochloride Hydrate in 100 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sam-
ple solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than procaterol from the sample solution is not larger than the peak area of procaterol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 0.87 g of sodium 1-pentanesulfonate in 1000 mL of water. To 760 mL of this solution add 230 mL of methanol and 10 mL of acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of procaterol is about 15 minutes.

Selection of column: Dissolve 20 mg each of Procaterol Hydrochloride Hydrate and threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2). To 15 mL of this solution add diluted methanol (1 in 2) to make 100 mL. Proceed with 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of procaterol and threoprocaterol in this order with the resolution of these peaks being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of procaterol obtained from 2 μL of the standard solution is not less than 3.

Time span of measurement: 2.5 times as long as the retention time of procaterol beginning after the solvent peak.

Water <2.46> 2.5 – 3.3% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.46> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Procaterol Hydrochloride Hydrate, add 2 mL of formic acid, dissolve by warming, and add exactly 15 mL of 0.1 mol/L perchloric acid VS. Add 1 mL of acetic anhydride, heat on a water bath for 3 minutes, cool, add 60 mL of acetic anhydride, and titrate <2.56> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 32.68 mg of C16H22N2O3.HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Prochlorperazine Maleate

プロクロルペラジンマレイン酸塩

C20H24ClN3S.2C4H4O4: 606.09
2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10H-phenothiazine dimaleate
[84-02-6]

Prochlorperazine Maleate, when dried, contains not less than 98.0% of C20H21ClN3S.2C4H4O4.

Description Prochlorperazine Maleate occurs as a white to yellow powder. It is odorless, and has a slightly bitter taste.

It is slightly soluble in acetic acid (100), very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It gradually acquires a red tint by light.

Melting point: 195 – 203°C (with decomposition).

Identification (1) Dissolve 5 mg of Prochlorperazine Maleate in 5 mL of sulfuric acid: a red color develops, which darkens slowly on standing. Warm a half of the solution: the color changes to red-purple. To the remainder add 1 drop of potassium dichromate TS: a green-brown color develops, which changes to brown on standing.

(2) Boil 0.5 g of Prochlorperazine Maleate with 10 mL of hydrobromic acid under a reflux condenser for 10 minutes. After cooling, add 100 mL of water, and filter through glass filter (G4). Wash the residue with three 10-mL portions of water, and dry at 105°C for 1 hour: it melts <2.60> between 195°C and 198°C (with decomposition).

(3) Dissolve 0.2 g of Prochlorperazine Maleate in 5 mL of a solution of sodium hydroxide (1 in 10), and extract with three 3-mL portions of diethyl ether [reserve the aqueous layer, and use for test (4)]. Evaporate the combined diethyl ether extracts on a water bath to dryness, dissolve the residue in 10 mL of methanol by warming, and pour into 30 mL of 10 mol/L hydrobromic acid under a reflux condenser for 10 minutes. Allow to stand for 1 hour, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 252°C and 258°C (with decomposition).

(4) To the aqueous layer reserved in (3) add boiling chips, and heat on a water bath for 10 minutes. Cool, add 2 mL of bromine TS, heat on a water bath for 10 minutes, and heat the solution to boil. After cooling, add 2 drops of this solution to 3 mL of a solution of resorcinol in sulfuric acid (1 in 300), and heat on a water bath for 15 minutes: a red-purple color is produced.

Purity Heavy metals <1.07>—Proceed with 1.0 g of Prochlorperazine Maleate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).
Loss on drying  <2.4/> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition  <2.4/> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Prochlorperazine Maleate, previously dried, dissolve in 60 mL of acetic acid (100) while stirring and warming. Cool, and titrate <2.5/> with 0.05 mol/L perchloric acid VS until the color of the solution changes from orange to green (indicator: 0.5 mL of p-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
15.15 mg of C₂₀H₂₄ClN₃S.₂C₄H₄O₄
Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Prochlorperazine Maleate Tablets
プロクロルペラジンマレイン酸塩錠

Prochlorperazine Maleate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of prochlorperazine maleate (C₂₀H₂₄ClN₃S.₂C₄H₄O₄: 606.09).

Method of preparation Prepare as directed under Tablets, with Prochlorperazine Maleate.

Identification (1) Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 5 mg of Prochlorperazine Maleate according to the labeled amount, add 15 mL of acetic acid (100), shake, and filter. To 5 mL of the filtrate add 3 mL of sulfuric acid, and shake: a light red color develops. To this solution add 1 drop of potassium dichromate TS: a green-brown color is produced and changes to brown on standing.

(2) Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 0.08 g of Prochlorperazine Maleate according to the labeled amount, add 15 mL of methanol and 1 mL of dimethylamine, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.08 g of Prochlorperazine Maleate RS in 15 mL of methanol and 1 mL of dimethylamine, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia TS (15:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly palladium (II) chloride TS on the plate: the spots obtained from the sample solution and the plate. Spray evenly palladium (II) chloride TS on the plate. Air-dry to a distance of about 10 cm, and air-dry the plate. Spray evenly palladium (II) chloride TS on the plate: the spots obtained from the sample solution and the plate. Spot 10 μL each of the standard solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia TS (15:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly palladium (II) chloride TS on the plate: the spots obtained from the sample solution and the plate. Spray evenly palladium (II) chloride TS on the plate: the spots obtained from the sample solution and the plate. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia TS (15:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly palladium (II) chloride TS on the plate: the spots obtained from the sample solution and the plate.

(3) To a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 0.04 g of Prochlorperazine Maleate according to the labeled amount, add 10 mL of 1 mol/L hydrochloric acid TS and 20 mL of diethyl ether, shake, and centrifuge. Transfer the diethyl ether layer to a separator, wash with 5 mL of 0.05 mol/L sulfuric acid TS, and evaporate on a water bath to dryness. Dissolve the residue in 5 mL of sulfuric acid TS, filter, if necessary, and add 1 to 2 drops of potassium permanganate TS: the red color of the test solution is discharged immediately.

Uniformity of dosage units  <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Prochlorperazine Maleate Tablets add 31/5 mL of a mixture of dilute phosphoric acid (1 in 500) and ethanol (99.5) (1:1), treat with ultrasonic waves until the tablet is disintegrated, and shake vigorously for 10 minutes. Add exactly V/20 mL of the internal standard solution, and make a mixture of dilute phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make V mL so that each mL contains about 80 μg of prochlorperazine maleate (C₂₀H₂₄ClN₃S.₂C₄H₄O₄). Centrifuge this solution, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of prochlorperazine maleate

\[
(C₂₀H₂₄ClN₃S.₂C₄H₄O₄) = Mₛ × Q₁/Q₂ × V/250
\]

Mₛ: Amount (mg) of Prochlorperazine Maleate RS
Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 1000).

Dissolution  <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Prochlorperazine Maleate Tablets is not less than 75%.

Start the test with 1 tablet of Prochlorperazine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add the dissolution medium to make exactly V mL so that each mL contains about 9 μg of prochlorperazine maleate (C₂₀H₂₄ClN₃S.₂C₄H₄O₄) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 18 mg of Prochlorperazine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the blank, and determine the absorbances, A₁ and Aₛ, at 255 nm.

Dissolution rate (%) with respect to the labeled amount of prochlorperazine maleate (C₂₀H₂₄ClN₃S.₂C₄H₄O₄)

\[
Mₛ: \text{Amount (mg) of Prochlorperazine Maleate RS}

C: \text{Labeled amount (mg) of prochlorperazine maleate (C₂₀H₂₄ClN₃S.₂C₄H₄O₄) in 1 tablet}

Assay Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Prochlorperazine Maleate Tablets, and powder in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 8 mg of prochlorperazine maleate (C₂₀H₂₄ClN₃S.₂C₄H₄O₄), add 60 mL of a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1), and shake vigorously for 10 minutes.
Add exactly 5 mL of the internal standard solution, and add a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Prochlorperazine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_t \) and \( Q_s \), of prochlorperazine to that of the internal standard.

**Internal standard solution**—A solution of butyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 1000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 257 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeckylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile (11:9).
Flow rate: Adjust the flow rate so that the retention time of prochlorperazine is about 5 minutes.

**System suitability**—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, prochlorperazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of prochlorperazine to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.
Storage—Light-resistant.

**Progesterone**

\[
C_{21}H_{30}O_2 \quad \text{Mol. wt.} \quad 314.46
\]

**Pregn-4-ene-3,20-dione**

[J7-83-0]

Progesterone, when dried, contains not less than 97.0% and not more than 103.0% of C_{21}H_{30}O_2.

**Description**—Progesterone occurs as white crystals or crystalline powder.

It is soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Progesterone in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Progesterone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Progesterone, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Progesterone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Progesterone and Progesterone RS in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

**Optical rotation** <2.49> \([\alpha]_D^20\) : +184° - +194° (after drying, 0.2 g, ethanol (99.5), 10 mL, 100 mm).

**Melting point** <2.60> 128 – 133°C or 120 – 122°C

**Purity**—Related substances—Dissolve 80 mg of Progesterone in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (0.5 g).

**Assay**—Weigh accurately about 10 mg each of Progesterone
and Progesterone RS, previously dried, and dissolve each in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL each of these solutions, add ethanol (99.5) to make exactly 50 mL, and use these solution as the sample solution and the standard solution, respectively. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at the wavelength of maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry $<2.24>$. Amount (mg) of progesterone ($C_{21}H_{30}O_2$) = $M_S \times A_T/A_S$

$M_S$: Amount (mg) of Progesterone RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

**Progesterone Injection**

プロゲステロン注射液

Progesterone Injection is an oily solution for injection. It contains not less than 95.0% and not more than 105.0% of the labeled amount of progesterone ($C_{21}H_{30}O_2$: 314.46).

**Method of preparation** Prepare as directed under Injections, with Progesterone.

**Description** Progesterone Injection is a clear, colorless to pale yellow, oily liquid.

**Identification** To 1 mL of Progesterone Injection add 1 mL of diluted ethanol (9 in 10), shake well, take the ethanol layer, shake well with 1 mL of petroleum benzin, and use the ethanol layer as the sample solution. Separately, dissolve about 5 mg of Progesterone RS in 1 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 2 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and diethylamine (19:1) to a distance of about 10 cm, and heat the plate at 105°C for 10 minutes: the principal spot obtained from the sample solution has the same $R_f$ value as the spot from the standard solution.

Extractable volume $<6.05>$ It meets the requirement.

Foreign insoluble matter $<6.06>$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter $<6.07>$ It meets the requirement.

Sterility $<4.06>$ Perform the test according to the Direct inoculation method: it meets the requirement.

**Assay** Measure the specific gravity of Progesterone Injection. Weigh accurately the mass of Progesterone Injection, equivalent to about 1 mL, mix with 2 mL of tetrahydrofuran, and add ethanol (99.5) to make exactly 3 mL so that each mL contains about 0.5 mg of progesterone ($C_{21}H_{30}O_2$). Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and ethanol (99.5) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Progesterone RS, previously dried in vacuum for 4 hours using phosphorus (V) oxide as the desiccant, dissolve in 2 mL of tetrahydrofuran, and add ethanol (99.5) to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and ethanol (99.5) to make 20 mL, and use this solution as the standard solution. Perform the test with 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of progesterone to that of the internal standard.

Amount (mg) of progesterone ($C_{21}H_{30}O_2$) = $M_S \times Q_T/Q_S \times V/20$

$M_S$: Amount (mg) of Progesterone RS

Internal standard solution—A solution of testosterone propionate in ethanol (99.5) (1 in 4000).

Operating conditions—Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of progesterone is about 6 minutes.

System suitability—System performance: When the procedure is run with 5 µL of the standard solution under the above operating conditions, progesterone and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of progesterone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

**Proglumide**

プログルミド

Proglumide occurs as white crystals or crystalline powder.

$C_{18}H_{26}N_2O_4$: 334.41

(4RS)-4-Benzoylamino-3N,N-dipropylglutaramic acid [6620-60-6]

Proglumide, when dried, contains not less than 98.5% of $C_{18}H_{26}N_2O_4$.

**Description** Proglumide occurs as white crystals or crystalline powder.
It is freely soluble in methanol, soluble in ethanol (95), sparingly soluble in diethyl ether, and very slightly soluble in water.

A solution of Proglumide in methanol (1 in 10) shows no optical rotation.

**Identification (1)** Put 0.5 g of Proglumide in a round-bottom tube, add 5 mL of hydrochloric acid, seal the tube, and heat the tube carefully at 120°C for 3 hours. After cooling, open the tube, filter the content to collect crystals separated out, wash the crystals with 50 mL of cold water, and dry at 100°C for 1 hour: the melting point 2.60°C of the crystals is between 121°C and 124°C.

(2) Determine the infrared absorption spectrum of Proglumide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Absorbance** $<2.24 > E_{1cm}^1(225 \text{ nm})$: 384 – 414 (after drying, 4 mg, methanol, 250 mL).

**Melting point** $<2.60 > 148 – 150°C$

**Purity (1)** Heavy metals $<1.07 >$—Proceed with 1.0 g of Proglumide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic $<1.17 >$—To 1.0 g of Proglumide add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) and 1.5 mL of hydrogen peroxide (30), burn the ethanol, and prepare the test solution according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Proglumide in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, ethyl acetate, acetic acid (100) and methanol (50:18:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** $<2.41 >$ Not more than 0.10% (1 g, reduced pressure, phosphorus (V) oxide, 60°C, 3 hours).

**Residue on ignition** $<2.44 >$ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.16 g of Proglumide, previously dried, dissolve in 40 mL of methanol, add 10 mL of water, and titrate $<2.50 >$ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 33.44 mg of C$_{18}$H$_{26}$N$_{2}$O$_{4}$

**Containers and storage** Containers—Well-closed containers.

---

**1-Proline**

**1-プロリン**

C$_{5}$H$_{9}$NO$_{2}$: 115.13

(2S)-Pyrrrolidine-2-carboxylic acid

[147-85-3]

1-Proline contains not less than 99.0% and not more than 101.0% of C$_{5}$H$_{9}$NO$_{2}$, calculated on the dried basis.

**Description** 1-Proline occurs as white crystals or crystalline powder. It has a slightly sweet taste.

It is very soluble in water and in formic acid, and slightly soluble in ethanol (99.5).

It is deliquescent.

**Identification** Determine the infrared absorption spectrum of l-Proline as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** $<2.49 >$ $[\alpha]_{D}$: $-84.0 – 86.0°$ (1 g, calculated on the dried basis, water, 25 mL, 100 mm).

**pH** $<2.54 >$ The pH of a solution of 1.0 g of 1-Proline in 10 mL of water is 5.9 to 6.9.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of 1-Proline in 10 mL of water: the solution is clear and colorless.

(2) Chloride $<1.02 >$—Perform the test with 0.5 g of 1-Proline. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate $<1.14 >$—Perform the test with 0.6 g of 1-Proline. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium $<1.02 >$—Perform the test with 0.25 g of 1-Proline. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metal $<1.07 >$—Proceed with 1.0 g of 1-Proline according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron $<1.10 >$—Prepare the test solution with 1.0 g of 1-Proline according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Weigh accurately about 0.5 g of 1-Proline, and dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount, equivalent to 2.5 mmol, of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, glycine, L-alanine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine and L-argi-
nine, dissolve them in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, and add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the mass percentage of each amino acid, using the mass of amino acid other than proline in 1 mL of the sample solution obtained from the height of the peaks obtained from the sample and standard solution: the amount of each amino acid other than proline is not more than 0.1%.

Operating conditions—
Detector: A visible absorption photometer (wavelength: 570 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated polystyrene (3 μm in particle diameter) (Na type).
Column temperature: A constant temperature of about 57°C.
Chemical reaction vessel temperature: A constant temperature of about 130°C.
Reaction time: About 1 minute.
Mobile phase: Prepare the mobile phases A, B, C, D and E according to the following table, and add 0.1 mL each of caprylic acid.

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid monohydrate</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>12.80 g</td>
<td>6.10 g</td>
<td>—</td>
</tr>
<tr>
<td>Trisodium citrate dihydrate</td>
<td>6.19 g</td>
<td>7.74 g</td>
<td>13.31 g</td>
<td>26.67 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>3.74 g</td>
<td>54.35 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>8.00 g</td>
</tr>
<tr>
<td>Ethanol (99.5)</td>
<td>130 mL</td>
<td>20 mL</td>
<td>4 mL</td>
<td>—</td>
<td>100 mL</td>
</tr>
<tr>
<td>Thiodiglycerol</td>
<td>5 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5 mL</td>
<td>—</td>
</tr>
<tr>
<td>Lauromacrogol solution (1 in 4)</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
</tr>
<tr>
<td>Water</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>amount</td>
</tr>
</tbody>
</table>

Total amount 1000 mL 1000 mL 1000 mL 1000 mL 1000 mL

Switching of mobile phase: Switch the mobile phases A, B, C, D and E sequentially so that when proceed with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and alanine is not less than 1.2.

System performance: When the test is run with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak height of each amino acid other than proline in the standard solution is not more than 5.0%, and the relative standard deviation of the retention time is not more than 1.0%.

(8) Residual solvent—Being specified separately.

Loss on drying 2.44 Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition 2.44 Not more than 0.1% (1 g).

Assay Weigh accurately about 0.12 g of L-Proline, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate 2.50 with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 11.51 mg of C₃H₇NO₂

Containers and storage Containers—Tight containers.

Promethazine Hydrochloride

プロメタジン塩酸塩

C₁₇H₂₀N₂S.HCl: 320.88
(2RS)-N,N-Dimethyl-1-(10H-phenothiazin-10-yl)propan-2-ylamine monohydrochloride

[58-33-3]

Promethazine Hydrochloride, when dried, contains not less than 98.0% of C₁₇H₂₀N₂S.HCl.

Description Promethazine Hydrochloride occurs as a white to light yellow powder.
It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.
It is gradually colored by light.
A solution of Promethazine Hydrochloride (1 in 25) shows on optical rotation.
Melting point: about 223°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Promethazine Hydrochloride (1 in 100,000) as
directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Promethazine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.5 g of Promethazine Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and filter. To 5 mL of the filtrate add dilute nitric acid to make acidic: the solution responds to the Qualitative Tests <1.00> (2) for chloride.

pH <2.54> The pH of a solution of Promethazine Hydrochloride (1 in 10) is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Promethazine Hydrochloride in 10 mL of water, protecting from direct sunlight: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Promethazine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Perform the test under the protection from sunlight. Dissolve 0.10 g of Promethazine Hydrochloride in exactly 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 20 mg of isopromethazine hydrochloride for thin-layer chromatography in ethanol (95) to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and diethylamine (19:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution corresponding to the spots from the standard solution (1) and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Promethazine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.09 mg of C17H20N2S.HCl

Containers and storage Containers—Tight containers. Storage—Light-resistant.

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**Propafenone Hydrochloride**

プロパフェノン塩酸塩

![Propafenone Hydrochloride structure](image)

C21H27NO3.HCl: 377.90

1-[(2RS)-2-Hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylpropan-1-one monohydrochloride

(A) 34183-22-7

Propafenone Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of C21H27NO3.HCl.

Description Propafenone Hydrochloride occurs as white crystals or a white crystalline powder.

It is freely soluble in formic acid, sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

A solution of Propafenone Hydrochloride in methanol (1 in 100) shows no optical rotation.

Identification (1) Dissolve 0.1 g of Propafenone Hydrochloride in 20 mL of water by warming. After cooling, to 3 mL of this solution add water to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave lengths.

(2) Determine the infrared absorption spectrum of Propafenone Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.1 g of Propafenone Hydrochloride in 20 mL of water by warming. After cooling, to 10 mL of this solution add 1 mL of dilute nitric acid, and filter to separate formed precipitate: the filtrate responds to the Qualitative Tests <1.09> (2) for chloride.

Melting point <2.60> 172 – 175°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Propafenone Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Propafenone Hydrochloride in 20 mL of the mobile phase in the operating conditions 1, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase in the operating conditions 1 to make exactly 50 mL. Pipet 2.5 mL of this solution, add 2.5 mL of a solution of diphenyl phthalate in methanol (1 in 2000), add the mobile phase in the operating conditions 1 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions 1 and 2. Determine each
peak area of both solutions by the automatic integration method: the area of each peak other than the peak of propafenone from the sample solution is not larger than the peak area of propafenone from the standard solution.

Operating conditions 1—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 900 mL of the filtrate add 600 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of diphenyl phthalate is about 39 minutes.
Time span of measurement: Beginning after the solvent peak to the retention time of diphenyl phthalate.

System suitability 1—
System performance: Dissolve 12 mg of Propafenone Hydrochloride and 50 mg of isopropyl benzoate in 100 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions 1, propafenone and isopropyl benzoate are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions 1, the relative standard deviation of the peak area of propafenone is not more than 2.0%.

Operating conditions 2—
Detector, column and column temperature: Proceed as directed in the operation conditions 1.
Mobile phase: Dissolve 7.33 g of sodium 1-decanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 700 mL of the filtrate add 700 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of diphenyl phthalate is about 11 minutes.
Time span of measurement: About 2.5 times as long as the retention time of diphenyl phthalate, beginning after the retention time of diphenyl phthalate.

System suitability 2—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions 2, propafenone and diphenyl phthalate are eluted in this order with the resolution between these peaks being not less than 21.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of propafenone is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Propafenone Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 50 mL of acetic anhydride, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 18.90 mg of C21H27NO3.HCl

Containers and storage Containers—Well-closed containers.

Propafenone Hydrochloride Tablets プロパフェノン塩酸塩錠

Propafenone Hydrochloride Tablets contain not less than 96.0% and not more than 104.0% of the labeled amount of propafenone hydrochloride (C21H27NO3.HCl: 377.90).

Method of preparation Prepare as directed under Tablets, with Propafenone Hydrochloride.

Identification To a quantity of Propafenone Hydrochloride Tablets, equivalent to 0.3 g of Propafenone Hydrochloride according to the labeled amount, add 60 mL of water, and disintegrate by warming. After cooling, centrifuge, and to 3 mL of the supernatant liquid add water to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24(2)>; it exhibits maxima between 247 nm and 251 nm, and between 302 nm and 306 nm. Separately, determine the both maximal absorbances, A1 and A2, of the solution, the ratio of A1/A2 is between 2.30 and 2.55.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propafenone Hydrochloride Tablets add 30 mL of a mixture of water and acetonitrile (1:1), shake well to disintegrate, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 6 mg of propafenone hydrochloride (C21H27NO3.HCl), add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of propafenone hydrochloride
(C21H27NO3.HCl) = M5 × Q5/Q3 × 10/V

M5: Amount (mg) of propafenone hydrochloride for assay

Internal standard solution—A solution of isopropyl benzoate in methanol (1 in 200).

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Propafenone Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Propafenone Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Dis-
card the first 10 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add water to make exactly $V'$ mL so that each mL contains about 67 $\mu$g of propafenone hydrochloride ($C_{21}H_{27}NO_3\cdot HCl$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 13 mg of propafenone hydrochloride for assay, previously dried at 105$^\circ$C for 2 hours, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, $A_1$ and $A_2$, of the sample solution and standard solution at 305 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3\cdot HCl$)

$$M_S = \frac{M_S \times A_1}{A_S} \times \frac{V}{V'} \times \frac{1}{C} \times 450$$

$M_S$: Amount (mg) of propafenone hydrochloride for assay
$C$: Labeled amount (mg) of propafenone hydrochloride ($C_{21}H_{27}NO_3\cdot HCl$) in 1 tablet

Assay To a quantity of Propafenone Hydrochloride Tablets, equivalent to 1.5 g of propafenone hydrochloride ($C_{21}H_{27}NO_3\cdot HCl$), add 70 mL of a mixture of water and acetonitrile (1:1), shake well to disintegrate, shake well for another 5 minutes, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL, and centrifuge. Pipet 4 mL of the supernatant liquid, and add methanol to make exactly 50 mL. Pipet 5 mL of the solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of propafenone hydrochloride for assay, previously dried at 105$^\circ$C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of propafenone to that of the internal standard.

Amount (mg) of propafenone hydrochloride ($C_{21}H_{27}NO_3\cdot HCl$)

$$\text{Amount} = M_S \times \frac{Q_T}{Q_S} \times \frac{50}{1}$$

$M_S$: Amount (mg) of propafenone hydrochloride for assay

Internal standard solution—A solution of isopropyl benzoate in methanol (1 in 200).

Operating conditions—
Detector: An ultraviolet absorption spectrophotometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).
Column temperature: A constant temperature of about 40$^\circ$C.
Mobile phase: Dissolve 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 $\mu$m. To 900 mL of the filtrate add 600 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of propafenone is about 8 minutes.
System suitability—
System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, propafenone and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of propafenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

### Propantheline Bromide

プロパンテリン臭化物

Propantheline Bromide, when dried, contains not less than 98.0% and not more than 102.0% of C$_{23}$H$_{30}$BrNO$_3$.

Description Propantheline Bromide occurs as a white to yellowish white, crystalline powder. It is odorless and has a very bitter taste.

It is very soluble in water, in ethanol (95), in acetic acid (100) and in chloroform, soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Propantheline Bromide (1 in 50) is between 5.0 and 6.0.

Melting point: about 161$^\circ$C (with decomposition, after drying).

Identification (1) To 5 mL of a solution of Propantheline Bromide (1 in 20) add 10 mL of sodium hydroxide TS, heat to boil for 2 minutes. Cool to 60$^\circ$C, and add 5 mL of dilute hydrochloric acid. After cooling, collect the precipitates, and wash with water. Recrystallize from dilute ethanol, and dry at 105$^\circ$C for 1 hour: the crystals melt <2.07> between 217$^\circ$C and 222$^\circ$C.

(2) Dissolve 0.01 g of the crystals obtained in (1) in 5 mL of sulfuric acid: a vivid yellow to yellow-red color develops.

(3) To 5 mL of a solution of Propantheline Bromide (1 in 10) add 2 mL of dilute nitric acid: this solution responds to the Qualitative Tests <1.09> (1) for bromide.

Purity Xanthene-9-carboxylic acid and xanthone—Dissolve 10 mg of Propanetheline Bromide in exactly 2 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 1.0 mg of xanthene-9-carboxylic acid and 1.0 mg of xanthone in exactly 40 mL of chloroform, and use this solution as the standard solution. Perform the test immediately with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 25 $\mu$L each of the sample solution and standard solution on a plate of silica gel with
fluorescent indicator for thin-layer chromatography, and air-dry the plate for 10 minutes. Develop the plate with a mixture of 1,2-dichloroethane, methanol, water and formic acid (56:24:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light: the spots from the sample solution corresponding to the spots from the standard solution are not more intense than those from the standard solution.

Loss on drying <2.4%> Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition <2.4%> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Propanteline Bromide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate Bromide, previously dried, dissolve in 50 mL of a mixture of 1,2-dichloroethane, methanol, water and formic acid (56:24:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light: the spots from the sample solution corresponding to the spots from the standard solution are not more intense than those from the standard solution.

Containers and storage Containers—Well-closed containers.

Propiverine Hydrochloride

プロピベリン塩酸塩

\[
C_{23}H_{29}NO_3\cdot HCl: 403.94
\]

1-Methylpiperidin-4-yl 2,2-diphenyl-2-propoxyacetate monohydrochloride [54556-98-8]

Propiverine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.5% of \( C_{23}H_{30}BrNO_3 \cdot HCl \).

Description Propiverine Hydrochloride occurs as white crystals or a white crystalline powder.

It is soluble in water and in ethanol (99.5).

Identification (1) Dissolve 50 mg of Propiverine Hydrochloride in 20 mL of water, and add acetonitrile to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Propiverine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the infrared absorption spectrum of Propiverine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Propiverine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Propiverine Hydrochloride (1 in 100) add 6 mL of ethyl acetate, and add 3 drops of silver nitrate TS: a white precipitate is formed, which does not dissolve on the addition of 0.5 mL of dilute nitric acid and shaking. The precipitate dissolves on the addition of 2 mL of ammonia TS and shaking.

Melting point <2.6%> 213 – 218°C

Purity (1) Sulfate <1.14%>—Perform the test with 0.40 g of Propiverine Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Heavy metals <1.0%>—Proceed with 1.0 g of Propiverine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 50 mg of Propiverine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time about 0.28 to propiverine, obtained from the sample solution is not larger than 3/10 times the peak area of propiverine from the standard solution, the area of the peak other than propiverine and above mentioned peak from the sample solution is not larger than 1/10 times the peak area of propiverine from the standard solution, and the total area of the peaks other than propiverine from the sample solution is not larger than 1/2 times the peak area of propiverine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of propiverine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of propiverine obtained with 15 \( \mu L \) of this solution is equivalent to 3.5 to 6.5% of that with 15 \( \mu L \) of the standard solution.

System performance: When the procedure is run with 15 \( \mu L \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 15 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propiverine is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Loss on drying <2.4%> Not more than 1.0% (1 g, 105°C, 1 hour).

Residue on ignition <2.4%> Not more than 0.1% (1 g).
Assay Weigh accurately about 50 mg each of Propiverine Hydrochloride and Propiverine Hydrochloride RS, both previously dried, and dissolve each in the mobile phase to make exactly 100 mL. Pipet 10 mL of each of these solutions, add the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas, \( A_T \) and \( A_S \), of propiverine from each solution.

Amount (mg) of propiverine hydrochloride
\[
(C_23H29NO3.HCl) = M_S \times A_T / A_S
\]

\( M_S \): Amount (mg) of Propiverine Hydrochloride RS

System suitability—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.21 g of potassium dihydrogen phosphate and 1.51 g of sodium 1-octane sulfonate in 650 mL of water, adjust to pH 3.2 with phosphoric acid, and add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of propiverine is about 17 minutes.

System suitability—

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 6000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propiverine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Propiverine Hydrochloride Tablets

プロピベリン塩酸塩錠

Propiverine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of propiverine hydrochloride (\( C_{23}H_{29}NO_3\cdot HCl \): 403.94).

Method of preparation Prepare as directed under Tablets, with Propiverine Hydrochloride.

Identification Shake vigorously a quantity of powdered Propiverine Hydrochloride Tablets, equivalent to 50 mg of Propiverine Hydrochloride according to the labeled amount, with 20 mL of water. Add acetonitrile to make 100 mL, centrifuge, and filter the supernatant liquid, if necessary. Determine the absorption spectrum of the supernatant liquid or the filtrate under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 257 nm and 261 nm.

Purity Related substances—Shake vigorously a quantity of powdered Propiverine Hydrochloride Tablets, equivalent to 50 mg of Propiverine Hydrochloride according to the labeled amount, with the mobile phase, add the mobile phase to make exactly 100 mL, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time about 0.28 to propiverine, obtained from the sample solution is not larger than 3/10 times the peak area of propiverine from the standard solution, the area of the peak other than propiverine and the peak mentioned above from the sample solution is not larger than 1/5 times the peak area of propiverine from the standard solution, and the total area of the peaks other than propiverine from the sample solution is not larger than 7/10 times the peak area of propiverine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Propiverine Hydrochloride.

Time span of measurement: About 2.5 times as long as the retention time of propiverine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of propiverine obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that with 15 μL of the standard solution.

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propiverine is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propiverine Hydrochloride Tablets add the mobile phase, shake vigorously, add the mobile phase to make exactly V mL so that each mL contains about 0.1 mg of propiverine hydrochloride (\( C_{23}H_{29}NO_3\cdot HCl \)), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Propireive Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Propiverine Hydrochloride.

Amount (mg) of propiverine hydrochloride
\[
(C_{23}H_{29}NO_3\cdot HCl) = M_S \times A_T / A_S \times V / 500
\]
M₅: Amount (mg) of Propiverine Hydrochloride RS

\[ M₅ = \frac{A₅}{A₅/A₃} \times \frac{V}{V/M \times 1/C × 36} \]

M₅: Amount (mg) of Propiverine Hydrochloride RS

Dissolution (6.10) When the test is performed at 50 revolutions per minute according to Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 20 minutes of Propiverine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Propiverine Hydrochloride Tablets, withdraw not less than 25 mL of the dissolved solution at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 11 μg of propiverine hydrochloride \( (C_{23}H_{29}NO_3 \cdot HCl) \) according to the labeled amount. Pipet 15 mL of this solution, add exactly 2 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Propiverine Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the dissolution medium to make exactly 100 mL. Further, pipet 15 mL of this solution, add exactly 2 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.10) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of propiverine of both solutions.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: To diluted 0.02 mol/L potassium dihydrogen phosphate TS (1 → 2) add phosphoric acid, and adjust to pH 2.0. To 560 mL of this solution add 440 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of propiverine is about 6 minutes.

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operations conditions, the relative standard deviation of the peak area of propiverine is not more than 2.0%.

Assay Weigh accurately and powder not less than 20 Propiverine Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of propiverine hydrochloride \( (C_{23}H_{29}NO_3 \cdot HCl) \), add the mobile phase, shake vigorously, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Propiverine Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Propiverine Hydrochloride.

Costanters and storage Container—Tight containers.

Propranolol Hydrochloride

Propranolol Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of \( C_{16}H_{21}NO_2 \cdot HCl \).

Description Propranolol Hydrochloride occurs as a white, crystalline powder.
It is freely soluble in methanol, soluble in water and in acetic acid (100), and sparingly soluble in ethanol (99.5).
A solution of Propranolol Hydrochloride in methanol (1 in 40) shows no optical rotation.
It is gradually colored to yellowish white to light brown by light.

Identification (1) Determine the absorption spectrum of a solution of Propranolol Hydrochloride in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Propranolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Propranolol Hydrochloride (1 in 50) responds to the Qualitative Tests (1.09) (2) for chloride.

pH < 2.5 The pH of a solution prepared by dissolving 0.5 g of Propranolol Hydrochloride in 50 mL of water is
Residue on ignition (2.20) Not more than 0.1% (1 g).

Loss on drying (2.21) Not more than 0.5% (1 g, 105°C, 4 hours).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Propranolol Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals (1.07)—Proceed with 1.0 g of Propranolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Propranolol Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.2.1D) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than propranolol from the sample solution is not larger than 1/2 times the peak area of propranolol from the standard solution, and the total area of the peaks other than the peak of propranolol is not larger than 2 times the peak area of propranolol from the standard solution.

System suitability—
Detector: An ultraviolet absorption photometer (wavelength: 292 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 1.6 g of sodium lauryl sulfate and 0.31 g of tetrabutylammonium phosphate in 450 mL of water, add 1 mL of sulfuric acid and 550 mL of acetonitrile for liquid chromatography, and adjust to pH 3.3 with 2 mol/L sodium hydroxide TS.
Flow rate: Adjust the flow rate so that the retention time of propranolol is about 4 minutes.
Time span of measurement: About 5 times as long as the retention time of propranolol.

System suitability—
Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of propranolol obtained with 20 µL of this solution is equivalent to 17 to 33% of that with 20 µL of the standard solution.

System performance: When the procedure is run with 20 mL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propranolol is not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propranolol is not more than 2.0%.

Melting point (2.60) 163 – 166°C

Propranolol Hydrochloride Tablets

Propranolol Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of propranolol hydrochloride (C16H21NO2.HCl: 295.80).

Method of preparation—Prepare as directed under Tablets, with Propranolol Hydrochloride.

Identification—Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry (2.2.24): it exhibits maxima between 288 nm and 292 nm, and between 317 nm and 321 nm.

Uniformity of dosage units (6.02)—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propranolol Hydrochloride Tablets add 20 mL of water, and shake until the tablet is completely disintegrated. Add 50 mL of methanol, shake vigorously for 10 minutes, then add methanol to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, add methanol to make exactly V mL so that each mL contains about 20 µg of propranolol hydrochloride (C16H21NO2.HCl), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A5, of the sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry (2.2.24).

\[
\text{Amount (mg) of propranolol hydrochloride (C}_{16}\text{H}_{21}\text{NO}_{2}.\text{HCl)} = M_S \times A_1/A_5 \times V/V \times 1/25
\]

M_S: Amount (mg) of propranolol hydrochloride for assay

Dissolution (6.10)—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Propranolol Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Propranolol Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the
specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V’ mL so that each mL contains about 10 μg of propranolol hydrochloride (C₁₆H₂₁NO₂.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₃, of the sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry.<sup>2.24</sup>

Dissolution rate (%) with respect to the labeled amount of propranolol hydrochloride (C₁₆H₂₁NO₂.HCl)

\[
M₅ = \frac{M_s \times A_1/A_3 \times V/V' \times 1/C \times 18}{9}
\]

M₅: Amount (mg) of propranolol hydrochloride for assay

C: Labeled amount (mg) of propranolol hydrochloride (C₁₆H₂₁NO₂.HCl) in 1 tablet

**Assay** Weigh accurately a portion of the powder, equivalent to about 20 mg of propranolol hydrochloride (C₁₆H₂₁NO₂.HCl), add 60 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Filter, discard the first 20 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₃, of the sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry.<sup>2.24</sup>

Amount (mg) of propranolol hydrochloride (C₁₆H₂₁NO₂.HCl)

\[
M₅ = \frac{M_s \times A_1/A_3 \times V/V' \times 1/C \times 18}{9}
\]

M₅: Amount (mg) of propranolol hydrochloride for assay

**Containers and storage** Containers—Well-closed containers and storage.

Storage—Light-resistant.

### Propyl Parahydroxybenzoate

**Description** Propyl Parahydroxybenzoate occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and very slightly soluble in water.<sup>•</sup>

**Identification** (1) The melting point < 2.60° of Propyl Parahydroxybenzoate is between 96°C and 99°C.

(2) Determine the infrared absorption spectrum of Propyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.<sup>•</sup>

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Propyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add water to make 1000 mL.

(2) Acidity—Dissolve 0.2 g of Propyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromoresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Propyl Parahydroxybenzoate in 25 mL of aceton, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of aceton, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).<sup>•</sup>

(4) Related substances—Dissolve 0.1 g of Propyl Parahydroxybenzoate in 10 mL of aceton, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add aceton to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with is not more intense than the spot with the standard solution.

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 1.0 g of Propyl Parahydroxybenzoate add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS = 180.2 mg of C₁₀H₁₂O₃

---

**Propyl Parahydroxybenzoate**

パラオキシ安息香酸プロピル

**C₁₀H₁₂O₃:** 180.20

Propyl 4-hydroxybenzoate

[94-13-3]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (*) •.
Propylene Glycol

**Description** Propylene Glycol is a clear, colorless, viscous liquid. It is odorless, and has a slightly bitter taste.

**Specific gravity** $<2.560$ $d_{20}^0 = 1.035 - 1.040$

**Purity (1)** Acidity—Mix 10.0 mL of Propylene Glycol with 50 mL of freshly boiled and cooled water, and add 5 drops of phenolphthalein TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: the solution has a red color.

**Identification (1)** Mix 2 to 3 drops of Propylene Glycol and with pyridine. It is hygroscopic.

**Identification (2)** Heat gently 1 mL of Propylene Glycol with 0.5 g of potassium hydrogen sulfate: a characteristic odor is evolved.

**Identification (3)** Sulfate $<1.140$—Triturate Propylthiouracil finely in a mortar. To 0.75 g of the powder add 25 mL of water, heat for 10 minutes on a water bath, cool, filter, and wash the residue with water until the volume of the filtrate becomes 30 mL. To 10 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

**Residue on ignition** $<2.440$—Perform the test with 1.0 g of Propylene Glycol according to Method 1, and perform the test (not more than 2 ppm).

**Glycerin—Heat** 1.0 g of Propylene Glycol with 0.5 g of potassium hydrogen sulfate and evaporate to dryness: no odor of acrolein is perceptible.

**Water** $<2.485$—Triturate Propylthiouracil with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

**Loss on drying** $<2.410$—Triturate Propylthiouracil with 2.5 mL of Standard Lead Solution (not more than 0.5% (1 g, 105°C, 2 hours)).

Assay Weigh accurately about 0.3 g of Propylthiouracil, previously dried, and add 30 mL of water. Add 30 mL of 0.1 mol/L hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

**Residue on ignition** $<2.440$—Not more than 0.1% (1 g).

**Distilling range** $<2.570$—184 – 189°C, not less than 95 vol%.

**Containers and storage** Containers—Tight containers.
mol/L sodium hydroxide VS from a burette, heat to boil, and
dissolve by stirring. Wash down the solid adhering to the
wall of the flask with a small amount of water, and add 50
mL of 0.1 mol/L silver nitrate VS with stirring. Boil gently
for 5 minutes, add 1 to 2 mL of bromothymol blue TS, and
titrates to blue with 0.1 mol/L sodium hydroxide VS until a
persistent blue-green color develops. Determine the total
volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 8.512 mg of C₂H₁₀N₂O₅

Containers and storage Containers—Well-closed contain-
ers.
Storage—Light-resistant.

Propylthiouracil Tablets
プロピルチオウラシル錠

Propylthiouracil Tablets contain not less than
93.0% and not more than 107.0% of the labeled
amount of propylthiouracil (C₇H₁₀N₂O₅: 170.23).

Method of preparation Prepare as directed under Tablets,
with Propylthiouracil.

Identification To a quantity of powdered Propylthiouracil
Tablets, equivalent to 0.3 g of Propylthiouracil according to
the labeled amount, add 5 mL of ammonia TS, allow to
stand for 5 minutes with occasional shaking, add 10 mL of
water, and centrifuge. To the supernatant liquid add acetic
acid (50%), collect the precipitate produced, recrystallize from
water, and dry at 105°C for 1 hour: it melts <2.60° between
218°C and 221°C. Proceed with the residue as directed in the
Identification under Propylthiouracil.

Uniformity of dosage units <6.02> Perform the test accord-
ing to the following method: it meets the requirement of the
Content uniformity test.

To 1 tablet of Propylthiouracil Tablets add 3V/4 mL of
2nd fluid for dissolution test, treat with ultrasonic waves
until the tablet is disintegrated, and add 2nd fluid for disso-
lution test to make exactly V mL so that each mL contains
about 0.25 mg of propylthiouracil (C₇H₁₀N₂O₅). Filter this
solution through a membrane filter with a pore size not
exceeding 0.45 μm, discard the first 5 mL of the filtrate,
pipet 2 mL of the subsequent filtrate, add 2nd fluid for disso-
lution test to make exactly 100 mL, and use this solution as the
sample solution. Proceed as directed in the Assay.

Amount (mg) of propylthiouracil (C₇H₁₀N₂O₅) = M₅ × A₁/As × V/V × 1/C × 9
M₅: Amount (mg) of propylthiouracil for assay

Dissolution <6.10> When the test is performed at 75 revolu-
tions per minute according to the Paddle method, using 900
mL of 2nd fluid for dissolution test as the dissolution me-
dium, the dissolution rate in 30 minutes of Propylthiouracil
Tablets is not less than 80%.

Start the test with 1 tablet of Propylthiouracil Tablets,
withdraw not less than 20 mL of the medium at the specified
minute after starting the test, and filter through a membrane
filter with a pore size not exceeding 0.8 μm. Discard the first
10 mL of the filtrate, pipet V mL of the subsequent filtrate,
add the dissolution medium to make exactly V mL so that
each mL contains about 5.6 μg of propylthiouracil (C₇H₁₀N₂O₅) according to the labeled amount, and use this
solution as the sample solution. Separately, weigh about 50
mg of propylthiouracil for assay, previously dried at 105°C
for 3 hours, and dissolve in the dissolution medium to make
exactly 1000 mL. Pipet 5 mL of this solution, add the disso-
lution medium to make exactly 50 mL, and use this solution
as the standard solution. Proceed as directed in the Assay.

Dissolution rate (%) with respect to the labeled amount
of propylthiouracil (C₇H₁₀N₂O₅) = M₅ × A₁/As × V/V × 1/C × 9
M₅: Amount (mg) of propylthiouracil for assay
C: Labeled amount (mg) of propylthiouracil (C₇H₁₀N₂O₅)
in 1 tablet

Assay Weigh accurately the mass of not less than 20
Propylthiouracil Tablets, and powder. Weigh accurately a
portion of the powder, equivalent to about 50 mg of propylthiouracil (C₇H₁₀N₂O₅), add 150 mL of 2nd fluid for
dissolution test, disperse finely the particles with the aid of
ultrasonic waves, and add 2nd fluid for dissolution test to
make exactly 200 mL. Filter this solution through a mem-
brane filter with a pore size not exceeding 0.45 μm, discard
the first 5 mL of the filtrate, pipet 2 mL of the subsequent
filtrate, add 2nd fluid for dissolution test to make exactly
100 mL, and use this solution as the sample solution. Sepa-
rately, weigh accurately about 50 mg of propylthiouracil for
assay, previously dried at 105°C for 2 hours, and dissolve in
2nd fluid for dissolution test to make exactly 200 mL. Pipet
2 mL of this solution, add 2nd fluid for dissolution test to
make exactly 100 mL, and use this solution as the standard
solution. Determine the absorbance at 274 nm, A₁ and A₅,
of the sample solution and standard solution as directed
under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of propylthiouracil (C₇H₁₀N₂O₅)
= M₅ × A₁/As
M₅: Amount (mg) of propylthiouracil for assay

Containers and storage Containers—Well-closed contain-
ers.
Storage—Light-resistant.

Protamine Sulfate
プロタミン硫酸塩

Protamine Sulfate is the sulfate of protamine pre-
pared from the mature sperm of fish belonging to the
family Salmonidae.

It has a property to bind with heparin.

It binds with not less than 100 Units of heparin per
mg, calculated on the dried basis.

Description Protamine Sulfate occurs as a white powder.

It is sparingly soluble in water.

Identification (1) Dissolve 1 mg of Protamine Sulfate in 2
mL of water, add 5 drops of a solution prepared by dissolv-
ing 0.1 g of 1-naphthol in 100 mL of diluted ethanol (7 in 10)
and 5 drops of sodium hypochlorite TS, then add sodium hydroxide TS until the solution becomes alkaline: a vivid red color develops.

(2) Dissolve 5 mg of Protamine Sulfate in 1 mL of water by warming, add 1 drop of a solution of sodium hydroxide (1 in 10) and 2 drops of copper (II) sulfate TS: a red-purple color develops.

(3) An aqueous solution of Protamine Sulfate (1 in 20) responds to the Qualitative Tests <1.06> for sulfate.

\[
\text{pH } < 2.54> \text{ Dissolve } 1.0 \, \text{g of Protamine Sulfate in } 100 \, \text{mL of water}: \text{the pH of this solution is between 6.5 and 7.5.}
\]

**Purity** (1) Clarity and color of solution—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Absorbance—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance between 260 nm and 280 nm is not more than 0.1.

**Loss on drying** <2.41> Not more than 5.0% (1 g, 105°C, 3 hours).

**Nitrogen content** Weigh accurately about 10 mg of Protamine Sulfate, and perform the test as directed under Nitrogen Determination <1.06>: the amount of nitrogen (N:14.01) is 22.5 – 25.5%, calculated on the dried basis.

**Heparin-binding capacity**

(i) Sample solution (a)—Weigh accurately about 15 mg of Protamine Sulfate, and dissolve in water to make exactly 100 mL. Repeat this procedure 3 times, and use the solutions so obtained as the sample solutions (a1), (a2) and (a3).

(ii) Sample solution (b)—Pipet 10 mL each of the sample solutions (a1), (a2) and (a3), add exactly 5 mL of water to them, and use these solutions as the sample solutions (b1), (b2) and (b3).

(iii) Sample solution (c)—Pipet 10 mL each of the sample solutions (a1), (a2) and (a3), add exactly 20 mL of water to them, and use these solutions as the sample solutions (c1), (c2) and (c3).

(iv) Standard solution—Dissolve Heparin Sodium RS in water to make a solution containing exactly about 20 Units per mL.

(v) Procedure—Transfer exactly 2 mL of the sample solution to a cell for spectrophotometer, add the standard solution dropwise while mixing, and determine the transmittance at 500 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. Continue the addition until a sharp change in the transmittance is observed, and note the volume, V mL, of the standard solution added. Repeat this procedure 2 times for each sample solution.

(vi) Calculation—Calculate the amount of heparin bound with 1 mg of the sample by the following formula from the volume of titrant on each sample solution, and calculate the average of 18 results obtained. The assay is not valid unless each relative standard deviation of 6 results obtained from the sample solution (a), sample solution (b) and sample solution (c) is not more than 5%, respectively, and also unless each relative standard deviation of 6 results obtained from 3 sets, (a1, b1, c1), (a2, b2, c2) and (a3, b3, c3) is not more than 5%, respectively.

\[
\text{Amount (heparin Unit) of heparin bound to } 1 \, \text{mg of Protamine Sulfate} = S \times V \times 50/M_F \times d
\]

\[
S: \text{Amount (heparin Unit) of heparin sodium in } 1 \, \text{mL of the standard solution}
\]

\[
M_F: \text{Amount (mg) of the sample, calculated on the dried basis}
\]

\[
d: \text{Dilution factor for each sample solution from the sample solution (a)}
\]

**Sulfate content** Weigh accurately about 0.15 g of Protamine Sulfate, dissolve in 75 mL of water, add 5 mL of 3 mol/L hydrochloric acid TS, and heat to boil. Add gradually 10 mL of barium chloride TS while boiling, and allow to stand for 1 hour while heating. Filter the precipitate formed, wash the precipitate with warm water several times, and transfer the precipitate into a tared crucible. Dry the precipitate, and incinerate by ignition to constant mass: the amount of sulfate (SO4) is 16 – 22%, calculated on the dried basis, where 1 g of the residue is equivalent to 0.4117 g of SO4.

**Containers and storage** Containers—Tight containers.

**Protamine Sulfate Injection**

プラタミン硫酸塩注射液

Protamine Sulfate Injection is an aqueous solution for injection.

It contains not less than 92.0% and not more than 108.0% of the labeled amount of Protamine Sulfate. It binds with not less than 100 Units of heparin per mg of the labeled amount.

**Method of preparation** Prepare as directed under Injections, with Protamine Sulfate.

**Description** Protamine Sulfate Injection is a colorless liquid. It is odorless or has the odor of preservatives.

**Identification** (1) Dilute a volume of Protamine Sulfate Injection, equivalent to 1 mg of Protamine Sulfate according to the labeled amount, with water to make 2 mL, and proceed as directed in the Identification (1) under Protamine Sulfate.

(2) Dilute a volume of Protamine Sulfate Injection, equivalent to 5 mg of Protamine Sulfate according to the labeled amount, with water to make 1 mL, and proceed as directed in the Identification (2) under Protamine Sulfate.

**pH** <2.54> 5.0 – 7.0

**Bacterial endotoxins** <4.06> Less than 6.0 EU/mg.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** (1) Protein—Pipet a volume of Protamine Sulfate Injection, equivalent to about 10 mg of Protamine Sulfate,
transfer to a Kjeldahl flask, evaporate on a water bath to
dryness with the aid of a current of air, determine the nitrogen
as directed under Nitrogen Determination <1.06>, and
calculate the amount of protein by converting 0.24 mg of
nitrogen (N: 14.01) to 1 mg of protein.

(2) Heparin-binding activity—Proceed the test as di-
rected in the Heparin-binding capacity under Protamine
Sulfate, changing the sample solution (a) as below, and de-
determine the amount of heparin bound to 1 mg of protein by
dividing by the amount of protein.

(i) Sample solution (a)—Pipet a volume of Protamine
Sulfate Injection, equivalent to 15.0 mg of Protamine Sul-
fate, into a 100-mL beaker and add water to make exactly
100 mL. Repeat this procedure two more times, and designate the solutions so
obtained as the sample solutions (a1), (a2) and (a3).

Containers and storage Containers—Hermetic containers.

Prothionamide

プロチオナミド

\[
\text{C}_9\text{H}_{12}\text{N}_2\text{S} : 180.27
\]

2-Propylpyridine-4-carbothioamide

[14222-60-7]

Prothionamide, when dried, contains not less than
98.0% of \(\text{C}_9\text{H}_{12}\text{N}_2\text{S}\).

Description Prothionamide occurs as yellow crystals or
crystalline powder. It has a slight, characteristic odor.

It is freely soluble in methanol and in acetic acid (100),
soluble in ethanol (95), slightly soluble in diethyl ether, and
practically insoluble in water.

It dissolves in dilute hydrochloric acid and in dilute sulfuric
acid.

Identification (1) Mix 0.05 g of Prothionamide with 0.1 g
of 1-chloro-2,4-dinitrobenzene, transfer about 10 mg of this
mixture to a test tube, and heat for several seconds over a
small flame until the mixture is fused. Cool, and add 3 mL
of potassium hydroxide-ethanol TS: a red to orange-red
color develops.

(2) Place 0.5 g of Prothionamide in a 100-mL beaker,
dissolve in 20 mL of sodium hydroxide TS by heating
while shaking occasionally: the gas evolved turns a
moistened red litmus paper to blue. Boil gently, and evapo-
rate the solution to 3 to 5 mL. After cooling, add gradually
20 mL of acetic acid (100), and heat on a water bath: the gas
evolved darkens moistened lead (II) acetate paper.

Evaporate the solution on a water bath to 3 to 5 mL with the
aid of a current of air, cool, add 10 mL of water, and mix
well. Filter the crystals by suction, recrystallize from water
immediately, and dry in a desiccator (in vacuum, silica gel)
for 6 hours: the crystals melt \(<2.60\) between 198°C and
203°C (with decomposition).

Melting point \(<2.60\) 142–145°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g
of Prothionamide in 20 mL of ethanol (95): the solution is
clear, and shows a yellow color.

(2) Acidity—Dissolve 3.0 g of Prothionamide in 20 mL
of methanol with warming. Add 100 mL of water to the so-
lution, cool in an ice water bath with agitation, and remove
any precipitate by filtration. Allow 80 mL of the filtrate to
to cool to room temperature, and add 0.8 mL of cresol red TS
and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color
develops.

(3) Heavy metals \(<1.07\)—Proceed with 1.0 g of Pro-
thionamide according to Method 2, and perform the test.
Prepare the control solution with 2.0 mL of Standard Lead
Solution (not more than 20 ppm).

(4) Arsenic \(<1.11\)—Prepare the test solution with 0.6 g
of Prothionamide according to Method 3, and perform the
test. To the test solution add 10 mL of a solution of magne-
sium nitrate hexahydrate in ethanol (95) (1 in 50), then add
1.5 mL of hydrogen peroxide (30), and ignite to burn (not
more than 3.3 ppm).

Loss on drying \(<2.41\) Not more than 0.5% (1 g, 80°C,
3 hours).

Residue on ignition \(<2.44\) Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Prothionamide,
previously dried, dissolve in 50 mL of acetic acid (100), and titrate
\(<2.50\) with 0.1 mol/L perchloric acid VS until the
color of the solution changes from orange-red to dark
orange-brown (indicator: 2 mL of \(p\)-naphtholbenzein TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 18.03 mg of \(\text{C}_9\text{H}_{12}\text{N}_2\text{S}\).

Containers and storage Containers—Well-closed contain-
ers.

Storage—Light-resistant.

Protirelin

プロチレリン

\[
\text{C}_{16}\text{H}_{22}\text{N}_6\text{O}_4 : 362.38
\]

5-Oxo-L-prolyl-L-histidyl-L-prolinamide

[24305-27-9]

Protirelin contains not less than 98.5% of
\(\text{C}_{16}\text{H}_{22}\text{N}_6\text{O}_4\), calculated on the dehydrated basis.

Description Protirelin occurs as a white powder.

It is freely soluble in water, in methanol, in ethanol (95)
and in acetic acid (100).

It is hygroscopic.

Identification (1) Take 0.01 g of Protirelin in a test tube
made of hard glass, add 0.5 mL of 6 mol/L hydrochloric
acid TS, seal the upper part of the tube, and heat carefully at
Protirelin Tartrate Hydrate

Protirelin Tartrate Hydrate, calculated on the anhydrous basis, contains not less than 98.5% of protirelin tartrate (C_{16}H_{22}N_{6}O_{4}C_{4}H_{6}O_{6}: 512.48).

**Description** Protirelin Tartrate Hydrate occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in acetic acid (100), and practically insoluble in ethanol (95) and in diethyl ether.

Melting point: about 187°C (with decomposition).

**Identification** (1) To 1 mL of a solution of Protirelin Tartrate Hydrate (1 in 1000) add 2 mL of a solution of 4-nitrobenzene diazonium fluoroborate (1 in 2000) and 2 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0: a red color develops.

(2) Dissolve 0.03 g of Protirelin Tartrate Hydrate in 5 mL of sodium hydroxide TS, add 1 drop of copper (II) sulfate TS: a purple color develops.

(3) To 0.20 g of Protirelin Tartrate Hydrate add 5.0 mL of 6 mol/L hydrochloric acid TS, and boil for 7 hours under a reflux condenser. After cooling, evaporate 2.0 mL of this solution on a water bath to dryness, dissolve the residue in 2.0 mL of water and use this solution as the sample solution. Separately, dissolve 22 mg of L-glutamic acid, 32 mg of L-histidine hydrochloride, 0.12 g of L-histidine dihydrochloride monohydrate and 0.06 g of L-proline in 20 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry the plates at 100°C for 30 minutes. Spray evenly a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS, and boil for 7 hours under a reflux condenser. After cooling, evaporate 2.0 mL of this solution on a water bath to dryness, dissolve the residue in 2.0 mL of 6 mol/L hydrochloric acid TS, and boil for 7 hours under a reflux condenser. After cooling, evaporate 2.0 mL of this solution on a water bath to dryness, dissolve the residue in 2.0 mL of water and use this solution as the sample solution. Separately, dissolve 22 mg of L-glutamic acid, 32 mg of L-histidine hydrochloride monohydrate and 17 mg of L-proline in 2.0 mL of 0.1 mol/L hydrochloric acid TS by heating, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of L-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and, heat at 80°C for 5 minutes: the three spots obtained from the sample solution show, respectively, the same color and the same Rf value as the corresponding spots obtained from the standard solution.
A solution of Protirelin Tartrate Hydrate (1 in 40) responds to the Qualitative Tests \( <1.0\% \) for tartrate.

**Optical rotation** \( <2.49 \) \( [\alpha]_D^20 = -50.0 \pm -53.0^\circ \) (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

**pH** \( <2.54 \) Dissolve 1.0 g of Protirelin Tartrate Hydrate in 100 mL of water: the pH of this solution is between 3.0 and 4.0.

**Purity (1)** Clarity and color of solution—Dissolve 0.10 g of Protirelin Tartrate Hydrate in 10 mL of water: the solution is clear and colorless.

**Heavy metals** \( <1.07 \)—Proceed with 1.0 g of Protirelin Tartrate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Identification (1)** Dissolve 10 g of Pullulan in 100 mL of water with stirring by adding in small portions: a viscous solution is produced.

**Identification (2)** Mix 10 mL of the viscous solution obtained in (1) with 0.1 mL of pullulanase TS, and allow to stand: the solution loses its viscosity.

**Identification (3)** To 10 mL of a solution of Pullulan (1 in 50) add 2 mL of macrogol 600: a white precipitate is formed immediately.

**Viscosity** \( <2.55 \) Take exactly 10.0 g of Pullulan, previously dried, dissolve in water to make exactly 100 g, and perform the test at 30 ± 0.1°C as directed in Method 1: the kinematic viscosity is between 100 and 180 mm²/s.

**pH** \( <2.54 \) Dissolve 1.0 g of Pullulan in 10 mL of freshly boiled and cooled water: the pH is between 4.5 and 6.5.

**Purity (1)** Heavy metals \( <1.07 \)—Proceed with 4.0 g of Pullulan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

**Monosaccharide and oligosaccharides**—Dissolve 0.8 g of Pullulan, previously dried, in 100 mL of water, and designate this solution as the sample stock solution. To 1 mL of the sample stock solution add 0.1 mL of potassium chloride saturated solution, and shake vigorously with 3 mL of methanol. Centrifuge, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of the sample stock solution, add water to make exactly 50 mL, and use this solution as the standard solution. Pipet 0.2 mL each of the sample solution, the standard solution and water, transfer them gently to each test tube containing 5 mL of a solution
of anthrone in diluted sulfuric acid (3 in 4) (1 in 500) and cooling in ice water, stir immediately, then heat at 90°C for 10 minutes, and cool immediately. Perform the test with these solutions so obtained as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbance at 620 nm, A₅, A₆ and A₇; the amount of monosaccharide and oligosaccharides is not more than 10.0%.

Amount (% of monosaccharide and oligosaccharides

\[
\frac{(A_7 - A_6)}{(A_5 - A_6)} \times 8.2
\]

**Loss on drying <2.41>** Not more than 6.0% (1 g, in vacuum, 90°C, 6 hours).

**Residue on ignition <2.44>** Not more than 0.3% (2 g).

Containers and storage Containers—Well-closed containers.

### Pyrantel Pamoate

ピランテルパモ酸塩

C₁₁H₁₄N₂S·C₂₃H₁₆O₆: 594.68

1-Methyl-2-[[(1E)-2-(thien-2-yl)vinyl]-1,4,5,6-tetrahydroxy-2-naphthoate (1/1) [22204-24-6]

Pyrantel Pamoate, when dried, contains not less than 98.0% of C₁₁H₁₄N₂S·C₂₃H₁₆O₆.

**Description** Pyrantel Pamoate occurs as a light yellow to yellow, crystalline powder. It is odorless and tasteless.

It is sparingly soluble in N,N-dimethylformamide, very slightly soluble in methanol and in ethanol (95), and practically insoluble in water, in ethyl acetate and in diethyl ether.

Melting point: 256 – 264°C (with decomposition).

**Identification (1)** To 0.05 g of Pyrantel Pamoate add 10 mL of methanol and 1 mL of a mixture of hydrochloric acid and methanol (1:1), and shake vigorously: a yellow precipitate is produced. Filter the solution, and use the filtrate as the sample solution. Use the precipitate for the test (2). To 0.5 mL of the sample solution add 1 mL of a solution of 2,3-indolinedione in sulfuric acid (1 in 1000): a red color develops.

(2) Collect the precipitate obtained in the test (1), wash with methanol, and dry at 105°C for 1 hour. To 0.01 g of the dried precipitate add 10 mL of methanol, shake well, and filter. To 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a green color develops.

(3) Dissolve 0.1 g of Pyrantel Pamoate in 50 mL of N,N-dimethylformamide, and add methanol to make 200 mL. To 2 mL of the solution add a solution of hydrochloric acid in methanol (9 in 1000) to make 100 mL. Determine the absorpti

**Purity (1)** Chloride <1.07>—To 1.0 g of Pyrantel Pamoate add 10 mL of dilute nitric acid and 40 mL of water, and heat on a water bath with shaking for 5 minutes. After cooling, add water to make 50 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute nitric acid and water to make 50 mL. Proceed the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate <1.14>—To 0.75 g of Pyrantel Pamoate add 5 mL of dilute hydrochloric acid and water to make 100 mL, and heat on a water bath for 5 minutes with shaking. After cooling, add water to make 100 mL, and filter. To 20 mL of the filtrate add water to make 50 mL. Proceed the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.144%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Pyrantel Pamoate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pyrantel Pamoate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 0.10 g of Pyrantel Pamoate in 10 mL of N,N-dimethylformamide, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add N,N-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the spot of pyrantel and the spot of pamoic acid from the sample solution are not more intense than the spot of pyrantel (Rf value: about 0.3) from the standard solution.

**Loss on drying <2.41>** Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition <2.44>** Not more than 0.3% (1 g).

**Assay** Weigh accurately about 0.5 g of Pyrantel Pamoate, previously dried, add 25 mL of chloroform and 25 mL of sodium hydroxide TS, shake for 15 minutes, and extract. Extract further with two 25-mL portions of chloroform. Filter each extract through 5 g of anhydrous sodium sulfate on a pledget of absorbent cotton. Combine the chloroform extracts, add 30 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal
Pyrazinamide

Pyrazinamide, when dried, contains not less than 99.0% and not more than 101.0% of C₅H₅N₃O.

Description
Pyrazinamide occurs as white crystals or crystalline powder. It is sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5) and in acetic anhydride.

Identification (1) Determine the absorption spectrum of a solution of Pyrazinamide in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrazinamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point 2.60° 188 – 193°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Pyrazinamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Pyrazinamide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the sample solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot is not more intense than the principal spot.

Loss on drying 2.41° Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Pyrazinamide, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 59.47 mg of C₁₁H₁₄N₂S.C₂₃H₁₆O₆

Containers and storage Containers—Tight containers.

Pyridostigmine Bromide

Pyridostigmine Bromide, when dried, contains not less than 98.5% of C₁₉H₁₅BrN₂O₂.

Description
Pyridostigmine Bromide occurs as a white, crystalline powder. It is odorless or has a slightly characteristic odor. It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of Pyridostigmine Bromide (1 in 10) is between 4.0 and 6.0. It is deliquescent.

Identification (1) Dissolve 0.02 g of Pyridostigmine Bromide in 10 mL of water, add 5 mL of Reinecke salt TS: the unpleasant odor of dimethylamine is perceptible.

(2) To 0.1 g of Pyridostigmine Bromide add 0.6 mL of sodium hydroxide TS: the unpleasant odor of dimethylamine is perceptible.

(3) Determine the absorption spectrum of a solution of Pyridostigmine Bromide in 0.1 mol/L hydrochloric acid TS (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Pyridostigmine Bromide (1 in 50) responds to the Qualitative Tests <1.09> for Bromide.

Melting point 2.60° 153 – 157°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pyridostigmine Bromide in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Pyridostigmine Bromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g

Containers and storage Containers—Well-closed containers.
of Pyridostigmine Bromide according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Pyridostigmine Bromide in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spots 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonium chloride TS (5:4:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution in color.

**Loss on drying** <2.41> Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 100°C, 5 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Pyridostigmine Bromide, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 26.11 mg of C₉H₁₄BrN₂O₂

Containers and storage Containers—Hermetic containers.

## Pyridoxine Hydrochloride

### Vitamin B₆

ビリドキシン塩酸塩

C₉H₁₅NO₃.HCl: 205.64
4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol monohydrochloride [58-56-0]

Pyridoxine Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of C₉H₁₅NO₃.HCl.

**Description** Pyridoxine Hydrochloride occurs as a white to pale yellow, crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetic anhydride and in acetic acid (100).

It is gradually affected by light.

Melting point: about 206°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Pyridoxine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pyridoxine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyridoxine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Pyridoxine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pyridoxine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

**pH** <2.54> The pH of a solution prepared by dissolving 1.0 g of Pyridoxine Hydrochloride in 50 mL of water is between 2.5 and 3.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Pyridoxine hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Pyridoxine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Related substances—Dissolve 1.0 g of Pyridoxine Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 2 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, hexane and ammonia solution (28) (65:13:13:9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate in diluted ethanol (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate, and air-dry: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.30% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Pyridoxine Hydrochloride, previously dried, add 5 mL of acetic acid (100) and 5 mL of acetic anhydride, dissolve by gentle boiling, cool, add 30 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 20.56 mg of C₉H₁₅NO₃.HCl

Containers and storage Containers—Tight containers.

Storage—Light-resistant.
Pyridoxine Hydrochloride Injection

Vitamin B₆ Injection

ピリドキシン塩酸塩注射液

Pyridoxine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pyridoxine hydrochloride (C₈H₁₁NO₃.HCl: 205.64).

Method of preparation Prepare as directed under Injections, with Pyridoxine Hydrochloride.

Description Pyridoxine Hydrochloride Injection is a colorless or pale yellow, clear liquid.

It is gradually affected by light.

pH: 3.0 – 6.0

Identification (1) To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.05 g of Pyridoxine Hydrochloride according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible spectrophotometry (2.24): it exhibits a maximum between 288 nm and 292 nm.

(2) To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.01 g of Pyridoxine Hydrochloride according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve to the labeled amount, add water to make 10 mL, and use this solution as the standard solution. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Pyridoxine Hydrochloride RS, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Pipet 1 mL each of the sample solution and standard solution, add 2.0 mL of barbital buffer solution, 9.0 mL of 2-propanol and 2.0 mL of a freshly prepared solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 4000), shake well, add 2-propanol to make exactly 25 mL, and allow to stand for 90 minutes. Determine the absorbances, A₁ and A₅, of the subsequent sample solution and subsequent standard solution, respectively, at 650 nm as directed under Ultraviolet-visible Spectrophotometry (2.24), using a solution, prepared in the same manner with 1 mL of water, as the blank.

Amount (mg) of pyridoxine hydrochloride

\[ \text{Amount (mg) of pyridoxine hydrochloride} = M_S \times \frac{A_1}{A_5} \times 1/5 \]

M₅: Amount (mg) of Pyridoxine Hydrochloride RS

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Pyroxylin

ピロキシリン

Pyroxylin is a nitric acid ester of cellulose. It is usually moistened with 2-propanol or some other solvent.

Description Pyroxylin occurs as a white cotton-like substance or white flakes.

It is freely soluble in acetone, and very slightly soluble in diethyl ether.

Upon heating or exposure to light, it is decomposed with the evolution of nitrous acid vapors.

Identification Ignite Pyroxylin: it burns very rapidly with a luminous flame.

Purity (1) Clarity of solution—Dissolve 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, in 25 mL of a mixture of diethyl ether and ethanol (95) (3:1): the solution is clear.

(2) Acidity—Shake 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, with 20 mL of water for 10 minutes: the filtrate is neutral.

(3) Water-soluble substances—Evaporate 10 mL of the filtrate obtained in (2) on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.5 mg.

(4) Residue on ignition—Weigh accurately about 2 g of Pyroxylin, previously dried at 80°C for 2 hours, and moisten with 10 mL of a solution of castor oil in acetone (1 in 20) to gelatinize the sample. Ignite the contents to carbonate the sample, heat strongly at about 500°C for 2 hours, and allow to cool in a desiccator (silica gel): the amount of the residue is not more than 0.30%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, packed loosely, remote from
fire, and preferably in a cold place.

**Pyrrolnitrin**

ピロールニトリン

\[
\text{C}_{10}\text{H}_{6}\text{Cl}_{2}\text{N}_{2}\text{O}_{2} : 257.07
\]

3-Chloro-4-(3-chloro-2-nitrophenyl)pyrrole

[1018-71-9]

Pyrrolnitrin contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Pyrrolnitrin is expressed as mass (potency) of pyrrolnitrin (C_{10}H_{6}Cl_{2}N_{2}O_{2}).

**Description**

Pyrrolnitrin occurs as yellow to yellow-brown, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

**Identification (1)**

Determine the absorption spectrum of a solution of Pyrrolnitrin in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pyrrolnitrin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrrolnitrin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Pyrrolnitrin RS: both spectra exhibit similar intensities of absorption at the same wavelenghts.

**Melting point** <2.60> 124–128°C

**Purity**

Related substances—Dissolve 0.10 g of Pyrrolnitrin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 3 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>, Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of xylene, ethyl acetate, and formic acid (18:2:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly diluted sulfuric acid (1 in 3) on the plate, and heat at 100°C for 30 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

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**Quinapril Hydrochloride**

キナプリル塩酸塩

\[
\text{C}_{25}\text{H}_{30}\text{N}_{2}\text{O}_{5} \cdot \text{HCl} : 474.98
\]

Quinapril Hydrochloride contains not less than 99.0% and not more than 101.0% of C_{25}H_{30}N_{2}O_{5}.
Quinapril Hydrochloride Tablets

Quinapril Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of quinapril hydrochloride (C_{25}H_{30}N_{2}O_{5}.HCl: 474.98).

Method of preparation
Prepare as directed under Tablets, with Quinapril Hydrochloride.

Identification
To a quantity of powdered Quinapril Hydrochloride Tablets, equivalent to 20 mg of Quinapril Hydrochloride according to the labeled amount, add 10 mL of methanol, shake for 5 minutes, and centrifuge. To 5 mL of the supernatant liquid add 0.5 mL of dilute hydrochloric acid, and add methanol to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 256 nm and 260 nm, between 262 nm and 266 nm, and between 269 nm and 273 nm.

Purity
To an amount of the supernatant liquid obtained in the Assay add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) so that

**Time span of measurement:** About 4 times as long as the retention time of quinapril, beginning after the solvent peak.

**System suitability—**
Test for required detectability: Pipet 10 mL of the standard solution, and add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 100 mL. Confirm that the peak area of quinapril obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of quinapril are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of quinapril is not more than 2.0%.

(3) Residual solvent—Being specified separately.

**Water**<2.44> Not more than 1.0% (0.2 g, coulometric titration).

**Residue on ignition**<2.44> Not more than 0.1% (1 g).

**Assay**
Start to titrate within 3 minutes after dissolving Quinapril Hydrochloride. Weigh accurately about 0.5 g of Quinapril Hydrochloride, dissolve in 70 mL of acetic acid (100), add 4 mL of bismuth nitrate TS, and titrate<2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 47.50 mg of C_{25}H_{30}N_{2}O_{5}.HCl

**Containers and storage**
Containers—Tight containers. Storage—In a cold place.
each mL contains 0.2 mg of Quinapril Hydrochloride according to the labeled amount, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 0.5 to quinapril obtained from the sample solution is not larger than 2 times the peak area of quinapril from the standard solution, and the area of the peak, having the relative retention time of about 2.0 to quinapril obtained from the sample solution is not larger than the peak area of quinapril from the standard solution.

Operating conditions—
Proceed as directed in the operating conditions in the Purity (2) under Quinapril Hydrochloride.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of quinapril are not less than 3000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of quinapril is not more than 2.0%

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Quinapril Hydrochloride Tablets add 3 V/5 mL of a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1), shake vigorously to disintegrate the tablet, shake again for 10 minutes, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly V mL so that each mL contains about 0.22 mg of quinapril hydrochloride (C25H30N2O5·HCl) and centrifuge. Pipet 15 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of quinapril hydrochloride (C25H30N2O5·HCl) 
\[
M_5 = M_S \times \frac{Q_6}{Q_5} \times \frac{V}{120}
\]

\(M_5\): Amount (mg) of quinapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) (1 in 800).

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Quinapril Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Quinapril Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly V mL so that each mL contains about 1.2 mg of quinapril hydrochloride (C25H30N2O5·HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 24 mg of quinapril hydrochloride for assay (separately, determine the water <2.40> in the same manner as Quinapril Hydrochloride), and dissolve in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL. Pipet 2 mL of this solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \(A_1\) and \(A_5\), of quinapril of both solutions.

Dissolution rate (%) with respect to the labeled amount of quinapril hydrochloride (C25H30N2O5·HCl) 
\[
M_5 = M_S \times \frac{A_1}{A_5} \times \frac{V}{V/\times \times 1/C \times 9/2}
\]

\(M_5\): Amount (mg) of quinapril hydrochloride for assay, calculated on the anhydrous basis
C: Labeled amount (mg) of quinapril hydrochloride (C25H30N2O5·HCl) in 1 tablet

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 214 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: While keeping the temperature not below 25°C, adjust to pH 2.0 of 0.1 mol/L potassium dihydrogen phosphate TS with perchloric acid. To 1000 mL of this solution add 1500 mL of acetonitrile for liquid chromatography.
Flow rate: Adjust the flow rate so that the retention time of quinapril is about 7 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of quinapril are not less than 2000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of quinapril is not more than 2.0%

Assay To 20 Quinapril Hydrochloride Tablets add 300 mL of a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1), shake vigorously to disintegrate the tablets, shake again for 10 minutes, and add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 500 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, equivalent to about 6.5 mg of quinapril hydrochlo-
ride (C_{25}H_{30}N_{2}O_{5}, HCl), add exactly 4 mL of the internal standard solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of quinapril hydrochloride for assay (separately, determine the water in the same manner as Quinapril Hydrochloride), and dissolve in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 100 mL. Pipet 25 mL of this solution, add exactly 4 mL of the internal standard solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions. Calculate the ratios, Q_{T} and Q_{S}, of the peak area of quinapril to that of the internal standard.

Amount (mg) of quinapril hydrochloride (C_{25}H_{30}N_{2}O_{5}, HCl) in 1 tablet

\[ M_S \times \frac{Q_T}{Q_S} \times \frac{1}{V} \times 25/4 \]

M_{S}: Amount (mg) of quinapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) (1 in 800).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 214 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: While keeping the temperature below 25°C, adjust to pH 2.0 of 0.2 mol/L potassium dihydrogen phosphate TS with perchloric acid. To 1000 mL of this solution add 1000 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of quinapril is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, quinapril and the internal standard are eluted in this condition with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of quinapril to that of the internal standard is not more than 1.0%.

Containers and storage Container—Tight containers.
phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method, and calculate their amount by the area percentage method: the amount of dihydroquinidine sulfate is not more than 15.0%, and those of quinine sulfate and dihydroquinidine sulfate are not more than 1.0%. The total area of the peaks other than the principal peak and the above peaks is not larger than the peak area of cinchonine from the standard solution.

**Operating conditions—**


Column: A column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).

Temperature: Room temperature.

Mobile phase: A mixture of water, acetonitrile, methane-sulfonic acid TS and a solution of diethylamine (1 in 10) (43:5:1:1).

Flow rate: Adjust the flow rate so that the retention time of quinidine is about 10 minutes.

Selection of column: Dissolve 0.01 g each of Quinidine Sulfate Hydrate and quinine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinidine in this order with a resolution between quinidine and quinine of at least 1.2, respectively.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonine obtained from 50 μL of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of quinidine begins after the solvent peak.

(3) Readily carbonizable substances <1.15>—Take 0.20 g of Quinidine Sulfate Hydrate and perform the test: the solution has no more color than Matching fluid M.

**Loss on drying <2.44>** Not more than 5.0% (1 g, 130 °C, 3 hours).

**Residue on ignition <2.44>** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Quinidine Sulfate Hydrate, previously dried, dissolve in 20 mL of acetic acid (100), and add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.90 mg of (C₂₃H₂₈N₂O₄)·H₂SO₄

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

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**Quinine Ethyl Carbonate**

キニーネエチル炭酸エステル

C₁₉H₂₈N₂O₅: 396.48

Ethyl (85,9R)-6′-methoxycinchonan-9-yl carbonate [83-75-0]

Quinine Ethyl Carbonate contains not less than 98.5% of C₂₃H₂₈N₂O₄, calculated on the dehydrated basis.

**Description** Quinine Ethyl Carbonate occurs as white crystals. It is odorless, and tasteless at first but slowly develops a bitter taste.

It is very soluble in methanol, freely soluble in ethanol (95) and in ethanol (99.5), soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

**Identification (1)** Determine the absorption spectrum of a solution of Quinine Ethyl Carbonate in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Quinine Ethyl Carbonate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [α]D: -42.2 - -44.0° (0.5 g, calculated on the dehydrated basis, methanol, 50 mL, 100 mm).

**Melting point** <2.60> 91 – 95 °C

**Purity (1)** Chloride—Dissolve 0.30 g of Quinine Ethyl Carbonate in 10 mL of dilute nitric acid and 20 mL of water. To 5 mL of the solution add 2 to 3 drops of silver nitrate TS: no color develops.

(2) Sulfate <1.10>—Dissolve 1.0 g of Quinine Ethyl Carbonate in 5 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS, 5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Quinine Ethyl Carbonate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 20 mg of Quinine Ethyl Carbonate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of quinine sulfate in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add mobile phase to
make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method, and calculate the amount of a main impurity in the sample solution which appears at about 1.2 times of the retention time of quinine ethyl carbonate by the area percentage method: it is not more than 10.0%. The total area of the peaks other than the principal peak and above mentioned peak from the sample solution is not larger than the peak area of quinine from the standard solution.

Operating conditions—
Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1.2 g of sodium 1-octanesulfonate in 1000 mL of a mixture of water and methanol (1:1), and adjust to pH 3.5 with diluted phosphoric acid (1 in 20).
Flow rate: Adjust the flow rate so that the retention time of the peak of quinine ethyl carbonate is about 20 minutes.
Selection of column: Dissolve 5 mg each of Quinine Ethyl Carbonate and quinine sulfate in the mobile phase to make 50 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinine, dihydroquinine, quinine ethyl carbonate and the main impurity of quinine ethyl carbonate in this order with the resolution between the peaks of quinine and dihydroquinine being not less than 2.7, and between the peaks of quinine and quinine ethyl carbonate being not less than 5.
Detection sensitivity: Adjust the detection sensitivity so that the peak height of quinine obtained from 10 μL of the standard solution is between 5 mm and 10 mm.
Time span of measurement: About 2 times as long as the retention time of quinine ethyl carbonate.

Water <2.48> Not more than 3.0% (0.5 g, volumetric titration, direct titration).
Residue on ignition <2.44> Not more than 0.1% (1 g).
Assay Weigh accurately about 0.3 g of Quinine Ethyl Carbonate, dissolve in 60 mL of acetic acid (100), add 2 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 19.82 mg of C23H28N2O4

Containers and storage Containers—Well-closed containers.

Quinine Hydrochloride Hydrate

キニーネ塩酸塩水和物

C20H24N2O2.HCl.2H2O: 396.91
(85,9R)-6'- Methoxycinchonan-9-ol monohydrochloride dihydrate [6119-47-7]

Quinine Hydrochloride Hydrate, when dried, contains not less than 98.5% of quinine hydrochloride (C20H24N2O2.HCl: 360.88).

Description Quinine Hydrochloride Hydrate occurs as white crystals. It is odorless, and has a very bitter taste.

It is very soluble in ethanol (99.5), freely soluble in acetic acid (100), in acetic anhydride and in ethanol (95), soluble in water, and practically insoluble in diethyl ether.

It, previously dried, is freely soluble in chloroform.
It gradually changes to brown by light.

Identification (1) A solution of Quinine Hydrochloride Hydrate (1 in 50) shows no fluorescence. To 1 mL of the solution add 100 mL of water and 1 drop of dilute sulfuric acid: a blue fluorescence is produced.

(2) To 5 mL of a solution of Quinine Hydrochloride Hydrate (1 in 1000) add 1 to 2 drops of bromine TS and 1 mL of ammonia TS: a green color develops.

(3) To 5 mL of a solution of Quinine Hydrochloride Hydrate (1 in 50) add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. Collect the precipitate, and add an excess of ammonia TS: it dissolves.

Optical rotation <2.49> [α]D: −245 – −255° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Quinine Hydrochloride Hydrate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 6.0 and 7.0.

Purity (1) Sulfate <1.14>—Perform the test with 1.0 g of Quinine Hydrochloride Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Barium—Dissolve 0.5 g of Quinine hydrochloride hydrate in 10 mL of water by warming, and add 1 mL of dilute sulfuric acid: no turbidity is produced.

(3) Chloroform-ethanol-insoluble substances—Warm 2.0 g of Quinine Hydrochloride Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2:1) at 50°C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), dry at 105°C for 1 hour, and weigh: the mass of the residue so obtained is not more than 2.0 mg.

(4) Related substances—Dissolve 20 mg of Quinine Hydrochloride Hydrate in the mobile phase to make exactly 100
mL, and use this solution as the sample solution. Separately, dissolve 25 mg of cinchonine in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method, and calculate the amount of dihydroquinine hydrochloride by the area percentage method: it is not more than 10.0%. The total area of the peaks other than the main peak and the above peaks is not larger than the peak area of cinchonine from the standard solution.

**Operating conditions—**
- Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).
- Column temperature: Room temperature.
- Mobile phase: A mixture of water, acetonitrile, methanesulfonic acid TS and a solution of diethylamine (1 in 10) (43:5:1:1).
- Flow rate: Adjust the flow rate so that the retention time of quinine is about 10 minutes.
- Selection of column: Dissolve 10 mg each of Quinine Hydrochloride and quinine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μL of this solution under the above operating conditions. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with the resolution between quinidine and quinine, and that between quinidine and quinine hydrochloride by the area percentage method: it is not more than 10.0%. The total area of the peaks other than the main peak and the above peaks is not larger than the peak area of cinchonine from the standard solution.

**Identification** (1) Determine the absorption spectrum of a solution of Quinine Sulfate Hydrate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Quinine Sulfate Hydrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.4 g of Quinine Sulfate Hydrate add 20 mL of water and 1 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for sulfate.

**Optical rotation** <2.49> [α]D: −235° to −245° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

**pH** <2.5> Shake 2.0 g of Quinine Sulfate Hydrate in 20 mL of freshly boiled and cooled water, and filter: the pH of this filtrate is between 5.5 and 7.0.

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 g of Quinine Sulfate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Chloroform–ethanol-insoluble substances—Warm 2.0 g of Quinine Sulfate Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2:1) at 50°C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), dry at 105°C for 1 hour, and weigh: the mass of the residue is not more than 2.0 mg.

Quinine Sulfate Hydrate contains not less than 98.5% of quinine sulfate [(C20H24N2O2)2·H2SO4·2H2O: 746.91], calculated on the dried basis.

**Description** Quinine Sulfate Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a very bitter taste. It is freely soluble in acetic acid (100), slightly soluble in water, in ethanol (95), in ethanol (99.5) and in chloroform, and practically insoluble in diethyl ether. It gradually changes to brown by light.
(3) Related substances—Dissolve 20 mg of Quinine Sulfate Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of cinchonidine in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 > \) according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of dihydroquinine sulfate by the area percentage method: it is not more than 5%. The total area of the peaks other than the main peak and the above peak is not larger than the peak area of cinchonidine from the standard solution.

**Operating conditions—**


Column: A column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).

Temperature: Room temperature.

Mobile phase: A mixture of water, acetonitrile, methane sulfonic acid TS and a solution of diethylamine (1 in 10) (43:5:1:1).

Flow rate: Adjust the flow rate so that the retention time of quinine is about 10 minutes.

Selection of column: Dissolve 0.01 g each of Quinine Sulfate Hydrate and quinidine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with the resolution between quinidine and quinine and between quinidine and quinidine sulfate in the mobile phase to make 50 mL. Proceed with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 > \) according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of dihydroquinine sulfate by the area percentage method: it is not more than 5%. The total area of the peaks other than the main peak and the above peak is not larger than the peak area of cinchonidine from the standard solution.

**Operating conditions—**


Column: A column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).

Temperature: Room temperature.

Mobile phase: A mixture of water, acetonitrile, methane sulfonic acid TS and a solution of diethylamine (1 in 10) (43:5:1:1).

Flow rate: Adjust the flow rate so that the retention time of quinine is about 10 minutes.

Selection of column: Dissolve 0.01 g each of Quinine Sulfate Hydrate and quinidine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with the resolution between quinidine and quinine and between quinidine and dihydroquinine being not less than 1.2.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonidine obtained from 50 μL of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of quinine beginning after the solvent peak.

**Loss on drying \( <2.41 > \) 3.0% - 5.0% (1 g, 105°C, 3 hours).

**Residue on ignition \( <2.44 > \) Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.5 g of Quinine Sulfate Hydrate, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate \( <2.50 > \) with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.90 mg of \( \text{C}_{26} \text{H}_{32} \text{~N}_2 \text{O}_4 \text{~S} \cdot \text{H}_2 \text{SO}_4 \)

**Containers and storage**

Containers—Well-closed containers.

Storage—Light-resistant.

Rabeprazole Sodium

ラベプラゾールナトリウム

C\text{\textsubscript{18}}H\text{\textsubscript{20}}N\text{\textsubscript{3}}NaO\text{\textsubscript{3}}S: 381.42

Monosodium (RS)-2-\{(4-(3-methoxypropoxy)-3-methylpyridin-2-yl)[methylsulfanyl]-1H-benzimidazolide

\[ 117976-90-6 \]

Rabeprazole Sodium contains not less than 98.0% and not more than 101.0% of \( \text{C}_{18} \text{H}_{20} \text{~N}_3 \text{NaO}_3 \text{~S} \), calculated on the dried basis.

**Description**

Rabeprazole Sodium occurs as a white to pale yellowish white powder.

It is very soluble in water, and freely soluble in ethanol (99.5).

It dissolves in 0.01 mol/L sodium hydroxide TS.

It is hygroscopic.

A solution of Rabeprazole Sodium (1 in 20) shows no optical rotation.

**Identification (1)**

Determine the absorption spectrum of a solution of Rabeprazole Sodium in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24 > \), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rabeprazole Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rabeprazole Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25 > \), and compare the spectrum with the Reference Spectrum or the spectrum of Rabeprazole Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample, or the sample and the RS separately in ethanol (99.5), evaporate the ethanol at 40°C, dry the residues in vacuum at 55°C for 24 hours, and perform the test with the residues.

(3) A solution of Rabeprazole Sodium (1 in 10) responds to the Qualitative Tests \( <1.09 > \) for sodium salt.

**Purity (1)**

Heavy metals \( <1.07 > \)—Proceed with 2.0 g of Rabeprazole Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Rabeprazole Sodium in 50 mL of a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 > \) according to the following con-
ditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 0.7 to rabeprazole from the sample solution is not larger than 4/5 times the peak area of rabeprazole from the standard solution, the area of the peak other than rabeprazole and other than the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of rabeprazole from the standard solution, and the total area of the peaks other than rabeprazole from the sample solution is not larger than the peak area of rabeprazole from the standard solution.

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of rabeprazole, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) to make exactly 100 mL. Confirm that the peak area of rabeprazole obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rabeprazole are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of rabeprazole and the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Ranitidine Hydrochloride

C₁₁H₁₂N₂O₃S.HCl: 350.86
(1EZ)-N-[2-{[(Dimethylamino)methyl][furan-2-yl][methyl]sulfanyl][ethyl]-N’-methyl-2-nitroethene-1,1-diamine monohydrochloride [66357-59-3]

Ranitidine Hydrochloride, when dried, contains not less than 97.5% and not more than 102.0% of
**Ranitidine Hydrochloride**

**C<sub>13</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>S.HCl.**

**Description** Ranitidine Hydrochloride occurs as a white to pale yellow, crystalline or fine granular powder.

- It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).
- It is hygroscopic.
- It is gradually colored by light.
- Melting point: about 140°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Ranitidine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ranitidine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ranitidine Hydrochloride as directed in the paste method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Ranitidine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ranitidine Hydrochloride (1 in 50) responds to the Qualitative Tests (1.09) for chlorides.

**pH** (2.54) The pH of a solution obtained by dissolving 1.0 g of Ranitidine Hydrochloride in 100 mL of water is between 4.5 and 6.0.

**Purity (1)** Clarity and color of solution—A solution of Ranitidine Hydrochloride (1 in 10) is clear and pale yellow to light yellow.

(2) Heavy metals (1.07)—Proceed with 2.0 g of Ranitidine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic (1.11)—Prepare the test solution with 1.0 g of Ranitidine Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.22 g of Ranitidine Hydrochloride in methanol to make exactly 10 mL, and use this solution as the standard solution. Pipet 0.5 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 6 mL, 4 mL, 2 mL and 1 mL of the standard solution (1), add to each methanol to make exactly 10 mL, and use these solutions as the standard solution (2), the standard solution (3), the standard solution (4) and the standard solution (5), respectively. Separately, dissolve 12.7 mg of ranitidinediamine in methanol to make exactly 10 mL, and use this solution as the standard solution (6). Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 µL each of the sample solution and standard solutions (1), (2), (3), (4) and (5) on a plate of silica gel for thin-layer chromatography. Separately, spot 10 µL of the sample solution on the plate, then spot 10 µL of the standard solution (6) on the spotted position of the sample solution. Immediately develop the plate with a mixture of ethyl acetate, 2-propanol, ammonia solution (28) and water (25:15:5:1) to a distance of about 15 cm, and air-dry the plate. Allow the plate to stand in iodine vapor until the spot from the standard solution (5) appears: the spot obtained from the standard solution (6) is completely separated from the principal spot from the sample solution. The spot having Rf value of about 0.7 from the sample solution is not more intense than the spot from the standard solution (1), the spots other than the principal spot and the spot of Rf value of about 0.7 from the sample solution are not more intense than the spot from the standard solution (2), and the total amount of these related substances, calculated by comparison with the spots from the standard solutions (1), (2), (3), (4) and (5), is not more than 1.0%.

**Loss on drying (2.41)** Not more than 0.75% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition (2.44)** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 20 mg of Ranitidine Hydrochloride and Ranitidine Hydrochloride RS, previously dried, dissolve each in the mobile phase to make exactly 200 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, A<sub>T</sub> and A<sub>S</sub>, of ranitidine.

Amount (mg) of C<sub>13</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>S.HCl = M<sub>S</sub> × A<sub>T</sub>/A<sub>S</sub>

M<sub>S</sub>: Amount (mg) of Ranitidine Hydrochloride RS

**Operating conditions**
- Detector: An ultraviolet absorption photometer (wavelength: 322 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 µm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 5) (17:3).
- Flow rate: Adjust the flow rate so that the retention time of ranitidine is about 5 minutes.

**System suitability**
- System performance: Dissolve 20 mg of Ranitidine Hydrochloride and 5 mg of benzalphthalide in 200 mL of the mobile phase. When the procedure is run with 10 µL of this solution under the above operating conditions, benzalphthalide and ranitidine are eluted in this order with the resolution between these peaks being not less than 2.0.
- System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ranitidine is not more than 1.0%.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.
Rape Seed Oil

Oleum Rapae

ナタネ油

Rape Seed Oil is the fixed oil obtained from the seed of *Brassica campestris* Linné subsp. *napus* Hooker filius et Anderson var. *nippo-oleifera* Makino (*Cruciferae*).

**Description**  Rape Seed Oil is a clear, pale yellow, slightly viscous oil. It is odorless or has a slight odor and a mild taste. It is miscible with diethyl ether and with petroleum diethyl ether. It is slightly soluble in ethanol (95).

Specific gravity *d*<sup>25</sup>: 0.906 – 0.920

**Acid value** *<1.13*> Not more than 0.2.

**Saponification value** *<1.13*> 169 – 195

**Unsaponifiable matters** *<1.13*> Not more than 1.5%.

**Iodine value** *<1.13*> 95 – 127

**Containers and storage** Containers—Tight containers.

Rebamipide

レバミピド

C<sub>19</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>4</sub>: 370.79

(2S,5)-2-(4-Chlorobenzoylamino)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid

[90098-04-7]

Rebamipide, when dried, contains not less than 99.0% and not more than 101.0% of C<sub>19</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>4</sub>.

**Description**  Rebamipide occurs as a white crystalline powder, and has a bitter taste. It is soluble in N,N-dimethylformamide, very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

A solution of Rebamipide in N,N-dimethylformamide (1 in 20) shows no optical rotation.

Melting point: about 291°C (with decomposition).

**Identification** (1)  Determine the absorption spectrum of a solution of Rebamipide in methanol (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry *<2.245*> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)  Determine the infrared absorption spectrum of Rebamipide as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.255*> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3)  Perform the test with Rebamipide as directed under Flame Coloration Test *<1.045*> (2): a green color appears.

**Purity** (1)  Chloride *<1.035*>—Dissolve 0.5 g of Rebamipide in 40 mL of *N*,*N*-dimethylformamide, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.40 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of *N*,*N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(2)  Heavy metals *<1.077*>—Proceeed with 2.0 g of Rebamipide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3)  Rebamipide *m*-chloro isomer—Dissolve 40 mg of Rebamipide in a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.016*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak of rebamipide *m*-chloro isomer, having the relative retention time of about 0.95 with respect to rebamipide, from the sample solution, is not larger than 3/8 times the area of the peak of rebamipide from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 300 mL of phosphate buffer solution, pH 6.2, add 750 mL of water. To 830 mL of this solution add 170 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 20 minutes.

**System suitability**—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make exactly 25 mL. Confirm that the peak area of rebamipide obtained from 10 µL of this solution is equivalent to 15 to 25% of that of rebamipide from 10 µL of the standard solution.

System performance: To 1 mL of the sample solution add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make 100 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rebamipide are not less than 11,000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times.
with 10 \mu L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rebamipide is not more than 2.0%.

(4) Related substances—Perform the test with exactly 10 \mu L each of the sample solution and standard solution obtained in (3) as directed under Liquid Chromatography \(<2.03\) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each area of the peaks of rebamipide \(o\)-chloro isomer and debenzoylated isomer, having the relative retention times of about 0.5 and about 0.7, respectively, with respect to rebamipide obtained from the sample solution, is not larger than 3/8 times the peak area of rebamipide from the standard solution, the area of each peak other than rebamipide and the peak mentioned above from the sample solution is not larger than 1/4 times the peak area of rebamipide from the standard solution, and the total area of the peaks other than rebamipide from the sample solution is not larger than the peak area of rebamipide from the standard solution.

For the calculation, use the peak area of rebamipide \(o\)-chloro isomer, after multiplying by the response factor, 1.4.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \mu m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.44 g of sodium 1-decanesulfonate in 1000 mL of water and to this solution add 1000 mL of methanol and 10 mL of phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 12 minutes.

Time span of measurement: About 3 times as long as the retention time of rebamipide, beginning after the solvent peak.

**System suitability—**

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make exactly 50 mL. Confirm that the peak area of rebamipide obtained from 10 \mu L of this solution is equivalent to 7 to 13% of that of rebamipide from 10 \mu L of the standard solution.

System performance: Dissolve 20 mg of 4-chlorobenzoate in methanol to make 50 mL. To 5 mL of this solution add 5 mL of the sample solution and a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make 50 mL. When the procedure is run with 10 \mu L of this solution under the above operating conditions, rebamipide and 4-chlorobenzoate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 \mu L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rebamipide is not more than 2.0%.

(5) Residual solvent—Being specified separately.

**Loss on drying \(<2.41\)** Not more than 3.0% (1 g, 105°C, 2 hours).

**Residue on ignition \(<2.44\)** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.6 g of Rebamipide, previously dried, dissolve in 60 mL of \(N, N\)-dimethylformamide, and titrate \(<2.50\) with 0.1 mol/L potassium hydroxide VS until the color of the solution changes from pale yellow to colorless (indicator: 2 drops of phenol red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide VS weighs 37.08 mg of \(C_{19}H_{15}ClN_2O_4\).

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

**Rebamipide Tablets**

レバミピド錠

Rebamipide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of rebamipide (\(C_{19}H_{15}ClN_2O_4\): 370.79).

**Method of preparation** Prepare as directed under Tablets, with Rebamipide.

**Identification** To a quantity of powdered Rebamipide Tablets, equivalent to 30 mg of Rebamipide according to the labeled amount, add 5 mL of a mixture of methanol and ammonia solution (28) (9:1), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 30 mg of rebamipide for assay in 5 mL of a mixture of methanol and ammonia solution (28) (9:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\). Spot 5 \mu L each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and formic acid (75:25:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same \(R_f\) value as the spot from the standard solution.

**Uniformity of dosage units \(<6.02\)** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Rebamipide Tablets add 10 mL of water, shake well for 10 minutes, add exactly 10 mL of the internal standard solution, add 10 mL of \(N, N\)-dimethylformamide, shake well for 5 minutes, and add \(N, N\)-dimethylformamide to make 50 mL. Centrifuge this solution, pipet 20 mL of the supernatant liquid, equivalent to 3 mg of rebamipide (\(C_{19}H_{15}ClN_2O_4\)), and add 20 mL of \(N, N\)-dimethylformamide and water to make 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 \mu m, discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.1 g of rebamipide for assay, previously dried at 105°C for 2 hours, dissolve in \(N, N\)-dimethylformamide, and add exactly 10 mL of the internal standard solution and \(N, N\)-dimethylformamide to make 50 mL. Pipet 1.5 mL of this solution, add 20 mL of \(N, N\)-dimethylformamide, add water to make 50 mL, and use this solution as the standard
solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of rebamipide (C}_{19}\text{H}_{15}\text{ClN}_{2}\text{O}_{4}) = M_s \times Q_s / Q_h \times 3 / 2V
\]

\(M_s\): Amount (mg) of rebamipide for assay

**Assay**

To 10 Rebamipide Tablets add exactly dimethylformamide to make 50 mL. To 2 mL of this solution add 0.1 g of rebamipide for assay, previously dried at 105 °C for 2 hours, dissolve in N,N-dimethylformamide to make exactly 50 mL. Filter, if necessary, through a membrane filter with a pore size not exceeding 0.5 \(\mu\)m. Discard the filtrate.

Start the test with 1 tablet of Rebamipide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \(\mu\)m. Discard the filtrate. Add the solution to the filtrate, pipet \(V\) mL of the subsequent filtrate, add the dissolution medium to make exactly \(V'\) mL so that each mL contains about 22 \(\mu\)g of rebamipide (C_{19}H_{15}ClN_{2}O_{4}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of rebamipide for assay, previously dried at 105 °C for 2 hours, and dissolve in N,N-dimethylformamide to make exactly 25 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry with 200 nm as the sample solution and 20 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of rebamipide to that of the internal standard is not more than 8.

Dissolution rate (%) with respect to the labeled amount of rebamipide (C_{19}H_{15}ClN_{2}O_{4})

\[
M_d = A_1 / A_3 \times V'/V \times 1/C \times 36
\]

\(M_d\): Amount (mg) of rebamipide for assay

C: Labeled amount (mg) of rebamipide (C_{19}H_{15}ClN_{2}O_{4}) in 1 tablet

\(A_1\) and \(A_3\), at 326 nm.

System suitability—

System performance: When the procedure is run with 20 \(\mu\)L of the standard solution under the above operating conditions, the ratio of the peak area of rebamipide to that of the internal standard is not more than 1.0%.

**Containers and storage**

Containers—Well-closed containers.

**Reserpine**

Reserpine, when dried, contains not less than 96.0% of C_{33}H_{40}N_{2}O_{9}.

Description Reserpine occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in acetic acid (100) and in chloroform, slightly soluble in acetonitrile, very slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether. It is affected by light.
Reserpine Injection / Official Monographs

Identification (1) To 1 mg of Reserpine add 1 mL of vanillin-hydrochloric acid TS, and warm: a vivid red-purple color develops.

(2) Determine the absorption spectrum of a solution of Reserpine in acetonitrile (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Reserpine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Reserpine, previously dried, as directed in the potassium bromide disk method under Infrared Spectroscopy <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Reserpine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D25 = −114−−127° (after drying, 0.25 g, chloroform, 25 mL, 100 mm).

Purity Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Reserpine in 50 mL of acetonitrile, and use this solution as the standard solution. Pipet 3 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution: both spectra exhibit similar intensities of absorption at the same wavelength.

System suitability—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate, pH 3.0 and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of reserpine is about 20 minutes.

Time span of measurement: About twice as long as the retention time of reserpine.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add acetonitrile to make exactly 50 mL. Confirm that the peak area of reserpine obtained from 10 μL of this solution is equivalent to 3 to 5% of that from 10 μL of the standard solution.

System performance: Dissolve 0.01 g of Reserpine and 4 mg of butyl parahydroxybenzoate in 100 mL of acetonitrile. To 5 mL of this solution add acetonitrile to make 50 mL. When the procedure is run with 20 μL of this solution according to the operating conditions in the Assay, reserpine and butyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of reserpine is not more than 2.0%.

Loss on drying <2.47> Not more than 0.5% (0.2 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.2 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 10 mg each of Reserpine and Reserpine RS, previously dried, and dissolve each in acetonitrile to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.61> according to the following conditions, and calculate the ratios, Qr and Qs, of the peak area of reserpine to that of the internal standard.

Amount (mg) of C33H40N2O9 = M3 × Qr/Qs

M3: Amount (mg) of Reserpine RS

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate, pH 3.0 and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of reserpine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, reserpine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of reserpine to that of the internal standard is not more than 2.0%.

Containers and Storage Containers—Well-closed containers.

Storage—Light-resistant.

Reserpine Injection

Reserpine Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of reserpine (C33H40N2O9; 608.68).

Method of preparation Prepare as directed under Injections, with Reserpine.

Description Reserpine Injection is a clear, colorless or pale yellow liquid.

pH: 2.5 – 4.0
Identification Measure a volume of Reserpin Injection, equivalent to 1.5 mg of Reserpin according to the labeled amount, add 10 mL of diethyl ether, shake for 10 minutes, and take the aqeous layer. If necessary, add 10 mL of diethyl ether to the aqeous layer, and shake for 10 minutes to repeat the process. To the aqeous layer add water to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \( <2.24\): it exhibits a maximum between 265 nm and 269 nm.

Extractable volume \(<6.05\) It meets the requirement.

Foreign insoluble matter \(<6.06\) Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter \(<6.07\) It meets the requirement.

Sterility \(<4.06\) Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Reserpin Injection, equivalent to about 4 mg of reserpin (C\(_{33}\)H\(_{40}\)N\(_2\)O\(_9\)). Separately, weigh accurately about 4 mg of Reserpin RS, previously dried in vacuum at 60 \(^\circ\)C for 3 hours. Transfer them to separate separator, add 10 mL each of water and 5 mL each of ammonia TS, and extract with one 20-mL portion of chloroform, then with three 10-mL portions of chloroform with shaking vigorously. Combine the chloroform extracts, wash with two 50-mL portions of diluted hydrochloric acid (1 in 100), and combine the all washings. Then wash the chloroform extract with two 50-mL portions of a solution of sodium bicarbonate (1 in 100), and combine the washings. Extract the combined washing with two 10-mL portions of chloroform, and combine the washings with the former chloroform extract. Transfer the chloroform solution to a 100-mL volumetric flask through a pledget of absorbent cotton previously wetted with chloroform, wash with a small amount of chloroform, dilute with chloroform to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, \(A_T\) and \(A_S\), of the sample solution and the standard solution, respectively, at 295 nm as directed under Ultraviolet-visible Spectrophotometry \( <2.24\).

\[
\text{Amount (mg) of reserpin (C}_{33}\text{H}_{40}\text{N}_2\text{O}_9) = M_S \times \frac{A_T}{A_S}
\]

\(M_S:\) Amount (mg) of Reserpin RS

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

0.1% Reserpin Powder

Reserpin Powder

0.1% Reserpin Powder contains not less than 0.09% and not more than 0.11% of reserpin (C\(_{33}\)H\(_{40}\)N\(_2\)O\(_9\): 608.68).

Reserpin Tablets

Reserpin Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of reserpin (C\(_{33}\)H\(_{40}\)N\(_2\)O\(_9\): 608.68).

Method of preparation Prepare as directed under Tablets, with Reserpin.

Identification Take a portion of powdered Reserpin Tablets, equivalent to 0.4 mg of Reserpin according to the labeled amount, add 20 mL of acetonitrile, shake for 30 minute, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry \( <2.24\): it exhibits maxima between 265 nm and 269 nm, and between 294 nm and 298 nm.

Uniformity of dosage units \(<6.02\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.
Conduct this procedure without exposure to daylight, using light-resistant vessels. To 1 tablet of Reserpine Tablets add 2 mL of water, disintegrate by warming at 50°C for 15 minutes while shaking. After cooling, add exactly 2 mL of the internal standard solution per 0.1 mg of reserpine (C₃₃H₄₀N₂O₉), add 2 mL of acetonitrile, warm at 50°C for 15 minutes while shaking, and after cooling, add water to make 10 mL. Centrifuge the solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of Reserpine RS, previously dried at 60°C in vacuum for 3 hours, dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Reserpine.

Amount (mg) of reserpine (C₃₃H₄₀N₂O₉) = \( M_S \times \frac{Q_T}{Q_S} \times \frac{C}{10} \)  
\( M_S \): Amount (mg) of Reserpine RS  
\( C \): Labeled amount (mg) of reserpine in 1 tablet

**Internal standard solution**—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

**Dissolution**<sup>6.16</sup> When the test is performed at 100 revolutions per minute according to the Paddle method, using 500 mL of a solution of polysorbate 80 (1 in 20,000) in diluted dulcet acid (1 in 200) as the dissolution medium, the dissolution rate in 30 minutes of Reserpine Tablets is not less than 70%.

Start the test with 1 tablet of Reserpine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, filter through a filter laminated with polyester fibers, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dry Reserpine RS at 60°C in vacuum for 3 hours, weigh accurately an amount 100 times the labeled amount of Reserpine Tablets, dissolve in 1 mL of chloroform and 80 mL of ethanol (95), and add the dissolution medium to make exactly 200 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, transfer to glass-stoppered brown test tubes T and S, respectively, add exactly 5 mL each of ethanol (99.5), shake well, add exactly 1 mL each of diluted vanadium (V) oxide TS (1 in 2), shake vigorously, and allow to stand for 30 minutes. Perform the test with these solutions as directed under Fluorometry <2.22>, and determine the intensity of fluorescence, \( F_T \) and \( F_S \), at the wavelength of excitation at 365 nm and at the wavelength of fluorescence at 450 nm.

Dissolution rate (%) with respect to the labeled amount of reserpine (C₃₃H₄₀N₂O₉) = \( M_S \times \frac{F_T}{F_S} \times \frac{1}{C} \)  
\( M_S \): Amount (mg) of Reserpine RS  
\( C \): Labeled amount (mg) of reserpine (C₃₃H₄₀N₂O₉) in 1 tablet

**Assay** Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately and powder not less than 20 Reserpine Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.5 mg of reserpine (C₃₃H₄₀N₂O₉), add 3 mL of water, and warm at 50°C for 15 minutes while shaking. After cooling, add exactly 10 mL of the internal standard solution, 10 mL of acetonitrile and warm at 50°C for 15 minutes while shaking. After cooling, add water to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of Reserpine RS, previously dried at 60°C in vacuum for 3 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Reserpine.

Amount (mg) of reserpine (C₃₃H₄₀N₂O₉) = \( M_S \times \frac{Q_T}{Q_S} \times \frac{1}{20} \)  
\( M_S \): Amount (mg) of Reserpine RS

**Internal standard solution**—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

**Retinol Acetate**

**Vitamin A Acetate**

レチノール酢酸エストル

\[
\text{C}_{32}\text{H}_{52}\text{O}_2: \quad 328.49 \\
(2\text{E},4\text{E},6\text{E},8\text{E})-3,7-\text{Dimethyl}-9-(2,6,6-\text{trimethylcyclohexyl})-1-\text{yl}n\text{onona}-2,4,6,8-\text{tetraen-1-yl} \text{acetate} \\
[127-47-9]
\]

Retinol Acetate is synthetic retinol acetate or synthetic retinol acetate diluted with fixed oil.

It contains not less than 2,500,000 Vitamin A Units per gram.

A suitable antioxidant may be added.

It contains not less than 95.0% and not more than 105.0% of the labeled Units.

**Description** Retinol Acetate occurs as pale yellow to yellow-red crystals or an ointment-like substance, and has a faint, characteristic odor, but has no rancid odor.

It is freely soluble in petroleum ether, soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air and by light.

**Identification** Dissolve Retinol Acetate and Retinol Acetate RS, equivalent to 15,000 Units each, in 5 mL of petroleum ether, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 \( \mu \)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a
distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution is the same in color tone and Rf value with the blue spot from the standard solution.

**Purity (1)** Acid value \(<1.13>—Take exactly 5.0 g of Retinol Acetate, and perform the test: not more than 2.0.

(2) Peroxide—Weigh accurately about 5 g of Retinol Acetate, transfer in a 250-mL glass-stoppered conical flask, add 50 mL of a mixture of acetic acid (100) and isooctane (3:2), and gently mix to dissolve completely. Replace the air of the inside gradually with about 600 mL of Nitrogen, then add 0.1 mL of saturated potassium iodide TS under a current of Nitrogen. Immediately stopper tightly, and shake vigorously for 5 to 10 seconds. Titrate with the blue spot from the standard solution.

\[
\text{Amount (mEq/kg) of peroxide} = \frac{V}{M} \times 10
\]

\(V:\) Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed
\(M:\) Amount (g) of the sample

**Assay** Proceed as directed in Method 1-1 under Vitamin A Assay \(<2.35>.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere, and in a cold place.

### Retinol Palmitate

**Vitamin A Palmitate**

レチノールパルミチン酸エステル

\[\text{C}_{36}\text{H}_{60}\text{O}_2: 524.86 \]

\((2E,4E,6E,8E)-3,7-\text{Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)}\text{nona-2,4,6,8-tetraen-1-yl} \text{ palmitate} \]

\[\text{[79-81-2]}\]

Retinol Palmitate is a synthetic retinol palmitate or a synthetic retinol palmitate diluted with fixed oil.

It contains not less than 1,500,000 Vitamin A Units per gram.

A suitable antioxidant may be added.

It contains not less than 95.0% and not more than 105.0% of the labeled Units.

**Description** Retinol Palmitate occurs as a light yellow to yellow-red, ointment-like or an oily substance. It has a faint, characteristic odor, but has no rancid odor.

It is very soluble in petroleum ether, slightly soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air and by light.

### Identification

Dissolve Retinol Palmitate and Retinol Palmitate RS, equivalent to 15,000 Units each, in 5 mL of petroleum ether, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03>.

Spot 5 \(\mu L\) of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution is the same in color tone and Rf value with the blue spot from the standard solution.

**Purity (1)** Acid value \(<1.13>—Take exactly 5.0 g of Retinol Palmitate, and perform the test: not more than 2.0.

(2) Peroxide—Weigh accurately about 5 g of Retinol Palmitate, transfer in a 250-mL glass-stoppered conical flask, add 50 mL of a mixture of acetic acid (100) and isooctane (3:2), and gently mix to dissolve completely. Replace the air of the inside gradually with about 600 mL of Nitrogen, then add 0.1 mL of saturated potassium iodide TS under a current of Nitrogen. Immediately stopper tightly, and mix with a swirling motion for 1 minute. Add 30 mL of water, stopper tightly, and shake vigorously for 5 to 10 seconds. Titrate with the blue spot from the standard solution.

\[
\text{Amount (mEq/kg) of peroxide} = \frac{V}{M} \times 10
\]

\(V:\) Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed
\(M:\) Amount (g) of the sample

**Assay** Proceed as directed in Method 1-1 under Vitamin A Assay \(<2.35>.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere, and in a cold place.

### Riboflavin

**Vitamin B₂**

リボフラビン

\[\text{C}_{17}\text{H}_{20}\text{N}_{4}\text{O}_{6}: 376.36 \]

\(7,8-\text{Dimethyl-10-[(23,35,4R)-2,3,4,5-tetrahydroxypropyl]benzo[6]pteridine-2,4(3H,10H)-dione} \]

\[\text{[83-89-5]}\]

Riboflavin, when dried, contains not less than 98.0% of \(\text{C}_{17}\text{H}_{20}\text{N}_{4}\text{O}_{6}\).
**Description**  Riboflavin occurs as yellow to orange-yellow crystals. It has a slight odor.

It is very slightly soluble in water, practically insoluble in ethanol (95), in acetic acid (100), and in diethyl ether.

It dissolves in sodium hydroxide TS.

A saturated solution of Riboflavin is neutral.

Melting point: about 290°C (with decomposition).

**Identification**  1. A solution of Riboflavin (1 in 100,000) is light yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the solution, and reappear on shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(2) To 10 mL of a solution of Riboflavin (1 in 100,000) placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, and after illumination with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, classify as yellow as the solution as directed in the Identification (1) and (2), and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.

(3) Determine the absorption spectrum of a solution of Riboflavin in phosphate buffer solution, pH 7.0 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Riboflavin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation**  \(\langle 2.49\rangle\)  \([\alpha]_D^{20} -128 -142^\circ\) Weigh accurately about 0.1 g of dried Riboflavin TS, dissolve in exactly 4 mL of dilute sodium hydroxide TS, add 10 mL of freshly boiled and cooled water, add exactly 4 mL of aldehyde-free ethanol while shaking, add freshly boiled and cooled water to make exactly 20 mL, and determine the rotation in a 100-mm cell within 30 minutes after preparing the solution.

**Purity**  Lumiflavin—Shake 25 mg of Riboflavin with 10 mL of ethanol-free chloroform for 5 minutes, and filter: the filtrate has no more color than the following control solution.

Control solution: To 2.0 mL of 1/60 mol/L potassium hydrochloric acid or sodium hydroxide TS, and after illumination with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, classify as yellow as the solution as directed in the Identification (1) and (2), and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.

**Residue on ignition**  \(\langle 2.44\rangle\) Not more than 1.5% (0.5 g, 105°C, 2 hours).

**Loss on drying**  \(\langle 2.47\rangle\) Not more than 0.2% (1 g).

**Assay**  Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 15 mg of Riboflavin, previously dried, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL, and use this solution as the sample solution. Dry Riboflavin RS at 105°C for 2 hours, weigh accurately about 15 mg, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and determine the absorbances, \(A_1\) and \(A_2\), at 445 nm. Add 0.02 g of sodium hydrosulfite to 5 mL of each solution, shake until decolorized, and immediately measure the absorbances, \(A_1\)

\[\text{Amount (mg) of } \text{C}_17\text{H}_20\text{N}_4\text{O}_6 = M_5 \times \frac{(A_1 - A'_1)}{(A_3 - A'_3)}\]

\[M_5: \text{Amount (mg) of Riboflavin RS}\]

**Riboflavin Powder**

**Vitamin B₂ Powder**

リポフラビン散

Riboflavin Powder contains not less than 95.0% and not more than 115.0% of the labeled amount of riboflavin (\(\text{C}_17\text{H}_20\text{N}_4\text{O}_6\)) 376.36.

**Method of preparation**  Prepare as directed under Granules or Powders, with Riboflavin.

**Identification**  Shake a portion of Riboflavin Powder, equivalent to 1 mg of Riboflavin according to the labeled amount, with 100 mL of water, filter, and proceed with the filtrate as directed in the Identification (1) and (2) under Riboflavin.

**Purity**  Rancidity—Riboflavin Powder is free from any unpleasant or rancid odor or taste.

**Assay**  The procedure should be performed under protection from direct sunlight and in light-resistant vessels. Weigh accurately Riboflavin Powder equivalent to about 15 mg of riboflavin (\(\text{C}_17\text{H}_20\text{N}_4\text{O}_6\)), add 800 mL of diluted acetic acid (100) (1 in 400), and extract by warming for 30 minutes with occasional shaking. Cool, dilute with water to make exactly 1000 mL, and filter through a glass filter (G4). Use this filtrate as the sample solution, and proceed as directed in the Assay under Riboflavin.

\[\text{Amount (mg) of riboflavin } = M_5 \times \frac{(A_1 - A'_1)}{(A_3 - A'_3)}\]

\[M_5: \text{Amount (mg) of Riboflavin RS}\]

**Containers and storage**  Containers—Tight containers. Storage—Light-resistant.
Riboflavin Butyrate
リボフラビン酪酸エステル

C_{33}H_{44}N_{4}O_{10}: 656.72
(2R,3S,4S)-5-(7,8-Dimethyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)pentan-1,2,3,4-tetrayl tetrabutanoate
[752-56-7]

Riboflavin Butyrate, when dried, contains not less than 98.5% of C_{33}H_{44}N_{4}O_{10}.

Description Riboflavin Butyrate occurs as orange-yellow crystals or crystalline powder. It has a slight, characteristic odor and a slightly bitter taste.

It is freely soluble in methanol, in ethanol (95) and in chloroform, slightly soluble in diethyl ether, and practically insoluble in water.

It is decomposed by light.

Identification (1) A solution of Riboflavin Butyrate in ethanol (95) (1 in 100,000) shows a light yellow-green color with a strong yellowish green fluorescence. To the solution add dilute hydrochloric acid or sodium hydroxide TS; the fluorescence disappears.

(2) Dissolve 0.01 g of Riboflavin Butyrate in 5 mL of ethanol (95), add 2 mL of a mixture of a solution of hydroxyammonium chloride (3 in 20) and a solution of sodium hydroxide (3 in 20) (1:1), and shake well. To this solution add 0.8 mL of hydrochloric acid and 0.5 mL of iron (III) chloride TS, and add 8 mL of ethanol (95); a deep red-brown color develops.

(3) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 146 – 150°C

Purity (1) Chloride—Dissolve 2.0 g of Riboflavin Butyrate in 10 mL of methanol, and add 24 mL of dilute nitric acid and water to make 100 mL. After shaking well, allow to stand for 10 minutes, filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. To 25 mL of the sample solution add water to make 50 mL, then add 1 mL of silver nitrate TS, and allow to stand for 5 minutes: the turbidity of the solution is not thicker than that of the following control solution.

Control solution: To 25 mL of the sample solution add 1 mL of silver nitrate TS, allow to stand for 10 minutes, and filter. Wash the precipitate with four 5-mL portions of water, and combine the washings with the filtrate. To this solution add 0.30 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL, add 1 mL of water, and mix (not more than 0.021%).

(2) Heavy metals <1.06>—Proceed with 2.0 g of Riboflavin Butyrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Free acid—To 1.0 g of Riboflavin Butyrate add 50 mL of freshly boiled and cooled water, shake, and filter. To 25 mL of the filtrate add 0.50 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: the solution shows a red color.

(4) Related substances—Dissolve 0.10 g of Riboflavin Butyrate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 50 mL. Pipet 5 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and 2-propanol (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 40 mg of Riboflavin Butyrate, previously dried, dissolve in ethanol (95) to make exactly 500 mL, and pipet 10 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Riboflavin RS, previously dried at 105°C for 2 hours, dissolve in 150 mL of diluted acetic acid (100) (2 in 75) by warming, and after cooling, add water to make exactly 500 mL. Pipet 5 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_{T} and A_{S}, of the sample solution and standard solution at 445 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of C_{33}H_{44}N_{4}O_{10} = M_{S} \times A_{T}/A_{S} \times 1/2 \times 1.745

M_{S}: Amount (mg) of Riboflavin RS

Containers and storage Containers—Tight containers.

Storage—Light-resistant.
Riboflavin Sodium Phosphate

Riboflavin Phosphate

Vitamin B2 Phosphate Ester

ライボフラビンリン酸エステルナトリウム

\[ \text{C}_{17}\text{H}_{20}\text{N}_{4}\text{NaO}_{9}\text{P} : 478.33 \]

Monosodium (2R,3S,4S)-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydronobenzo[g]pteridin-10(2H)-yl)-2,3,4-trihydroxypentyl monohydrogenphosphate

[130-40-5]

Riboflavin Sodium Phosphate contains not less than 92% of C_{17}H_{20}N_{4}NaO_{9}P, calculated on the anhydrous basis.

Description Riboflavin Sodium Phosphate is a yellow to orange-yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is soluble in water, and practically insoluble in ethanol (95), in chloroform and in diethyl ether.

It is decomposed on exposure to light.

It is very hygroscopic.

Identification (1) A solution of Riboflavin Sodium Phosphate (1 in 100,000) is light yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the solution, and reappear on shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS. It is decomposed on exposure to light. It is very hygroscopic.

(2) To 10 mL of a solution of Riboflavin Sodium Phosphate (1 in 100,000) placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, and after illumination with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31), and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.

(3) Determine the absorption spectrum of a solution of Riboflavin Sodium Phosphate in phosphate buffer solution, pH 7.0, (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry.

(4) To 0.05 g of Riboflavin Sodium Phosphate add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite. Boil the residue with 10 mL of nitric acid (1 in 50) for 5 minutes, after cooling, neutralize this solution with ammonium TS, and filter, if necessary: the solution responds to the Qualitative Tests <1.00> for sodium salt and phosphate.

Optical rotation <2.49> \([\alpha]_D^{20} +38^\circ \pm 43^\circ \text{ (0.3 g, calculated on the anhydrous basis, 5 mol/L hydrochloric acid TS, 20 mL, 100 mm).} \]

pH <2.54> Dissolve 0.20 g of Riboflavin Sodium Phosphate in 20 mL of water: the pH of the solution is between 5.0 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Riboflavin Sodium Phosphate in 10 mL of water: the solution is clear and yellow to orange-yellow in color.

(2) Lumiflavin—To 35 mg of Riboflavin Sodium Phosphate add 10 mL of ethanol-free chloroform, and shake for 5 minutes, then filter: the filtrate has no more color than the control solution.

Control solution: To 3.0 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

(3) Free phosphoric acid—Weigh accurately about 0.4 g of Riboflavin Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Measure exactly 5 mL each of the sample solution and Phosphoric Acid Standard Solution, transfer to separate 25-mL volumetric flasks, add 2.5 mL of hexammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS to each of these flasks, mix, and add water to make 25 mL. Allow to stand for 30 minutes at 20 ± 1°C, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of water in the same manner as a blank. Determine the absorbances, \(A_1\) and \(A_2\), of the subsequent solutions of the sample solution and standard phosphoric acid solution at 740 nm: the free phosphoric acid content is not more than 1.5%.

Content (% of free phosphoric acid (H_{3}PO_{4})

\[ M = \frac{1}{A_1 - A_2} \times 258.0 \]

Water <2.48> Place 25 mL of a mixture of methanol for Karl Fischer method and ethylene glycol for Karl Fischer method (1:1) in a dry flask for titration, and titrate with water determination TS to the end point. Weigh accurately about 0.1 g of Riboflavin Sodium Phosphate, place quickly into the flask, add a known excess volume of Karl Fischer TS, mix for 10 minutes, and perform the test: the water content is not more than 10.0%.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. To about 0.1 g of Riboflavin Sodium Phosphate, accurately weighed, dissolve in dilute acetic acid (100) (1 in 500) to make exactly 1000 mL, then pipet 10 mL of this solution, and add dilute acetic acid (100) (1 in 500) to make exactly 50 mL. Use this solution as the sample solution. Separately, dry Riboflavin RS at 105°C for 2 hours, weigh accurately about 15 mg, dissolve in 800 mL of dilute acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and determine the absorbances, \(A_1\) and \(A_2\), at 445 nm. Add 0.02 g of sodium hydrosulfite to 5 mL of each solution, shake until decolorized, and immediately measure the absorbances, \(A_{1,\text{a}}\) and \(A_{2,\text{a}}\), of the solutions.
Ribostamycin Sulfate

Riboflavin Sodium Phosphate Injection

Riboflavin Phosphate Injection
Vitamin B2 Phosphate Ester Injection

Riboflavin Sodium Phosphate Injection
Riboflavin Phosphate Injection
Vitamin B2 Phosphate Ester Injection

Riboflavin Sodium Phosphate Injection is an aqueous solution for injection. It contains not less than 95.0% and not more than 120.0% of the labeled amount of riboflavin (C17H20N4O6: 376.36). The concentration of Riboflavin Sodium Phosphate Injection should be stated as the amount of riboflavin (C17H20N4O6).

Method of preparation Prepare as directed under Injections, with Riboflavin Sodium Phosphate.

Description Riboflavin Sodium Phosphate Injection is a clear, yellow to orange-yellow liquid. pH: 5.0 – 7.0

Identification (1) To a measured volume of Riboflavin Sodium Phosphate Injection, equivalent to 1 mg of Riboflavin according to the labeled amount, add water to make 100 mL, and proceed with this solution as directed in the Identification (1) and (2) under Riboflavin Sodium Phosphate.

(2) To a measured volume of Riboflavin Sodium Phosphate Injection, equivalent to 0.05 g of Riboflavin according to the labeled amount, and evaporate on a water bath to dryness. Proceed with this residue as directed in the Identification (4) under Riboflavin Sodium Phosphate.

Bacterial endotoxins <4.01> Less than 10 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. To an accurately measured volume of Riboflavin Sodium Phosphate Injection, equivalent to about 15 mg of riboflavin (C17H20N4O6), add diluted acetic acid (100) (1 in 500) to make exactly 1000 mL, and use this solution as the sample solution. Proceed as directed in the Assay under Riboflavin Sodium Phosphate.

Ribostamycin Sulfate

Ribostamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of Streptomyces ribosidificus.

It contains not less than 680 μg (potency) and not more than 780 μg (potency) per mg, calculated on the dried basis. The potency of Ribostamycin Sulfate is expressed as mass (potency) of ribostamycin (C17H34N4O10: 454.47).

Description Ribostamycin Sulfate occurs as a white to yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification (1) Dissolve 20 mg of Ribostamycin Sulfate in 2 mL of phosphate buffer solution, pH 6.0, add 1 mL of ninhydrin TS, and boil: a blue-purple color develops.

(2) Dissolve 0.12 g each of Ribostamycin Sulfate and Ribostamycin Sulfate RS in 20 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <<2.03>>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and standard solution show a purple-brown color and the same Rf value.

(3) To 2 mL of a solution of Ribostamycin Sulfate (1 in
5) add 1 drop of barium chloride TS: a white turbidity is produced.

Optical rotation <\(2.49\)\> [\(\alpha\)]\(_D^2\): +42 – +49° (after drying, 0.25 g, water, 25 mL, 100 mm).

\(\text{pH} <2.54\) The \(\text{pH}\) of a solution obtained by dissolving 1.0 g of Ribostamycin Sulfate in 20 mL of water is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ribostamycin Sulfate in 5 mL of water: the solution is clear, and colorless or pale yellow.

(2) Heavy metals <\(1.07\)>—Proceed with 1.0 g of Ribostamycin Sulfate according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic <\(1.11\)>—Prepare the test solution with 1.0 g of Ribostamycin Sulfate according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.12 g of Ribostamycin Sulfate in water to make exactly 20 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <\(2.06\)>. Spot 5 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <\(2.41\)> Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <\(2.44\)> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <\(4.02\)> according to the following conditions.

(i) Test organism—\(\text{Bacillus subtilis}\) ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Ribostamycin Sulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 20 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 \(\mu\)g (potency) and 5 \(\mu\)g (potency), and use these solutions as the high concentration solution and the low concentration sample solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Ribostamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 \(\mu\)g (potency) and 5 \(\mu\)g (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Rice Starch

\(\text{Amylum Oryzae}\)

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (•).

•Description Rice Starch occurs as a white mass or powder. It is practically insoluble in water and in ethanol (99.5).

Identification (1) Examined under a microscope <\(5.01\)> using a mixture of water and glyc erin (1:1), Rice Starch presents polyhedral, simple grains 1 - 10 \(\mu\)m, mostly 4 - 6 \(\mu\)m, in size. These simple grains often gather in ellipsoidal, compound grains 50 - 100 \(\mu\)m in diameter. The granules have a poorly visible central hilum and there are no concentric striations. Between orthogonally orientated polarizing plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Rice Starch add 50 mL of water, boil for 1 minute, and allow to cool: a thin, cloudy mucilage is formed.

(3) To 1 mL of the mucilage obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): an orange-red to dark-blue color is produced which disappears on heating.

\(\text{pH} <2.54\) To 5.0 g of Rice Starch add 25 mL of freshly boiled and cooled water, and mix gently for 1 minute to achieve suspension. Allow to stand for 15 minutes: the \(\text{pH}\) of the solution is 5.0 to 8.0.

Purity (1) Iron—To 1.5 g of Rice Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use this solution as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (1 in 5) and 0.1 mL of mercapto acetic acid, and mix. Add ammonia solution (28) to these solutions until the color of a litmus paper to change from red to blue, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Rice Starch add 50 mL of water, shake for 5 minutes, and centrifuge. To 30 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes in the dark. Add 1 mL of starch TS, and titrate <\(2.50\)> with 0.002 mol/L sodium thiosulfate VS until the starch-iodine color disappears. Perform a blank determi-
nation in the same manner, and make any necessary correction. Not more than 1.4 mL of 0.002 mol/L sodium thiosulfate VS is required (not more than 20 ppm, calculated as H₂O₂).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Rice Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Open the tap of the funnel and stop the flow of carbon dioxide and also the heating and the cooling water. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat on a water-bath for 15 minutes and allow to cool. Add 0.1 mL of bromophenol blue TS, and titrate with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination in the same manner, and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

\[ \text{Amount (ppm) of sulfur dioxide} = \frac{V}{M} \times 1000 \times 3.203 \]

\( M \): Amount (g) of the sample

\( V \): Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

*Foreign matter—Under a microscope <5.01>, Rice Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant. |

**Loss on drying <2.41>** Not more than 15.0% (1 g, 130°C, 90 minutes).

**Residue on ignition <2.44>** Not more than 0.6% (1 g).

*Containers and storage* Containers—Well-closed containers.

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**Rifampicin**

リファンピシン

C₄₃H₅₈N₄O₁₂: 822.94

Rifampicin is a derivative of a substance having antibacterial activity produced by the growth of Streptomyces mediterranei.

It contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Rifampicin is expressed as mass (potency) of rifampicin (C₄₃H₅₈N₄O₁₂).

**Description** Rifampicin occurs as orange-red to red-brown, crystals or crystalline powder.

It is slightly soluble in water, in acetonitrile, in methanol and in ethanol (95).

**Identification** (1) To 5 mL of a solution of Rifampicin in methanol (1 in 5000) add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rifampicin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rifampicin as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and
compare the spectrum with the Reference Spectrum or the spectrum of Rifampicin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity (1)** Heavy metals \(<1.07\)—Proceed with 1.0 g of Rifampicin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \(<1.17\)—Prepare the test solution with 1.0 g of Rifampicin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Perform the test immediately after preparing of the sample and standard solutions. Dissolve 0.10 g of Rifampicin in 50 mL of acetonitrile, and use this solution as the sample stock solution. Pipet 5 mL of the sample stock solution, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 1 mL of the sample stock solution, and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\> according to the following conditions, and determine the peak areas, \(A_v\) and \(A_s\), of rifampicin.

\[
M_S: \text{Amount (mg (potency)) of Rifampicin RS} \\
\text{System suitability—} \\
\begin{align*}
\text{System performance: } & \text{Proceed as directed in the system suitability in the Assay.} \\
\text{System repeatability: } & \text{When the test is repeated 5 times with 50 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 1.0\%.}
\end{align*}
\]

**Containers and storage** Containers—Tight containers.

### Rifampicin Capsules

**リファンビシンカプセル**

Rifampicin Capsules contain not less than 93.0% and not more than 105.0% of rifampicin (C\(_{43}\)H\(_{58}\)N\(_4\)O\(_{12}\): 822.94). 

**Method of preparation** Prepare as directed under Capsules, with Rifampicin.

**Identification** Take out the content of Rifampicin Capsules, mix well, and powder, if necessary. Dissolve an amount of the content, equivalent to 20 mg (potency) of Rifampicin according to the labeled amount, in methanol to make 100 mL, and filter. To 5 mL of the filtrate add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\> it exhibits maxima between 234 nm and 238 nm, between 252 nm and 256 nm, between 331 nm and 335 nm, and between 472 nm and 476 nm.

**Purity** Related substances—Perform the test quickly after
the sample solution and standard solution are prepared. Open the capsules of not less than 20 Rifampicin Capsules, carefully take out the content, weigh accurately, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg (potency) of Rifampicin according to the labeled amount, and dissolve in acetonitrile to make exactly 10 mL. Pipet 2 mL of this solution, and add the mixture of acetonitrile and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Rifampicin RS, equivalent to about 20 mg (potency), and dissolve in acetonitrile to make exactly 10 mL. Pipet 1 mL of this solution, add the mixture of acetonitrile and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the amount of the peaks of quinone substance and N-oxide substance, having the relative retention time of about 0.5 and about 1.2 with respect to rifampicin, obtained from the sample solution are not more than 4.0% and not more than 1.5%, respectively. The amount of the peak other than the peaks mentioned above is not more than 1.0%, and the total amount of these related substances is not more than 2.0%. For these calculations, use the areas of the peaks of the quinone substance and N-oxide substance after multiplying by their relative response factors, 1.24 and 1.16, respectively.

Amount (mg) of quinone substance

\[ M_s/M_t \times A_{Tb}/A_S \times 2.48 \]

Amount (mg) of N-oxide substance

\[ M_s/M_t \times A_{Ta}/A_S \times 2.32 \]

Each amount (mg) of related substances other than quinone and N-oxide substances

\[ M_s/M_t \times A_{Tc}/A_S \times 2 \]

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octysilized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.1 g of sodium perchlorate, 6.5 g of citric acid monohydrate and 2.3 g of potassium dihydrogen phosphate in 1100 mL of water, and add 900 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rifampicin is about 12 minutes.

Time span of measurement: About 2.5 times as long as the retention time of rifampicin.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of rifampicin obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rifampicin is not less than 2500 and not more than 4.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 2.0%.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method using a sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Rifampicin Capsules is not less than 80%.

Start the test with 1 capsule of Rifampicin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 17 μg (potency) of rifampicin (C43H58N4O12) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 17 mg (potency) of Rifampicin RS, dissolve in 5 mL of methanol, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 334 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Dissolution rate (%): with respect to the labeled amount of rifampicin (C43H58N4O12)

\[ M_s \times A_{Tc}/A_S \times V/V \times 1/C \times 90 \]

C: Labeled amount [mg (potency)] of rifampicin (C43H58N4O12) in 1 capsule

Assay: Open the capsules of not less than 20 Rifampicin Capsules, take out the content, weigh accurately the mass of the content, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg (potency) of Rifampicin, dissolve in a mixture of acetonitrile and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of this solution, and add acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution prepared by dissolving 2.1 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3:1) to make exactly 50 mL, and use this solution as the standard solution. Separately, weigh accurately an amount of Rifampicin RS, equivalent to about 30 mg (potency), dissolve in 20 mL of a mixture of acetonitrile and methanol (1:1), and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the solution prepared by dissolving 2.1 g of citric acid monohydrate,
27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of the mixture of water and acetonitrile (3:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of rifampicin.

\[
\text{Amount [mg (potency)] of rifampicin (C_{43}H_{58}N_{4}O_{12}) = M_S \times A_T / A_S \times 5/2}
\]

\( M_S \): Amount [mg (potency)] of Rifampicin RS

**Operating conditions—**
Proceed as directed in the operating conditions in the Assay under Rifampicin.

**System suitability**—
System performance: Dissolve 30 mg (potency) of Rifampicin RS in 20 mL of the mixture of acetonitrile and methanol (1:1), and add acetone to make 100 mL. To 5 mL of this solution add 2 mL of a solution of butyl parahydroxybenzoate in the mixture of acetonitrile and methanol (1:1) (1 in 5000), then add the solution prepared by dissolving 2.1 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of acetonitrile and methanol (3:1) to make exactly 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and rifampicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 1.0%.

**Containers and storage**—
Containers—Tight containers.

## Ringer’s Solution

### リンゲル液

Ringer’s Solution is an aqueous solution for injection.

It contains not less than 0.53 w/v% and not more than 0.58 w/v% of chlorine [as (Cl: 35.45)], and not less than 0.030 w/v% and not more than 0.036 w/v% of calcium chloride hydrate (CaCl₂·2H₂O: 147.01).

### Method of preparation

**Sodium Chloride**

8.6 g

**Potassium Chloride**

0.3 g

**Calcium Chloride Hydrate**

0.33 g

**Water for Injection or Sterile Water**

for Injection in Containers—a sufficient quantity

To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

No preservative may be added.

### Description

Ringer’s Solution is a clear and colorless liquid. It has a slightly saline taste.

### Identification (1)

Evaporate 10 mL of Ringer’s Solution to 5 mL: the solution responds to the Qualitative Tests <1.09> for potassium salt and calcium salt.

(2) Ringer’s Solution responds to the Qualitative Tests <1.09> for sodium salt and chloride.

### pH

\(< 2.54\) 5.0 – 7.5

### Purity (1)

Heavy metals <1.07>—Evaporate 100 mL of Ringer’s Solution to about 40 mL on a water bath. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Control solution: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 0.3 ppm).

(2) Arsenic <1.11>—Perform the test with 20 mL of Ringer’s Solution as the test solution (not more than 0.1 ppm).

### Bacterial endotoxins <4.01>

Less than 0.50 EU/mL.

### Extractable volume <6.05>

It meets the requirement.

### Foreign insoluble matter <6.06>

Perform the test according to Method 1: it meets the requirement.

### Insoluble particulate matter <6.07>

It meets the requirement.

### Sterility <4.06>

Perform the test according to the Membrane filtration method: it meets the requirement.

### Assay (1)

Chlorine—To 20 mL of Ringer’s Solution, accurately measured, add 30 mL of water. Titrate <2.50> with 0.1 mol/L silver nitrate VS while shaking vigorously (indicator: 3 drops of sodium fluorescein TS).

Each mL of 0.1 mol/L silver nitrate VS

\[\text{= 3.545 mg of Cl}\]

(2) Calcium chloride Hydrate—To 50 mL of Ringer’s Solution, exactly measured, add 2 mL of 8 mol/L potassium hydroxide TS and 0.05 g of NN indicator, and titrate <2.50> immediately with 0.01 mol/L disodium hydrogen ethylenediamine tetraacetate VS, until the color of the solution changes from red-purple to blue.

Each mL of 0.01 mol/L disodium hydrogen ethylenediamine tetraacetate VS

\[\text{= 1.470 mg of CaCl}_2·2\text{H}_2\text{O}\]

### Containers and storage

Containers—Hermetic containers.

Plastic containers for aqueous infusions may be used.
Risperidone

リスペリドン

C23H27FN4O2: 410.48
3-[2-[4-(6-Fluoro-1,2-benzoisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one [106266-06-2]

Risperidone contains not less than 98.5% and not more than 101.0% of C23H27FN4O2, calculated on the dried basis.

Description Risperidone occurs as a white to yellowish white crystalline powder.

It is sparingly soluble in methanol and in ethanol (99.5), very slightly soluble in 2-propanol, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Risperidone in 2-propanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Risperidone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 169 – 173°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Risperidone according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Risperidone in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than 1.5 times the peak area of risperidone from the standard solution.

Operating conditions—Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecysilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: A solution of ammonium acetate (1 in 200).

Mobile phase B: Methanol.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 2</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>2 – 17</td>
<td>70 → 30</td>
<td>30 → 70</td>
</tr>
<tr>
<td>17 – 22</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>

Flow rate: 1.5 mL per minute.

Time span of measurement: About 1.6 times as long as the retention time of risperidone.

System suitability—Test for required detectability: Pipet 2 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of risperidone obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 10,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 80°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.16 g of Risperidone, dissolve in 70 mL of a mixture of 2-butanol and acetic acid (100) (7:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 20.52 mg of C23H27FN4O2

Containers and storage Containers—Tight containers.

Risperidone Fine Granules

リスペリドン細粒

Risperidone Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of risperidone (C23H27FN4O2: 410.48).

Method of preparation Prepare as directed under Granules, with Risperidone.
Risperidone Fine Granules / Official Monographs

Identification  To an amount of Risperidone Fine Granules, equivalent to 2 mg of Risperidone according to the labeled amount, add 100 mL of 2-propanol, shake thoroughly, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits maxima between 277 nm and 281 nm and between 283 nm and 287 nm.

Purity  Related substances—To an amount of Risperidone Fine Granules, equivalent to 2 mg of Risperidone according to the labeled amount, add 20 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than 1/2 times the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than the peak area of risperidone from the standard solution. For these calculations use the area of the peaks, having the relative retention time of about 0.4 and about 1.6 to risperidone, after multiplying by their relative response factors, 1.9 and 1.5, respectively.

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of risperidone, beginning after the solvent peak.

System suitability—
Test for required detectability: To exactly 5 mL of the standard solution add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Confirm that the peak area of risperidone obtained with 10 μL of this solution is equivalent to 7.5 to 12.5% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.5%.

Dissolution <6.10>  When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Risperidone Fine Granules is not less than 75%.

Start the test with an accurately weighed amount of Risperidone Fine Granules, equivalent to about 3 mg of risperidone (C_{23}H_{27}FN_{4}O_{2}) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add diluted hydrochloric acid (1 in 137) to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of risperidone for assay (separately determine the loss on drying <2.41> under the same conditions as Risperidone), and dissolve in methanol to make exactly 50 mL. Pipet 15 mL of this solution, add methanol to make exactly 25 mL. Pipet 2 mL of this solution, and add water to make exactly 200 mL. Pipet 3 mL of this solution, add exactly 3 mL of diluted hydrochloric acid (1 in 137), and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{6} and A_{S}, of risperidone of both solutions.

Dissolution rate (%) with respect to the labeled amount of risperidone (C_{23}H_{27}FN_{4}O_{2})  

\[
M_{S} = \frac{M_{f}}{S} \times A_{S} \times \frac{1}{C} \times 54/5
\]

C: Labeled amount (mg) of risperidone (C_{23}H_{27}FN_{4}O_{2}) in 1 g

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 237 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: To 1000 mL of a mixture of water and acetonitrile (13:7) add 1 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).
Flow rate: Adjust the flow rate so that the retention time of risperidone is about 3 minutes.

System suitability—
System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 3500 and not more than 2.5, respectively.
System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.0%.

Particle size <6.03>  It meets the requirements of Fine granules.

Assay  If necessary powder Risperidone Fine Granules, and weigh accurately an amount, equivalent to about 2 mg of risperidone (C_{23}H_{27}FN_{4}O_{2}), add 8 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 20 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately
about 50 mg of risperidone for assay (separately determine the loss on drying <2.4>) under the same conditions as Risperidone, and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \( A_1 \) and \( A_5 \), of risperidone from each solution.

\[
\text{Amount (mg) of risperidone (C}_{23}\text{H}_{27}\text{FN}_{4}\text{O}_{2}) = M \times A_1 / A_5 \times 1/25
\]

\( M \): Amount (mg) of risperidone for assay, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 1000 mL of a mixture of water and acetonitrile (4:1) add 1.5 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 13 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 1.0%.

Containers and storage Containers—Tight containers.

Risperidone Oral Solution

リスペリドン内服液

Risperidone Oral Solution contains not less than 95.0% and not more than 105.0% of the labeled amount of risperidone (C\(_{23}\)H\(_{27}\)FN\(_{4}\)O\(_{2}\) : 410.48).

Method of preparation Prepare as directed under Liquids and Solutions for Oral Administration, with Risperidone.

Description Risperidone Oral Solution occurs as a clear and colorless liquid.

Identification To a volume of Risperidone Oral Solution, equivalent to 2 mg of Risperidone according to the labeled amount, add 50 mg of sodium hydrogen carbonate and 10 mL of diethyl ether, shake, centrifuge, and evaporate the supernatant liquid to dryness in lukewarm water. Determine the absorption spectrum of a solution of the residue in 100 mL of 2-propanol as directed under Ultraviolet-visible Spectrophotometry <2.24> : it exhibits maxima between 277 nm and 281 nm and between 283 nm and 287 nm.

pH Being specified separately.

Purity Related substances—To a volume of Risperidone Oral Solution, equivalent to 2 mg of Risperidone according to the labeled amount, add methanol to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and water (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than 1/2 times the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than the peak area of risperidone from the standard solution. For these calculations use the area of the peaks, having the relative retention time of about 0.4 and about 1.6 to risperidone, after multiplying by their relative response factors, 1.9 and 1.5, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of risperidone, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of methanol and water (9:1) to make exactly 50 mL. Confirm that the peak area of risperidone obtained with 10 μL of this solution is equivalent to 7.5 to 12.5% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.5%.

Microbial limit <4.02> The acceptance criteria of TAMC and TYMC are 10⁴ CFU/mL and 10¹ CFU/mL, respectively. 

Escherichia coli is not observed.

Uniformity of dosage units <6.02> The granules in single-unit containers meet the requirement of the Mass variation test.

Assay To an exact volume of Risperidone Oral Solution, equivalent to about 2 mg of risperidone (C\(_{23}\)H\(_{27}\)FN\(_{4}\)O\(_{2}\)) according to the labeled amount, add methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of risperidone for assay (separately determine the loss on drying <2.4>) under the same conditions as Risperidone, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add
10 mL of water, then add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07>\) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of risperidone from each solution.

\[
\text{Amount (mg) of risperidone (C}_{23}\text{H}_{27}\text{FN}_{4}\text{O}_{2}) = M_S \times A_T / A_S \times 1/25
\]

\( M_S \): Amount (mg) of risperidone for assay, calculated on the dried basis

**Operating conditions**—

- **Detector:** An ultraviolet absorption photometer (wavelength: 275 nm).
- **Column:** A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 \( \mu \text{m} \) in particle diameter).
- **Column temperature:** A constant temperature of about 25°C.
- **Mobile phase:** To 1000 mL of a mixture of water and acetonitrile (4:1) add 1.5 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).
- **Flow rate:** Adjust the flow rate so that the retention time of risperidone is about 13 minutes.

**System suitability**—

- **System performance:** When the procedure is run with 10 \( \mu L \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.
- **System repeatability:** When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 1.0%.

**Containers and storage**—

Containers—Tight containers.

**Risperidone Tablets**

### Risperidone Tablets

Risperidone Tablets contain not less than 95.0\% and not more than 105.0\% of the labeled amount of risperidone (\( \text{C}_{23}\text{H}_{27}\text{FN}_{4}\text{O}_{2} \); 410.48).

**Method of preparation**—

Prepare as directed under Tablets, with Risperidone.

**Identification**—

Powder Risperidone Tablets. To a portion of the powder, equivalent to 2 mg of Risperidone according to the labeled amount, add 100 mL of 2-propanol, shake thoroughly, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\); it exhibits maxima between 277 nm and 281 nm and between 283 nm and 287 nm.

**Purity**—

Related substances—Powder Risperidone Tablets. To a portion of the powder, equivalent to 2 mg of Risperidone according to the labeled amount, add 20 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \text{m} \). Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07>\) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than 1/2 times the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than the peak area of risperidone from the standard solution. For these calculations use the area of the peaks, having the relative retention time of about 0.4 and about 1.6 to risperidone, after multiplying by their relative response factors, 1.9 and 1.5, respectively.

**Operating conditions**—

- **Detector, column, column temperature, mobile phase, and flow rate:** Proceed as directed in the operating conditions in the Assay.
- **Time span of measurement:** About 2.5 times as long as the retention time of risperidone, beginning after the solvent peak.

**System suitability**—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Confirm that the peak area of risperidone obtained with 10 \( \mu L \) of this solution is equivalent to 7.5 to 12.5\% of that with 10 \( \mu L \) of the standard solution.

System performance: When the procedure is run with 10 \( \mu L \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.5\%.

**Uniformity of dosage units** \(<6.02>\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Risperidone Tablets add \( 3V/5 \) mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly \( V/3 \) mL so that each mL contains 0.1 mg of risperidone (\( \text{C}_{23}\text{H}_{27}\text{FN}_{4}\text{O}_{2} \)). Filter this solution through a membrane filter with a pore size not exceeding 0.45 \( \mu \text{m} \), discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of risperidone (C}_{23}\text{H}_{27}\text{FN}_{4}\text{O}_{2}) = M_S \times A_T / A_S \times V/500
\]

\( M_S \): Amount (mg) of risperidone for assay, calculated on the dried basis

**Dissolution** \(<6.10>\) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Risperidone Tablets is not less than 75\%.

Start the test with 1 tablet of Risperidone Tablets, with-
draw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 1 mL of the subsequent filtrate, add diluted hydrochloric acid (1 in 137) to make exactly 1 mL so that each mL contains about 0.56 μg of risperidone (C23H27FN4O2) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of risperidone for assay (separately determine the loss on drying <2.4L> under the same conditions as Risperidone), and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 25 mL. Pipet 2 mL of this solution, and add water to make exactly 200 mL. Pipet 3 mL of this solution, add exactly 3 mL of diluted hydrochloric acid (1 in 137), and use this solution as the standard solution. Perform the test with exactly 100 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.8D> according to the following conditions, and determine the peak areas, A1 and A3, of risperidone from each solution.

\[ M_S = \frac{M_S \times A_T}{A_S \times V/V \times 1/C \times 9/5} \]

M5: Amount (mg) of risperidone for assay, calculated on the dried basis
C: Labeled amount (mg) of risperidone (C23H27FN4O2) in 1 tablet

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 237 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: To 1000 mL of a mixture of water and acetonitrile (13:7) add 1 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).
Flow rate: Adjust the flow rate so that the retention time of risperidone is about 3 minutes.

**System suitability—**
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.0%.

**Assay**
Weigh accurately the mass of not less than 20 Risperidone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 2 mg of risperidone (C23H27FN4O2), add 8 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 20 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of risperidone for assay (separately determine the loss on drying <2.4L> under the same conditions as Risperidone), and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.8D> according to the following conditions, and determine the peak areas, A1 and A3, of risperidone from each solution.

Amount (mg) of risperidone (C23H27FN4O2)
\[ M_S = \frac{M_S \times A_T}{A_S \times 1/25} \]

M5: Amount (mg) of risperidone for assay, calculated on the dried basis

**Containers and storage**
Containers—Tight containers.

**Ritodrine Hydrochloride**
リトドリン塩酸塩

\[
\text{C}_{17}H_{21}NO_3 \cdot \text{HCl}: \text{323.81} \\
\text{(RS,2SR)}-1-(4-Hydroxyphenyl)-2-\{2-(4-hydroxyphenyl)ethylamino\}propan-1-ol monohydrochloride \\
[23239-51-2]
\]

Ritodrine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of C17H21NO3.HCl.

**Description**
Ritodrine Hydrochloride occurs as a white crystalline powder.
It is freely soluble in water, in methanol and in ethanol.
It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of Ritodrine Hydrochloride (1 in 10) shows no optical rotation.

It is gradually colored to a light yellow by light.

Melting point: about 196°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Ritodrine Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ritodrine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ritodrine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum or the spectrum of Ritodrine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ritodrine Hydrochloride (1 in 50) responds to the Qualitative Tests 1.00 (2) for chloride.

**Purity (1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Ritodrine Hydrochloride in 10 mL of water is clear and colorless.

(2) Heavy metals 1.07—Proceed with 2.0 g of Ritodrine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Ritodrine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak of ritodrine threo-isomer is not larger than 3/10 times the peak area of ritodrine from the standard solution, and the total area of the peaks other than ritodrine and ritodrine threo-isomer is not larger than 3/10 times the peak area of ritodrine from the standard solution.

**Operating conditions**—

Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Flow rate: Adjust the flow rate so that the retention time of ritodrine is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of ritodrine beginning after the solvent peak.

**System suitability**—

Test for required detectability: To exactly 5 mL of the standard solution add exactly 50 mL of the mobile phase. Confirm that the peak area of ritodrine obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: To 20 mg of Ritodrine Hydrochloride and Ritodrine Hydrochloride RS, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 25 mL of these solutions, add exactly 5 mL of the internal standard solution, then add water to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q₁ and Q₃, of the peak area of ritodrine to that of the internal standard.

\[
M₅ = \frac{M_x \times Q₁/Q₃}{10}
\]

M₅: Amount (mg) of C₁₇H₂₂NO₃.HCl

\[
M_x = \text{Amount (mg) of Ritodrine Hydrochloride RS}
\]

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.6 g of diammonium hydrogen phosphate and 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust to pH 3.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of ritodrine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ritodrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ritodrine is not more than 2.0%.

**Loss on drying** 2.41—Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition** 2.44—Not more than 0.2% (1 g).

**Assay** Weigh accurately about 30 mg each of Ritodrine Hydrochloride and Ritodrine Hydrochloride RS, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 25 mL of these solutions, add exactly 5 mL of the internal standard solution, then add water to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q₁ and Q₃, of the peak area of ritodrine to that of the internal standard.

\[
M₅ = \frac{M_x \times Q₁/Q₃}{10}
\]

M₅: Amount (mg) of Ritodrine Hydrochloride RS

**System performance**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ritodrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operat-
Ritodrine Hydrochloride Tablets

Ritodrine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of ritodrine hydrochloride (C₁₇H₂₁NO₃.HCl: 323.81).

Method of preparation  Prepare as directed under Tablets, with Ritodrine Hydrochloride.

Identification  To 10 mL of the filtrate obtained in the Assay add 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits a maximum between 272 nm and 276 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ritodrine Hydrochloride Tablets add 9 mL of 0.01 mol/L hydrochloric acid TS, shake until the tablet is completely disintegrated, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL, and filter through a membrane filter having pore size of 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 5.6 μg of ritodrine hydrochloride (C₁₇H₂₁NO₃.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Ritodrine Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 80 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of ritodrine.

\[
M_5 = \frac{V × A_1}{A_5} × \frac{V}{V} × \frac{C}{C_18}
\]

Operating conditions—
Proceed as directed in the operating conditions in the Assay.

System suitability—
System performance: When the procedure is run with 80 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ritodrine are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 80 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ritodrine is not more than 1.5%.

Assay  To 20 Ritodrine Hydrochloride Tablets add 150 mL of 0.01 mol/L hydrochloric acid TS, shake for 20 minutes, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Filter through a glass filter (G4), and discard the first 20 mL of the filtrate. Pipet 30 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution and 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ritodrine Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 80 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> ac-

Containers and storage  Contain—Tight containers.
Storage—Light-resistant.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Ritodrine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Ritodrine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a glass filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 5.6 μg of ritodrine hydrochloride (C₁₇H₂₁NO₃.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Ritodrine Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 80 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of ritodrine.

\[
M_5 = \frac{V × A_1}{A_5} × \frac{V}{V} × \frac{C}{C_18}
\]

Operating conditions—
Proceed as directed in the operating conditions in the Assay.

System suitability—
System performance: When the procedure is run with 80 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ritodrine are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 80 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ritodrine is not more than 1.5%.

Assay  To 20 Ritodrine Hydrochloride Tablets add 150 mL of 0.01 mol/L hydrochloric acid TS, shake for 20 minutes, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Filter through a glass filter (G4), and discard the first 20 mL of the filtrate. Pipet 30 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution and 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ritodrine Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 80 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> ac-
according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of ritodrine to that of the internal standard.

$$\text{Amount (mg) of ritodrine hydrochloride (C}_{17}\text{H}_{21}\text{NO}_{3}\cdot\text{HCl}} = M_S \times \frac{Q_T}{Q_S} \times 4$$

$M_S$: Amount (mg) of Ritodrine Hydrochloride RS

**Internal standard solution**—A solution of methyl parahydroxybenzoate in methanol (3 in 5000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 274 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25 °C.
Mobile phase: Dissolve 6.6 g of diammonium hydrogen phosphate and 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust to pH 3.0 with phosphoric acid.
Flow rate: Adjust the flow rate so that the retention time of ritodrine is about 6 minutes.

**System suitability**—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ritodrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ritodrine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

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### Rokitamycin

Rokitamycin is a derivative of leucomycin A₅, which is a macrolide antibiotic produced by the growth of the mutants of *Streptomyces kitasatoensis*.

It contains not less than 900 μg (potency) and not more than 1050 μg (potency) per mg, calculated on the anhydrous basis. The potency of Rokitamycin is expressed as mass (potency) of rokitamycin (C₄₂H₆₉NO₁₅).

**Description** Rokitamycin occurs as a white to yellowish white powder.

It is very soluble in methanol and in chloroform, freely soluble in ethanol (99.5) and in acetonitrile, and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Rokitamycin in methanol (1 in 50,000), as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rokitamycin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rokitamycin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Rokitamycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ¹H spectrum of a solution of Rokitamycin in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 20), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.27>: it exhibits single signals A, B, C and D at around δ 1.4 ppm, at around δ 2.5 ppm, at around δ 3.5 ppm and at around δ 9.8 ppm, respectively. The ratio of integrated intensity of these signals, A:B:C:D, is...
Liquid Chromatography

Sample solution and standard solution as directed under Assay.

Retention time of about 0.86 and 3 times the peak of rokitamycin are not less than 3000 and not more than 1.5, respectively.

System suitability

- Detector: An ultraviolet absorption photometer (wavelength: 232 nm).
- Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 55°C.
- Mobile phase: A mixture of methanol, diluted 0.5 mol/L ammonium acetate TS (2 in 5) and acetonitrile (124:63:13).
- Flow rate: Adjust the flow rate so that the retention time of rokitamycin is about 11 minutes.
- Time span of measurement: About 2.5 times as long as the retention time of rokitamycin beginning after the solvent peak.

For required detectability: To exactly 1 mL of the standard solution add acetonitrile to make exactly 10 mL. Confirm that the peak area of rokitamycin obtained with 5 μL of this solution is equivalent to 7 to 13% of that with 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rokitamycin are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rokitamycin is not more than 2.0%.

Water Not more than 3.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics according to the following conditions.

Test organism

(i) Micrococcus luteus ATCC 9341

(ii) Culture medium—Use the medium in 3 Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH 2.5 to 5.4 of the medium so that it will be 7.8 to 8.0 after sterilization.

Standard solutions—Weigh accurately an amount of Rokitamycin RS equivalent to about 40 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 10 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 containing 0.01% of polysorbate 80 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

Sample solutions—Weigh accurately an amount of Rokitamycin equivalent to about 40 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 containing 0.01% of polysorbate 80 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage

Containers—Tight containers.

Rokitamycin Tablets

ロキタマイシン錠

Rokitamycin Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of rokitamycin (C_{48}H_{60}NO_{13}: 827.99).

Method of preparation

Prepare as directed under Tablets, with Rokitamycin.

Identification

Take an amount of powdered Rokitamycin Tablets, equivalent to 10 mg (potency) of Rokitamycin according to the labeled amount, add 20 mL of methanol, and centrifuge if necessary. To 1 mL of this solution add methanol to make 25 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 230 nm and 233 nm.

Uniformity of dosage units

Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Add 50 mL of water to 1 tablet of Rokitamycin Tablets, and disintegrate. Then add 10 mL of methanol, shake well, and add water to make exactly 100 mL. Centrifuge this solution if necessary, filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard 5 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 20 μg (potency) of Rokitamycin, and use this solution as the
Dissolution <6.10>  When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Roxatidine Acetate Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Roxatidine Acetate Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V’ mL so that each mL contains about 22 μg (potency) of Roxatidine Acetate Hydrochloride according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Roxatidine Acetate Hydrochloride, dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of the solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A5, at 232 nm of the sample solution and standard solution using water as the blank, as directed under Ultraviolet-visible Spectrophotometry 2.24>.

Amount [mg (potency)] of rokitamycin (C42H69NO15) = MS × A1/A5 × V’/V × 1/10

MS: Amount [mg (potency)] of Roxatidine Acetate Hydrochloride RS

Amount [mg (potency)] of rokitamycin (C42H69NO15) in 1 tablet = M5 × A1/A5 × V’/V × 1/C × 90

M5: Amount [mg (potency)] of Roxatidine Acetate Hydrochloride RS

C: Labeled amount [mg (potency)] of rokitamycin (C42H69NO15) in 1 tablet

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics 4.02> under the following conditions.

(i) Test organism, culture medium and standard solutions—Proceed as directed in the Assay under Roxatidine Acetate Hydrochloride.

(ii) Sample solutions—Weigh accurately not less than 20 tablets of Roxatidine Acetate Hydrochloride Tablets, and powder. Weigh accurately an amount of contents, equivalent to about 40 mg (potency) of Roxatidine Acetate Hydrochloride, add 50 mL of methanol, shake vigorously, then add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL, and centrifuge if necessary. Measure exactly a suitable quantity of this solution, add polysorbate 80 solution, prepared by adding 0.1 mol/L phosphate buffer solution, pH 8.0 to 0.1 g of polysorbate 80 to make 1000 mL, so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Expiration date 24 months after preparation.

Roxatidine Acetate Hydrochloride

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C19H28N2O4·HCl: 384.90
(3-[(Piperidin-1-yl)methyl]phenoxy)propylcarbamoylmethyl acetate monohydrate [93793-83-0]

Roxatidine Acetate Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C19H28N2O4·HCl.

Description Roxatidine Acetate Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Roxatidine Acetate Hydrochloride in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry 2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Roxatidine Acetate Hydrochloride RS prepared in the same manner as the sample solution; both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the infrared absorption spectrum of Roxatidine Acetate Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry 2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Roxatidine Acetate Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Roxatidine Acetate Hydrochloride (1 in 50) responds to the Qualitative Tests 1.09> (2) for chloride.

pH 2.5> Dissolve 1.0 g of Roxatidine Acetate Hydrochloride in 20 mL of water: the pH of this solution is between 4.0 and 6.0.

Melting point 2.6> 147 – 151°C (after drying).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Roxatidine Acetate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Roxatidine Acetate Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Roxatidine Acetate Hydrochloride in 10 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01>
Roxatidine Acetate Hydrochloride Extended-release Capsules

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Roxatidine Acetate Hydrochloride Extended-release Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of roxatidine acetate hydrochloride (C_{19}H_{28}N_{2}O_{4}.HCl: 384.90).

**Method of preparation** Prepare as directed under Capsules, with Roxatidine Acetate Hydrochloride.

**Identification** To 1 mL of the filtrate obtained in the Assay add ethanol (99.5) to make 20 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry (<2.24>): it exhibits maxima between 275 nm and 278 nm, and between 282 nm and 285 nm.

**Uniformity of dosage units** (<0.2>) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Roxatidine Acetate Hydrochloride Extended-release Capsules, add exactly V mL of ethanol (99.5) so that each mL contains about 2.5 mg of roxatidine acetate hydrochloride (C_{19}H_{28}N_{2}O_{4}.HCl), disperse the particles with the aid of ultrasonic wave, and filter through a membrane filter with a pore size of not more than 1.0 μm. To exactly 8 mL of the filtrate add exactly 2 mL of the internal standard solution, mix, and use this solution as the sample solution. Proceed as directed in the Assay.

\[
M_{3} = \text{Amount (mg) of Roxatidine Acetate Hydrochloride (C}_{19}\text{H}_{28}\text{N}_{2}\text{O}_{4}.\text{HCl)} = M_{2} \times Q_{r}/Q_{s} \times V/20
\]

**Internal standard solution**—A solution of benzoic acid in ethanol (99.5) (1 in 500).

**Dissolution** (<6.10>) When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rates of a 37.5-mg capsule in 45 minutes, in 90 minutes and in 8 hours are 20 – 50%, 35 – 65%, and not less than 70%, respectively.

Start the test with 1 capsule of Roxatidine Acetate Hydrochloride Extended-release Capsules, withdraw exactly 20 mL of the medium at the specified minute after starting the test, and supply exactly 20 mL of warm water to 37 ± 0.5°C immediately after withdrawing of the medium every time, and filter the media withdrawn through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 42 μg of roxatidine acetate hydrochloride (C_{19}H_{28}N_{2}O_{4}.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in water to make exactly 50 mL of the standard solution add ethanol (99.5) to make exactly 10 mL, Confirmation that the test solution is equivalent to 7 to 13 times the peak area of roxatidine acetate from the standard solution, and the total area of the peaks other than roxatidine acetate is not larger than 1/2 times the peak area of roxatidine acetate from the standard solution.

**System suitability**—

Test for required detectability: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add ethanol (99.5) to make exactly 10 mL. Confirm that the peak area of roxatidine acetate obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the solution for system suitability test.

System performance: Dissolve 50 mg of roxatidine acetate hydrochloride and 10 mg of benzoic acid in 25 mL of ethanol (99.5). When the procedure is run with 10 μL of this solution under the above operating conditions, benzoic acid and roxatidine acetate are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxatidine acetate is not more than 1.0%.

Loss on drying (<2.41) Not more than 0.3% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition (<2.44) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Roxatidine Acetate Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate (<2.50) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 38.49 mg of C_{19}H_{28}N_{2}O_{4}.HCl

Containers and storage Containers—Tight containers.

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according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than roxatidine acetate obtained from sample solution is not larger than 1/5 times the peak area of roxatidine acetate from the standard solution, and the total area of the peaks other than roxatidine acetate is not larger than 1/2 times the peak area of roxatidine acetate from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with cyanopropylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of hexane, ethanol (99.5), triethylamine, and acetic acid (100) (384:16:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 10 minutes.

Time span of measurement: About 1.5 times as long as the retention time of roxatidine acetate beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add ethanol (99.5) to make exactly 10 mL. Confirm that the peak area of roxatidine acetate obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the solution for system suitability test.

System performance: Dissolve 50 mg of roxatidine acetate hydrochloride and 10 mg of benzoic acid in 25 mL of ethanol (99.5). When the procedure is run with 10 μL of this solution under the above operating conditions, benzoic acid and roxatidine acetate are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxatidine acetate is not more than 1.0%.

Loss on drying (<2.41) Not more than 0.3% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition (<2.44) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Roxatidine Acetate Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate (<2.50) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 38.49 mg of C_{19}H_{28}N_{2}O_{4}.HCl

Containers and storage Containers—Tight containers.
mg of roxatidine acetate hydrochloride (C19H28N2O4.HCl), accurately a portion of the powder, equivalent to about 75 of Roxatidine Acetate Hydrochloride Extended-release Capsules, weigh Assay

Take out the contents of not less than 20 Roxatidine Acetate Hydrochloride Extended-release Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of roxatidine acetate hydrochloride (C19H28N2O4.HCl), add exactly 30 mL of ethanol (99.5), shake, and filter through a membrane filter with pore size of not more than 1.0 \( \mu \)m. To exactly 8 mL of the filtrate add exactly 2 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in ethanol (99.5) to make exactly 20 mL. To exactly 8 mL of this solution add exactly 2 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( C.07 \) according to the following conditions, and calculate the ratios, \( Q_2 \) and \( Q_3 \), of the peak area of roxatidine acetate to that of the internal standard.

$$
M_S = M_s \times \left[ \frac{A_{280}}{A_5} + \sum_{i=1}^{n-1} \left( \frac{A_{280}}{A_5} \times \frac{1}{45} \right) \right] \times \frac{V \times 180}{C}
$$

$$
M_S = \text{Amount (mg) of Roxatidine Acetate Hydrochloride RS}
$$

C: Labeled amount (mg) of roxatidine acetate hydrochloride (C19H28N2O4.HCl) in 1 capsule

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile, triethylamine and acetic acid (100): (340:60:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxatidine acetate is not less than 1.0%.

System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of roxatidine acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Roxatidine Acetate Hydrochloride Extended-release Tablets

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Roxatidine Acetate Hydrochloride Extended-release Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of roxatidine acetate hydrochloride (C19H28N2O4.HCl: 384.90).

Method of preparation Prepare as directed under Tablets, with Roxatidine Acetate Hydrochloride.

Identification Powder Roxatidine Acetate Hydrochloride Extended-release Tablets. To a portion of the powder, equivalent to 37.5 mg of Roxatidine Acetate Hydrochloride according to the labeled amount, add 40 mL of ethanol (99.5), and disperse the particles for 10 minutes with the aid of ultrasonic waves with occasional shaking. After shaking thoroughly, add ethanol (99.5) to make 50 mL. Filter the solution, and to 4 mL of the filtrate add ethanol (99.5) to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \( C.24 \): it exhibits maxima between 274 nm and 278 nm and between 281 nm and 285 nm.
ing to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Roxatidine Acetate Hydrochloride Extended-release Tablets add 5 mL of a mixture of water, triethylamine and acetic acid (100) (340:2:1), agitate for 5 minutes with the aid of ultrasonic waves with occasional shaking, then add 7.5 mL of acetonitrile, then agitate again for 5 minutes with the aid of ultrasonic waves. Add 5 mL of a mixture of water, triethylamine and acetic acid (100) (340:2:1), agitate for 5 minutes with the aid of ultrasonic waves, shake thoroughly, add a mixture of water, triethylamine and acetic acid (100) (340:2:1) to make exactly 50 mL, centrifuge, and filter the supernatant liquid. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, equivalent to 6 mg of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl), add exactly 3 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl) = Mₛ × Qₛ / Qₘ \times V

Mₛ: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—A solution of sodium benzoate in the mobile phase (3 in 2000).

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Roxatidine Acetate Hydrochloride Extended-release Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 6 mg of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl), add 40 mL of the mobile phase, and agitate to about 37.5 mg of roxatidine acetate hydrochloride powder. Weigh accurately a portion of the powder, equivalent as the standard solution. Perform the test with 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl) = Mₛ × Qₛ / Qₘ

Mₛ: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—A solution of sodium benzoate in the mobile phase (3 in 2000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wave-length: 274 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of water, acetonitrile, triethylamine and acetic acid (100) (340:60:2:1).
Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 8 minutes.

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the internal standard and roxatidine acetate are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of roxatidine acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Roxatidine Acetate Hydrochloride for Injection

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Roxatidine Acetate Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl: 384.90).

Method of preparation Prepare as directed under Injections, with Roxatidine Acetate Hydrochloride.

Description It occurs as white masses or powder.

Identification To an amount of Roxatidine Acetate Hydrochloride for Injection, equivalent to 75 mg of Roxatidine Acetate Hydrochloride according to the labeled amount, add 30 mL of ethanol (99.5), shake, and filter through a membrane filter with a pore size not exceeding 0.45 µm. To 1 mL of the filtrate add ethanol (99.5) to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 275 nm and 279 nm and between 282 nm and 286 nm.

pH Being specified separately.

Purity Clarity and color of solution Dissolve an amount of Roxatidine Acetate Hydrochloride for Injection, equivalent to 75 mg of Roxatidine Acetate Hydrochloride according to the labeled amount, in 20 mL of isotonic sodium chloride solution: the solution is clear and colorless.

Bacterial endotoxins <4.01> Less than 4.0 EU/mg.

Uniformity of dosage units <6.02> It meets the requirements of the Mass variation test.
Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dissolve with water each content of 10 Roxatidine Acetate Hydrochloride for Injection, wash the containers with water, combine the solution of the content and washings, and add water to make exactly V mL so that each mL contains about 3.75 mg of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄·HCl). Pipet 5 mL of this solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorous (V) oxide) for 4 hours, dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Qₐ and Qₐ₀, of the peak area of roxatidine acetate to that of the internal standard.

\[ Mₐ = \frac{Qₐ}{Qₐ₀} \times \frac{V}{50} \]

\[ Mₐ₀: \text{Amount (mg) of Roxatidine Acetate Hydrochloride RS} \]

Internal standard solution—Dissolve 20 mg of guanine in 10 mL of 2 mol/L hydrochloric acid TS, add 50 mL of water, then add 20 mL of a solution of sodium hydroxide (1 in 25) and water to make 100 mL. To 10 mL of this solution add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter). Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile, triethylamine and acetic acid (100) (340:60:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 14 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and roxatidine acetate are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of roxatidine acetate to that of the internal standard is not more than 1.0%.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Roxithromycin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve exactly 40 mg of Roxithromycin in the mobile phase A to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve exactly 20 mg of Roxithromycin RS in the mobile phase A to make exactly 10 mL. Pipet 1 mL of this solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak of Roxithromycin is a derivative of erythromycin.

It contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Roxithromycin is expressed as mass (potency) of roxithromycin (C₉₃H₇₆N₄O₁₃).

Description Roxithromycin occurs as a white crystalline powder.

It is freely soluble in ethanol (95) and in acetone, soluble in methanol, sparingly soluble in acetonitrile, and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Roxithromycin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Roxithromycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D₂⁰ = −93° to −96° (0.5 g calculated on the anhydrous basis, acetone, 50 mL, 100 mm).

Containers and storage Containers—Hermetic containers.
areas by the automatic integration method: the area of a peak having the relative retention time of about 1.05 with respect to roxithromycin from the sample solution is not larger than 2 times of the peak area of roxithromycin from the standard solution. The area of the peak other than the peak of roxithromycin and the peak having the relative retention time of about 1.05 is not larger than the peak area of roxithromycin from the standard solution, and the total area of the peaks other than roxithromycin from the sample solution is not larger than 6 times of the peak area of roxithromycin from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: To 200 mL of a solution of ammonium dihydrogenphosphate (17 in 100) add 510 mL of water, and adjust to pH 5.3 with 2 mol/L sodium hydroxide TS. To this solution add 315 mL of acetonitrile.

Mobile phase B: A mixture of acetonitrile and water (7:3).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 38</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>38 – 39</td>
<td>100 → 90</td>
<td>0 → 10</td>
</tr>
<tr>
<td>39 – 80</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of roxithromycin is about 21 minutes.

**System suitability—**

- Test for required detection: To exactly 2 mL of the standard solution add the mobile phase A to make exactly 10 mL. Confirm that the peak area of roxithromycin obtained from 20 μL of this solution is equivalent to 15 to 25% of that from 20 μL of the standard solution.

- System performance: Dissolve 5 mg each of Roxithromycin RS and N-demethylroxithromycin in the mobile phase A to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, N-demethylroxithromycin and roxithromycin are eluted in this order with the resolution between these peaks being not less than 6 and the symmetry factor of the peak of roxithromycin is not more than 1.5.

- System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of roxithromycin is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

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**Freeze-dried Live Attenuated Rubella Vaccine**  
乾燥弱毒生風しんワクチン

Freeze-dried Live Attenuated Rubella Vaccine is a preparation for injection which is dissolved before use. It contains live attenuated rubella virus. It conforms to the requirements of Freeze-dried Live Attenuated Rubella Vaccine in the Minimum Requirements for Biological Products.

**Description** Freeze-dried Live Attenuated Rubella Vaccine becomes a colorless, yellowish or reddish clear liquid on addition of solvent.
Saccharated Pepsin

含糖ペプシン

Saccharated Pepsin is a mixture of pepsin obtained from the gastric mucosa of hog or cattle and Lactose Hydrate, and it is an enzyme drug having a proteolytic activity.

It contains not less than 3800 units and not more than 6000 units per g.

**Description** Saccharated Pepsin occurs as a white powder. It has a characteristic odor, and has a slightly sweet taste.

It dissolves in water to give a slightly turbid liquid, and does not dissolve in ethanol (95) and in diethyl ether.

It is slightly hygroscopic.

**Purity**

1. **Rancidity**—Saccharated Pepsin has no unpleasant or rancid odor.
2. **Acidity**—Dissolve 0.5 g of Saccharated Pepsin in 50 mL of water, and add 0.50 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: the solution is red in color.

**Loss on drying** Not more than 1.0% (1 g, 80°C, 4 hours).

**Residue on ignition** Not more than 0.5% (1 g).

**Assay**

(i) **Substrate solution**—Use the substrate solution 1 hour.

(ii) **Sample solution**—Weigh accurately an amount of Saccharated Pepsin equivalent to about 1250 units, dissolve in ice-cold 0.01 mol/L hydrochloric acid TS to make exactly 50 mL.

(iii) **Standard solution**—Weigh accurately a suitable amount of Saccharated Pepsin RS, and dissolve in ice-cold 0.01 mol/L hydrochloric acid TS to make a solution containing about 25 units per mL.

(iv) **Procedure**—Proceed as directed in (2) Assay for protein digestive activity under the Digestion Test after adjusting the pH to 2.0.

Separately, determine the absorbances, using trichloroacetic acid TS A as the precipitation reagent.

Units in 1 g of Saccharated Pepsin

\[ \text{Units} = \frac{U_5 \times (A_T - A_{TB})}{(A_S - A_{SB})} \times \frac{1}{M} \]

\( U_5 \): Units per mL of the standard solution

\( M \) : Amount (g) of Saccharated Pepsin per mL of the sample solution

**Containers and storage** Containers—Tight containers.

Storage—Not exceeding 30°C.

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Saccharin

サッカリン

C₆H₅NO₃S: 183.18
1,2-Benzo[d]isothiazol-3(2H)-one 1,1-dioxide [81-07-2]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (* •).

Saccharin contains not less than 99.0% and not more than 101.0% of C₆H₅NO₃S, calculated on the dried basis.

*Description* Saccharin occurs as colorless or white crystals or a white crystalline powder. It has a very sweet taste.

It is sparingly soluble in ethanol (95), and slightly soluble in water.

It dissolves in sodium hydroxide TS.

**Identification** Determine the infrared absorption spectrum of Saccharin as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** 226 – 230°C

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of Saccharin in 30 mL of hot water or in 50 mL of ethanol (95): the solution is clear and colorless in each case.

(2) Heavy metals—Proceed with 2.0 g of Saccharin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Benzoate and salicylate—To 10 mL of a saturated solution of Saccharin in hot water add 3 drops of iron (III) chloride TS: no precipitate is formed, and no red-purple to purple color develops.

(4) o-Toluene sulfonamide—Dissolve 10 g of Saccharin in 70 mL of sodium hydroxide TS, and extract with three 30-mL portions of ethyl acetate. Combine all the ethyl acetate extracts, wash with 30 mL of a solution of sodium chloride (1 in 4), dehydrate with 5 g of anhydrous sodium sulfate, then evaporate the solvent. To the residue add exactly 5 mL of the internal standard solution to dissolve, and use this solution as the sample solution. Separately, dissolve 0.10 g of o-toluene sulfonamide in ethyl acetate to make exactly 100 mL. Pipet 1 mL of this solution, evaporate to dryness on a water bath, dissolve the residue in exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak height of o-toluene sulfonamide to that of the internal standard: \( Q_T \) is
not more than $Q_s$.

*Internal standard solution*—A solution of caffeine in ethyl acetate (1 in 500).

*Operating conditions*—
- Detector: A hydrogen flame-ionization detector.
- Column: A glass column 3 mm in inside diameter and 1 m in length, packed with siliceous earth for gas chromatography coated 3% with diethylene glycol succinate polyester for gas chromatography (180 - 250 μm in particle diameter).
- Column temperature: A constant temperature of about 200°C.
- Temperature of injection port: A constant temperature of about 225°C.
- Temperature of detector: A constant temperature of about 250°C.
- Carrier gas: Nitrogen.
- Flow rate: Adjust the flow rate so that the retention time of caffeine is about 6 minutes.

*System suitability*
- System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, the internal standard and o-toluene sulfonamide are eluted in this order with the resolution between these peaks being not less than 2.0.
- System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of o-toluene sulfonamide to that of the internal standard is not more than 2.0%.

(5) Readily carbonizable substances 1.15—Perform the test with 0.20 g of Saccharin, by warming at 48 to 50°C for 2 hours: the color of the solution is not more intense than the matching fluid A.

*Loss on drying* 2.41 Not more than 1.0% (1 g, 105°C, 2 hours).

*Residue on ignition* 2.44 Not more than 0.2% (1 g).

*Assay* Weigh accurately about 0.5 g of Saccharin, dissolve in 40 mL of ethanol (95), add 40 mL of water, and titrate 2.50 with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 18.32 mg of C$_7$H$_5$NO$_3$S

*Containers and storage* Containers—Well-closed containers.

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**Saccharin Sodium Hydrate**

**Saccharin Sodium**

サッカリンナトリウム水和物

C$_7$H$_4$NNaO$_3$S\(\cdot\)2H$_2$O: 241.20

2-Sodio-1,2-benzo[d]isothiazol-3(2H)-one 1,1-dioxide dihydrate

[6155-57-3]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (•).

Saccharin Sodium Hydrate contains not less than 99.0% and not more than 101.0% of saccharin sodium (C$_7$H$_4$NNaO$_3$S: 205.17), calculated on the anhydrous basis.

*Description* Saccharin Sodium Hydrate occurs as colorless crystals or a white, crystalline powder. It has an intensely sweet taste, even in 10,000 dilutions.

It is freely soluble in water and in methanol, and sparingly soluble in ethanol (95) and in acetic acid (100).

It effloresces slowly and loses about half the amount of water of crystallization in air.

*Identification* *(1)* Determine the infrared absorption spectrum of Saccharin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Saccharin Sodium Hydrate (1 in 10) responds to the Qualitative Tests 1.09 for sodium salt.

*Purity* *(1)* Clarity and color of solution—Dissolve 1.0 g of Saccharin Sodium Hydrate in 1.5 mL of water or in 50 mL of ethanol (95): the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 1.0 g of Saccharin Sodium Hydrate in 10 mL of water, and add 1 drop of phenolphthalein TS: the solution is colorless. Add 1 drop of 0.1 mol/L sodium hydroxide VS to the solution: the color changes to red.

*(3)* Heavy metals 1.07—Dissolve 2.0 g of Saccharin Sodium Hydrate in 40 mL of water, add 0.7 mL of dilute hydrochloric acid, dilute with water to make 50 mL, and rub the inner wall of the vessel with a glass rod until crystallization begins. Allow the solution to stand for 1 hour after the beginning of crystallization, and then filter through dry filter paper. Reject the first 10 mL of the filtrate, and take 25 mL of the subsequent filtrate. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test, using this solution as the test solution. To 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL, and use this solution as the control solution (not more than 10 ppm).

*(4)* Benzoate and salicylate—Dissolve 0.5 g of Saccharin

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 JP XVI  Official Monographs  /  Saccharin Sodium Hydrate  1367
Sodium Hydrate in 10 mL of water, add 5 drops of acetic acid (31) and 3 drops of iron (III) chloride TS: no turbidity is produced, and no red-purple to purple color develops.  

*5* o-Toluene sulfonamide—Dissolve 10 g of Saccharin Sodium Hydrate in 50 mL of water, and extract with three 30-mL portions of ethyl acetate. Combine all the ethyl acetate extracts, wash with 30 mL of a solution of sodium chloride (1 in 4), dehydrate with 5 g of anhydrous sodium sulfate, and evaporate ethyl acetate. To the residue add exactly 5 mL of the internal standard solution to dissolve, and use this solution as the sample solution. Separately, dissolve 0.10 g of o-toluene sulfonamide in ethyl acetate to make exactly 100 mL. Pipet 1 mL of this solution, evaporate on a water bath to dryness, dissolve the residue in exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 µL of each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, QT and Q2, of the peak height of o-toluene sulfonamide to that of the internal standard: QT is not more than Q2.

**Internal standard solution**—A solution of caffeine in ethyl acetate (1 in 500).

**Operating conditions**—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, packed with siliceous earth for gas chromatography (180 to 250 µm in diameter), coated with diethyleneglycol succinate polyester for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 200°C.

Injection port temperature: A constant temperature of about 225°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of caffeine is about 6 minutes.

**System suitability**—

System performance: When the procedure is run with 1 µL of the standard solution under the above operating conditions, the internal standard and o-toluene sulfonamide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 1 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of o-toluene sulfonamide to that of the internal standard is not more than 2.0%.

6 Readily carbonizable substances <1.15>—Perform the test with 0.20 g of Saccharin Sodium Hydrate. Allow the solution to stand between 48°C and 50°C for 10 minutes: the solution has no more color than Matching Fluid A.

**Water** <2.48> Not more than 15.0% (0.1 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 0.15 g of Saccharin Sodium Hydrate, dissolve in 50 mL of acetic acid (100), heat slightly if necessary, and titrate <2.90> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 20.52 mg of C18H17NNaO4S

**Containers and storage** Containers—Well-closed containers.

### Salazosulfapyridine

**Sulfasalazine**

![Chemical Structure](image)

C18H14N4O5S: 398.39

2-Hydroxy-5-[4-(pyridin-2-ylsulfamoyl)phenylazo]benzoic acid

[599-79-1]

Salazosulfapyridine, when dried, contains not less than 96.0% of C18H17NNaO4S.

**Description** Salazosulfapyridine occurs as a yellow to yellow-brown, fine powder. It is odorless and tasteless.

It is sparingly soluble in pyridine, slightly soluble in ethanol (95), practically insoluble in water, in chloroform and in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: 240 - 249°C (with decomposition).

**Identification** (1) Dissolve 0.1 g of Salazosulfapyridine in 20 mL of dilute sodium hydroxide TS: a red-brown color develops. This color gradually fades upon gradual addition of 0.5 g of sodium hydrosulfite with shaking. Use this solution in the following tests (2) to (4).

(2) To 1 mL of the solution obtained in (1) add 40 mL of water, neutralize with 0.1 mol/L hydrochloric acid TS, and add water to make 50 mL. To 5 mL of this solution add 2 to 3 drops of dilute iron (III) chloride TS: a red color develops and changes to purple, then fades when dilute hydrochloric acid is added dropwise.

(3) The solution obtained in (1) responds to the Qualitative Tests <1.09> for primary aromatic amines.

(4) To 1 mL of the solution obtained in (1) add 1 mL of pyridine and 2 drops of copper (II) sulfate TS, and shake. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a green color develops in the chloroform layer.

(5) Determine the absorption spectrum of a solution of Salazosulfapyridine in dilute sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity** (1) Chloride <1.03>—Dissolve 2.0 g of Salazosulfapyridine in 12 mL of sodium hydroxide TS and 36 mL of water, add 2 mL of nitric acid, shake, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of
directed under Thin-layer Chromatography standard solution. Perform the test with these solutions as pyridine to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the test solution (not more than 10 ppm).

Solution, and proceed in the same manner as the test with to a generator bottle, add exactly 2 mL of Standard Arsenic than that of the following color standard. Make 25 mL. Perform the test with 5 mL of this solution as white fumes are evolved again. After cooling, add water to tion of ammonium oxalate monohydrate, and heat until slightly yellow. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved again. After cooling, add water to make 25 mL. Perform the test with 5 mL of this solution as the test solution: the color of the test solution is not deeper than that of the following color standard.

Color standard: Proceed in the same manner without Salazosulfapyridine, transfer 5 mL of the obtained solution to a generator bottle, add exactly 2 mL of Standard Arsenic Solution, and proceed in the same manner as the test with the test solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.20 g of Salazosulfapyridine in 20 mL of pyridine, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add pyridine to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with diluted methanol (9 in 10) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(6) Salicylic acid—To 0.10 g of Salazosulfapyridine add 15 mL of diethyl ether, and shake vigorously. Add 5 mL of dilute hydrochloric acid, shake vigorously for 3 minutes, collect the diethyl ether layer, and filter. To the water layer add 15 mL of diethyl ether, shake vigorously for 3 minutes, collect the diethyl ether layer, filter, and combine the filtrates. Wash the residue on the filter paper with a small quantity of diethyl ether, and combine the washings and the filtrate. Evaporate the diethyl ether with the aid of air-stream at room temperature. To the residue add dilute ammonium iron (III) sulfate TS, shake, and filter, if necessary. Wash the residue on the filter paper with a small quantity of dilute ammonium iron (III) sulfate TS, combine the washings and the filtrate, add dilute ammonium iron (III) sulfate TS to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, dissolve in dilute ammonium iron (III) sulfate TS to make exactly 400 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₅, at 535 nm of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry: salicylic acid content is not more than 0.5%.

Content (mg) of salicylic acid (C₇H₆O₃)

\[ M_S = \frac{A_1}{A_2} \times \frac{M_5}{M_S} \]

M₅: Amount (mg) of salicylic acid for assay

Loss on drying Not more than 2.0% (1 g, 105°C, 4 hours).

Residue on ignition Not more than 0.2% (1 g).

Assay Weigh accurately about 20 mg of Salazosulfapyri-

dine, previously dried, and perform the test as directed in the procedure of determination for sulfur under the Oxygen Flask Combustion Method, using 10 mL of diluted hydrogen peroxide (30) (1 in 40) as an absorbing liquid.

Each mL of 0.005 mol/L barium perchlorate VS = 1.992 mg of C₁₂H₁₅N₃O₇S

Salbutamol Sulfate サルブタモール硫酸塩

\[
\text{C}_{13}\text{H}_{21}\text{NO}_3\cdot\text{H}_2\text{SO}_4: \text{576.70} \\
\text{(1RS)}\cdot\text{2-(1,1-Dimethylethyl)amino-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol hemisulfate} \\
\text{[51022-70-9]}
\]

Salbutamol Sulfate, when dried, contains not less than 98.0% of (C₁₃H₂₁NO₃)₂·H₂SO₄.

Description Salbutamol Sulfate occurs as a white powder. It is freely soluble in water, slightly soluble in ethanol (95), and in acetic acid (100) and practically insoluble in diethyl ether.

A solution of Salbutamol Sulfate (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Salbutamol Sulfate in 0.1 mol/L hydrochloric acid TS (1 in 12,500) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Salbutamol Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Salbutamol Sulfate (1 in 20) responds to the Qualitative Tests for sulfate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g
of Salbutamol Sulfate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals \( <1.0 \% \) —Proceed with 1.0 g of Salbutamol Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Salbutamol Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.0 \% \) . Spot 5 \( \mu \) L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water and ammonia solution (28) (25:15:8:2) to a distance of about 15 cm, and air-dry the plate. Leave the plate in a well-closed vessel saturated with diethylamine vapor for 5 minutes, and spray evenly 4-nitrobenzenediazonium chloride TS: the spots other than the principal spot from the sample solution are not more intense than the principal spot from the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution (28) (25:15:8:2) to a distance of about 15 cm, and air-dry the plate. Leave the plate in a well-closed vessel saturated with diethylamine vapor for 5 minutes, and spray evenly 4-nitrobenzenediazonium chloride TS: the spots other than the principal spot from the sample solution are not more intense than the principal spot from the sample solution.

(4) Boron—Take 50 mg of Salbutamol Sulfate and 5.0 mL of the Standard Boron Solution, and transfer to a platinum crucible. Add 5 mL of potassium carbonate-sodium carbonate solution (28) (25:15:8:2) to a distance of about 15 cm, and air-dry the plate. Leave the plate in a well-closed vessel saturated with diethylamine vapor for 5 minutes, and spray evenly 4-nitrobenzenediazonium chloride TS: the spots other than the principal spot from the sample solution are not more intense than the principal spot from the sample solution.

Loss on drying \( <2.41 \% \) —Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 3 hours).

Residue on ignition \( <2.44 \% \) —Not more than 0.1% (1 g).

Assay Weigh accurately about 0.9 g of Salbutamol Sulfate, previously dried, and dissolve in 50 mL of acetic acid (100) by warming. After cooling, titrate \( <2.50 \% \) with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 57.67 mg of \((C_{13}H_{21}NO_3)_2\cdot H_2SO_4\).

Containers and storage Containers—Tight containers.

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**Salicylic Acid**

サリチル酸

C\(\text{C}_7\text{H}_6\text{O}_3\): 138.12
2-Hydroxybenzoic acid [69-72-7]

Salicylic Acid, when dried, contains not less than 99.5% and not more than 101.0% of \(\text{C}_7\text{H}_6\text{O}_3\).

Description Salicylic Acid occurs as white crystals or crystalline powder. It has a slightly acid, followed by an acrid taste.

It is freely soluble in ethanol (95) and in acetone, and slightly soluble in water.

Identification (1) A solution of Salicylic Acid (1 in 500) responds to the Qualitative Tests \( <1.09 \% \) (1) and (3) for salicylate.

(2) Determine the absorption spectrum of a solution of Salicylic Acid in ethanol (95) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24 \% \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave-lengths.

(3) Determine the infrared absorption spectrum of Salicylic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25 \% \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point \( <2.60 \% \) 158 – 161°C

Purity (1) Chloride \( <1.07 \% \)—Dissolve 5.0 g of Salicylic Acid in 90 mL of water by heating, cool, dilute with water to 100 mL, and filter. Discard the first 20 mL of the filtrate, take subsequent 30 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.008%).

(2) Sulfate \( <1.14 \% \)—To 20 mL of the filtrate obtained in (1) add 1 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(3) Heavy metals \( <1.07 \% \)—Dissolve 2.0 g of Salicylic Acid in 25 mL of acetone, add 4 mL of sodium hydroxide TS, 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Related substances—Dissolve 0.50 g of Salicylic Acid in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve exactly 10 mg of phenol, exactly 25 mg of 4-hydroxyisophthalic acid and exactly 50 mg of parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution,
add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.61> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid, and phenol obtained from the sample solution are not larger than the area of each respective peak from the standard solution, the area of the peak other than salicylic acid and other than the substances mentioned above is not larger than the peak area of 4-hydroxisophthalic acid from the standard solution, and the total area of peaks other than salicylic acid is not larger than 2 times the peak area of parahydroxybenzoic acid from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of water, methanol and acetic acid (100) (60:40:1).

Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 17 minutes.

Time span of measurement: About 2 times as long as the retention time of salicylic acid, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol obtained from 10 µL of this solution are equivalent to 14 to 26% of the area of each respective peak from 10 µL of the standard solution.

System performance: Dissolve 10 mg of phenol, 25 mg of 4-hydroxyisophthalic acid and 50 mg of parahydroxybenzoic acid in 100 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make 10 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol are eluted in this order with the resolution between the peaks of 4-hydroxyisophthalic acid and phenol being not less than 4.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol is not more than 2.0%, respectively.

Loss on drying <2.41> Not more than 0.5% (2 g, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Salicylic Acid, previously dried, dissolve in 25 mL of neutralized ethanol, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS = 13.81 mg of C₇H₆O₃.

Containers and storage Containers—Well-closed containers.

Salicylated Alum Powder

サリチル・ミョウバン散

Salicylated Alum Powder contains not less than 2.7% and not more than 3.3% of salicylic acid (C₇H₆O₃: 138.12).

Method of preparation

Salicylic Acid, finely powdered 30 g
Dried Aluminum Potassium Sulfate, very finely powdered 640 g
Talc, very finely powdered a sufficient quantity

To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Salicylated Alum Powder occurs as a white powder.

Identification (1) The colored solution obtained in the Assay has a red-purple color and exhibits an absorbance maximum <2.24> between 520 nm and 535 nm (salicylic acid).

(2) Shake 0.3 g of Salicylated Alum Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g of salicylic acid in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.67>. Spot 5 µL each of the sample solution and standard solution on the plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, aceton and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same Rf value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal a purple color.

Assay Weigh accurately about 0.33 g of Salicylated Alum Powder, add 80 mL of ethanol (95), and shake vigorously. Dilute with ethanol (95) to make exactly 100 mL, filter, and discard the first 10 mL of the filtrate. Use the subsequent filtrate as the sample solution. Dissolve about 0.1 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours and accurately weighed, in sufficient ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, dilute with ethanol (95) to make exactly 100 mL, and use the solution as the standard solution. Pipet 10 mL of this solution, dilute with ethanol (95) to make exactly 100 mL, and use the solution as the standard solution. Pipet 10 mL of this solution, dilute with ethanol (95) to make exactly 100 mL, and use the solution as the standard solution. Pipet 10 mL of this solution, dilute with ethanol (95) to make exactly 100 mL, and use the solution as the standard solution. Pipet 10 mL of this solution, dilute with ethanol (95) to make exactly 100 mL, and use the solution as the standard solution. Pipet 10 mL of this solution, dilute with ethanol (95) to make exactly 100 mL, and use the solution as the standard solution. Pipet 10 mL of this solution, dilute with ethanol (95) to make exactly 100 mL, and use the solution as the standard solution.
solution, as the blank.

\[
\text{Amount (mg) of salicylic acid (C}_7\text{H}_6\text{O}_3) = M_S \times \frac{A_T}{A_S} \times \frac{1}{10}
\]

- \(M_S\): Amount (mg) of salicylic acid for assay

**Containers and storage** Containers—Well-closed containers.

### Salicylic Acid Adhesive Plaster

**サリチル酸絆創膏**

**Method of preparation**

Adhesive Plaster consists of a mixture of the below ingredients with carefully selected rubber, resins, zinc oxide and other substances. It has adhesive properties. It spreads evenly on a fabric.

- Salicylic Acid, finely powdered 500 g
- Adhesive plaster base a sufficient quantity

To make 1000 g

**Description** The surface of Salicylic Acid Adhesive Plaster is whitish in color and adheres well to the skin.

**Containers and storage** Containers—Well-closed containers.

### Salicylic Acid Spirit

**サリチル酸精**

Salicylic Acid Spirit contains not less than 2.7 w/v% and not more than 3.3 w/v% of salicylic acid (C\(_7\)H\(_6\)O\(_3\)): 138.12).

**Method of preparation**

- Salicylic Acid 30 g
- Glycerin 50 mL
- Ethanol a sufficient quantity

To make 1000 mL

Prepare as directed under Spirits, with the above ingredients.

**Description** Salicylic Acid Spirit is a clear, colorless liquid.

- Specific gravity \(d_{20}^{20}\): about 0.86

**Identification**

1. To 1 mL of Salicylic Acid Spirit add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 200 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

2. To 1 mL of Compound Salicylic Acid Spirit add 20 mL of water and 5 mL of dilute hydrochloric acid, and extract with 20 mL of diethyl ether. Wash the diethyl ether extract with two 5-mL portions of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, allow to stand for 10 minutes, and add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

3. To 0.5 mL of Compound Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chloroform, and use the extract as the sample solution (1). To 2
mL of Compound Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chloroform, wash the extract with two 5-mL portions of sodium hydrogen carbonate TS, and use the chloroform extract as the sample solution (2). Separately, dissolve 0.01 g each of salicylic acid and phenol in 5 mL each of chloroform, and use both solutions as the standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 5 µL each of the sample solutions (1) and (2) and the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the standard solution, pH 7.0, and methanol (3:1).

The standard solution and the ratios, Ta and Tb, of the peak area of salicylic acid and phenol to that of the internal standard in the sample solution as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for liquid chromatography, 5 mm in diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the standard solution, pH 7.0, and methanol (3:1).

Alcohol number <1.01> Not less than 7.5 (Method 2). Assay Measure accurately 2 mL of Compound Salicylic Acid Spirit, add exactly 5 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and about 50 mg of phenol for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the standard solution. Perform the test with 15 µL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, QSa and QSb, of the peak area of salicylic acid and phenol to that of the internal standard in the sample solution, and the ratios, QSa and QSb, of the peak area of salicylic acid and phenol to that of the internal standard in the standard solution.

\[
\text{Amount (mg) of salicylic acid (C_7H_6O_3)} = M_{Sb} \times Q_{Ta} / Q_{Sa} \times 1 / 5
\]

\[
\text{Amount (mg) of phenol (C_6H_5O)} = M_{Sb} \times Q_{Ta} / Q_{Sa} \times 1 / 5
\]

Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 0.05 g of theophylline in 100 mL of diluted methanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with 10 µL of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.

Santonin

サントニン

\[
[\alpha]_D^{20} = \text{–}-170 \text{ to } -175^\circ \quad (0.2 \text{ g}, \text{ chloroform, } 10 \text{ mL, } 100 \text{ mm})
\]

Santonin, when dried, contains not less than 98.5% and not more than 101.0% of C_{15}H_{18}O_3.

Description Santonin occurs as colorless crystals, or a white, crystalline powder.

It is freely soluble in chloroform, sparingly soluble in ethanol (95), and practically insoluble in water.

It becomes yellow by light.

Identification (1) Determine the absorption spectrum of a solution of Santonin in ethanol (95) (3 in 250,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Santonin as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation 2.49 [α]_D^20: –170 to –175° (0.2 g, chloroform, 10 mL, 100 mm).

Melting point 2.60 172 – 175°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Santonin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Alkaloids—Boil 0.5 g of Santonin with 20 mL of diluted sulfuric acid (1 in 100), cool, and filter. Dilute 10 mL of the filtrate with water to 30 mL, add 3 drops of iodine TS, and allow to stand for 3 hours: no turbidity is produced.

(3) Artemisinin—Dissolve 1.0 g of powdered Santonin in 2 mL of chloroform by slight warming: the solution is clear and colorless, or any yellow color produced is not darker than Matching Fluid A.

(4) Phenols—Boil 0.20 g of Santonin with 10 mL of water, cool, and filter. To the filtrate add bromine TS until...
the color of the solution becomes yellow: no turbidity is produced.

(5) Acid-coloring substances—Moisten 10 mg of Santonin with nitric acid: no color develops immediately. Moisten Santonin with sulfuric acid, previously cooled to 0°C: no color is produced immediately.

**Loss on drying** <2.4> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.4> Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.25 g of Santonin, previously dried, dissolve in 10 mL of ethanol (95) by warming, add exactly 20 mL of 0.1 mol/L sodium hydroxide VS, and ously dried, dissolve in 10 mL of ethanol (95) by warming, and filter. To 1 mL of the filtrate add 1 mL of dilute nitric acid. This solution responds to the Qualitative Tests <1.09> for chloride.

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 g of Sarpogrelate Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Sarpogrelate Hydrochloride according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Carry out the determination within 3 hours after preparing the sample solution. Dissolve 20 mg of Sarpogrelate Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of decomposed substance A, having the relative retention time about 0.82 to sarpogrelate, obtained from the sample solution is not larger than 1/5 times that of sarpogrelate from the standard solution, the area of the peak other than sarpogrelate and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of sarpogrelate from the standard solution, and the total area of the peaks other than sarpogrelate from the sample solution is not larger than 1/2 times the peak area of sarpogrelate from the standard solution. For this calculation use the peak area of the decomposed substance A after multiplying by the relative response factor, 0.78.

**Operating conditions**—Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of sarpogrelate, beginning after the solvent peak.

**System suitability**—Test for required detectability: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 50 mL, and confirm that the peak area of sarpogrelate with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: Dissolve 50 mg of Sarpogrelate Hydrochloride in 20 mL of water, and use as the sarpogrelate hydrochloride stock solution. To 1 mL of the sarpogrelate hydrochloride stock solution add 2 mL of sodium hydroxide TS, shake thoroughly, allow to stand for 10 minutes, and add 3 mL of 1 mol/L hydrochloric acid TS. To this solution add 1 mL of the sarpogrelate hydrochloride stock solution,
and add the mobile phase to make 50 mL. When the procedure is run with 10 \( \mu L \) of this solution under the above operating conditions, the decomposed substance A and sarpogrelate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sarpogrelate is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Water \(<2.48\) Not more than 0.5% (1 g, coulometric titration).

Residue on ignition \(<2.48\) Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Sarpogrelate Hydrochloride and Sarpogrelate Hydrochloride RS (separately determine the water \(<2.48\) in the same manner as Sarpogrelate Hydrochloride), add to them exactly 2.5 mL of the internal standard solution, and dissolve them with the mobile phase to make 50 mL. To 5 mL each of these solutions add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, \( Q_2 \) and \( Q_3 \), of the peak area of sarpogrelate to that of the internal standard.

\[
M_S = \frac{M_s \times Q_1}{Q_3}
\]

\( M_S \): Amount (mg) of sarpogrelate hydrochloride (\( C_{24}H_{31}NO_6 \cdot HCl \))

\( M_s \): Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (3 in 1000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 272 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of water, acetonitrile and trifluoroacetic acid (1300:700:1).
Flow rate: Adjust the flow rate so that the retention time of sarpogrelate is about 8 minutes.
System suitability—
System performance: When the procedure is run with 10 \( \mu L \) of the standard solution under the above operating conditions, sarpogrelate and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sarpogrelate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
µL of this solution is equivalent to 7 to 13% of that with 10 µL of the standard solution.

System performance: Dissolve 50 mg of sarpogrelate hydrochloride in 20 mL of water, and use this solution as the sarpogrelate hydrochloride stock solution. To 1 mL of the sarpogrelate hydrochloride stock solution add 2 mL of sodium hydroxide TS, shake thoroughly, allow to stand for 10 minutes, and add 3 mL of 1 mol/L hydrochloric acid TS. To this solution add 1 mL of the sarpogrelate hydrochloride stock solution, and add the mobile phase to make 50 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, the decomposed substance A and sarpogrelate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sarpogrelate is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: Sarpogrelate Hydrochloride Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Sarpogrelate Hydrochloride Fine Granules add exactly V/10 mL of the internal standard solution, and add 4V/5 mL of the mobile phase, disperse the particles with the aid of ultrasonic waves, then add the mobile phase to make V mL so that each mL contains about 1 mg of sarpogrelate hydrochloride (C24H31NO6.HCl), and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sarpogrelate hydrochloride (C24H31NO6.HCl)
\[ M_5 = M_S \times Q_T/Q_S \times V/50 \]

M5: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000).

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Sarpogrelate Hydrochloride Fine Granules is not less than 85%.

Start the test with an accurately weighed amount of Sarpogrelate Hydrochloride Fine Granules, equivalent to about 50 mg of sarpogrelate hydrochloride (C24H31NO6.HCl) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Sarpogrelate Hydrochloride RS (separately determine the water <2.48> in the same manner as Sarpogrelate Hydrochloride), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A3, of the sample solution and standard solution at 270 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (% with respect to the labeled amount of sarpogrelate hydrochloride (C24H31NO6.HCl)
\[ M_5/M_T \times A_1/A_3 \times 1/C \times 180 \]

M5: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

M_T: Amount (g) of sample

C: Labeled amount (mg) of sarpogrelate hydrochloride (C24H31NO6.HCl) in 1 g

Particle size <6.03> It meets the requirements of Fine granules.

Assay Powder Sarpogrelate Hydrochloride Fine Granules. Weigh accurately a portion of the powder, equivalent to about 0.25 g of sarpogrelate hydrochloride (C24H31NO6. HCl), add exactly 25 mL of the internal standard solution, add about 200 mL of the mobile phase, and disperse the particles with the aid of ultrasonic waves. To this solution add the mobile phase to make 250 mL, and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Sarpogrelate Hydrochloride RS (separately determine the water <2.48> in the same manner as Sarpogrelate Hydroxide), add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sarpogrelate to that of the internal standard.

Amount (mg) of sarpogrelate hydrochloride (C24H31NO6.HCl)
\[ M_5 = M_S \times Q_T/Q_S \times 5 \]

M5: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Sarpogrelate Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, sarpogrelate and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sarpogrelate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Sarpogrelate Hydrochloride Tablets

**Sarpogrelate Hydrochloride Tablets**

サルポグレラート塩酸塩錠

Sarpogrelate Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sarpogrelate hydrochloride (C$_{24}$H$_{31}$NO$_6$. HCl: 465.97).

**Method of preparation** Prepare as directed under Tablets, with Sarpogrelate Hydrochloride.

**Identification** Powder Sarpogrelate Hydrochloride Tablets. To a portion of the powder, equivalent to 50 mg of Sarpogrelate Hydrochloride according to the labeled amount, add 10 mL of 0.01 mol/L hydrochloric acid TS, allow to stand at room temperature for 10 minutes, then add 0.01 mol/L hydrochloric acid TS to make 100 mL, and disperse the particles with the aid of ultrasonic waves. Centrifuge this solution, and 5 mL of the supernatant liquid add 0.01 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry. It exhibits maxima between 269 nm and 273 nm, and between 274 nm and 278 nm.

**Purity** Related substances—Perform the procedure within 12 hours after preparing the sample solution. Powder Sarpogrelate Hydrochloride Tablets. To a portion of the powder, equivalent to 0.10 g of Sarpogrelate Hydrochloride according to the labeled amount, add 50 mL of mobile phase, and disperse the particles with the aid of ultrasonic waves. Filter the solution through a membrane filter with a pore size not exceeding 0.45 m. Disperse the particles with the aid of ultrasonic waves, then add the mobile phase to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry. It exhibits maxima between 269 nm and 273 nm, and between 274 nm and 278 nm.

**System suitability**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Sarpogrelate Hydrochloride.

Time span of measurement: About 2.5 times as long as the retention time of sarpogrelate, beginning after the solvent peak.

**System suitability**—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 50 mL. Confirm that the peak area of sarpogrelate obtained with 10 µL of this solution is equivalent to 7 to 13% of that with 10 µL of the standard solution.

System performance: Dissolve 50 mg of sarpogrelate hydrochloride in 20 mL of water, and use this solution as the sarpogrelate hydrochloride stock solution. To 1 mL of the stock solution add 2 mL of sodium hydroxide TS, shake thoroughly, allow to stand for 10 minutes, and add 5 mL of 0.01 mol/L hydrochloric acid TS. To this solution add 1 mL of the sarpogrelate hydrochloride stock solution, and add the mobile phase to make 50 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, the decomposed substance A and sarpogrelate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sarpogrelate is not more than 2.0%.

**Uniformity of dosage units**

Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Sarpogrelate Hydrochloride Tablets add exactly V/10 mL of the internal standard solution, and disintegrate the tablet. Add 4V/5 mL of the mobile phase, disperse the particles with the aid of ultrasonic waves, then add the mobile phase to make V mL so that each mL contains about 1 mg of sarpogrelate hydrochloride (C$_{24}$H$_{31}$NO$_6$.HCl), and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sarpogrelate hydrochloride

(C$_{24}$H$_{31}$NO$_6$.HCl) = \( M_S \times \frac{Q_S}{Q_i} \times \frac{V}{50} \)

\( M_S \) : Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000).

**Dissolution** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Sarpogrelate Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Sarpogrelate Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 55.6 µg of sarpogrelate hydrochloride (C$_{24}$H$_{31}$NO$_6$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Sarpogrelate Hydrochloride RS (separately determine the water 2.48 in the same manner as Sarpogrelate Hydrochloride), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_o \), of the sample solution and standard solution at 270 nm as directed under Ultraviolet-visible Spectrophotometry.
Scopolamine Butylbromide

ブチルスコポラミン臭化物

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₂₃H₃₄BrNO₄</td>
<td>440.37</td>
</tr>
</tbody>
</table>

Scopolamine Butylbromide, when dried, contains not less than 98.5% of C₂₃H₃₄BrNO₄.

**Description** Scopolamine Butylbromide occurs as white crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), soluble in ethanol (95), sparingly soluble in methanol, slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 140°C (with decomposition).

**Identification (1)** To 1 mg of Scopolamine Butylbromide add 3 to 4 drops of fuming nitric acid, and evaporate on a water bath to dryness. After cooling, dissolve the residue in 1 mL of N,N-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Scopolamine Butylbromide (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Scopolamine Butylbromide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Scopolamine Butylbromide (1 in 20) responds to the Qualitative Tests <1.09> for bromide.

**Optical rotation** <2.49> \([\alpha]_D^0\): -18.0 to -20.0° (after drying, 1 g, water, 10 mL, 100 mm).

**pH** <2.54> Dissolve 1.0 g of Scopolamine Butylbromide in 10 mL of water: the pH of this solution is between 5.5 and 6.5.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Scopolamine Butylbromide in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 0.5 mL of Matching Fluid F add diluted hydrochloric acid (1 in 40) to make 20 mL.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Scopolamine Butylbromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
Scopolamine Hydrobromide Hydrate

スコポラミン臭化水素酸塩水和物

\[
\text{C}_{17}\text{H}_{21}\text{NO}_4\cdot\text{HBr}\cdot3\text{H}_2\text{O}: 438.31
\]

(1S,2S,4R,5R,7S)-9-Methyl-3-oxa-9-azatricyclo-[3.3.1.0^2,6]non-7-yl (25)-3-hydroxy-2-phenylpropanoate monohydrobromide trihydrate [6533-68-2]

Scopolamine Hydrobromide Hydrate, when dried, contains not less than 98.5% of scopolamine hydrobromide (C\text{\textsubscript{17}}H\text{\textsubscript{21}}NO\textsubscript{4}•HBr: 384.26).

**Description** Scopolamine Hydrobromide Hydrate occurs as colorless or white crystals, or white granules or powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

**Identification** (1) To 1 mg of Scopolamine Hydrobromide Hydrate add 3 to 4 drops of fuming nitric acid, evaporate on a water bath to dryness, and cool. Dissolve the residue in 1 mL of N\textsubscript{2}N-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color is produced.

(2) A solution of Scopolamine Hydrobromide Hydrate (1 in 20) responds to the Qualitative Tests \(<\text{1.00}\) for bromide.

**Optical rotation** \(<\text{2.44}\): \([\alpha]_D^\circ = -24.0 - 26.0^\circ\) (after drying, 0.5 g, water, 10 mL, 100 mm).

**Melting point** \(<\text{2.60}\) 195 - 199°C (after drying; previously heat the bath to 180°C).

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Scopolamine Hydrobromide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.50 g of Scopolamine Hydrobromide Hydrate in 15 mL of water, and add 0.50 mL of 0.02 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.

(3) Apomorphine—Dissolve 0.20 g of Scopolamine Hydrobromide Hydrate in 20 mL of water, add 0.60 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color in the solution does not disappear.

(4) Related substances—Dissolve 0.15 g of Scopolamine Hydrobromide Hydrate in 3 mL of water, and use this solution as the sample solution.

(i) To 1 mL of the sample solution add 2 to 3 drops of ammonia TS: no turbidity is produced.

(ii) To 1 mL of the sample solution add 2 to 3 drops of potassium hydroxide TS: a transient white turbidity might be produced, and disappears clearly in a little while.
Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Scopolamine Hydrobromide Hydrate, previously dried, in 10 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 38.43 mg of C₁₇H₂₁NO₄.HBr

Containers and storage Containers—Tight containers.

L-Serine

L-セリン

C₃H₇NO₃: 105.09
(2S)-2-Amino-3-hydroxypropanoic acid [36-45-7]

L-Serine, when dried, contains not less than 98.5% and not more than 101.0% of C₃H₇NO₃.

Description L-Serine occurs as white crystals or a crystalline powder. It has a slight sweet taste. It is freely soluble in water and in formic acid, and practically insoluble in ethanol (99.5). It dissolves in 2 mol/L hydrochloric acid TS.

Identification Determine the infrared absorption spectrum of L-Serine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]D: +14.0 to +16.0° (After drying, 2.5 g, 2 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.50> The pH of a solution prepared by dissolving 1.0 g of L-Serine in 10 mL of water is between 5.2 and 6.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Serine in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.05>—Perform the test with 0.5 g of L-Serine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Serine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Serine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 2.0 g of L-Serine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <1.10>—Prepare the test solution with 1.0 g of L-Serine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Serine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid acid (100) (97:3) (1 in 100) on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on Ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.11 g of L-Serine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 10.51 mg of C₃H₇NO₃

Containers and storage Containers—Tight containers.

Serrapeptase

セラベプターゼ

Serrapeptase is the enzyme preparation having proteolytic activity, produced by the growth of Serratia species.

Usually, it is diluted with Lactose Hydrate. It contains not less than 2000 serratopeptase Units and not more than 2600 serratopeptase Units per mg.

It is hygroscopic.

Description Serrapeptase occurs as a grayish white to light brown powder, having a slight characteristic odor.

Identification Dissolve 0.4 g of Serrapeptase in 100 mL of acetic acid-sodium acetate buffer solution, pH 5.0, transfer exactly 1 mL of each of this solution into three tubes, and refer to them as A, B and C. To tube A add exactly 1 mL of water, to tubes B and C add exactly 1 mL of 0.04 mol/L sodium dihydrogen ethylenediamine tetraacetate TS, mix gently, and allow them to stand in a water bath at 4 ± 1°C for about 1 hour. Then, to the tube B add exactly 2 mL of 0.04 mol/L zinc chloride TS, to the tube A and C add ex-
actly 2 mL of water, mix gently, and allow them to stand in a
water bath at 4 ± 1°C for about 1 hour. Pipet 1 mL each of
these solutions, add borate-hydrochloric acid buffer solu-
tion, pH 9.0 to the solutions A and B to make exactly 200
mL, to the solution C to make exactly 50 mL, and use these
solutions as the sample solutions. Proceed with these sample
solutions as directed in the Assay: the activities of the solu-
tions A and B are almost the same, and the activity of the so-
lution C is not more than 5% of that of the solution A.

Activity of solutions A, B or C = A2/ A × 1/20 × D × 176
A2: Absorbance of the standard solution
A: Absorbance of the sample solution
20: Reaction time (minute)
D: Dilution rate (200 for solution A and B, 50 for solution
C)
176: Conversion factor (Total volume of enzyme reaction
solution/volume of filtrate taken × amount of tyro-
sine in 2 mL of tyrosine standard solution)

Purity (1) Heavy metals <1.07>—Put 1.0 g of Serrape-
tase in a porcelain crucible, add 2 drops each of sulfuric acid
and nitric acid, and incinerate by ignition. After cooling, to
the residue add 2 mL of hydrochloric acid, evaporate to dry-
ness on a water bath, add 10 mL of a solution of hydroxyla-
mine hydrochloride (3 in 100) and 2 mL of dilute acetic acid,
and heat on a water bath for 5 minutes. After cooling, filter
if necessary, wash the filter paper with 10 mL of water, put
the filtrate and washing in a Nessler tube, add water to make
50 mL, and use this solution as the test solution. Prepare the
control solution as follows: Evaporate to dryness 2 drops
each of sulfuric acid and nitric acid on a sand bath, add 2
mL of hydrochloric acid to the residue, evaporate to dryness
on a water bath, add 2.0 mL of Standard Lead Solution, 10
mL of a solution of hydroxylamine hydrochloride (3 in 100)
and 2 mL of dilute acetic acid, and heat on a water bath for
5 minutes. Proceed in the same manner as directed for the
preparation of the test solution, and add water to make 50
mL (not more than 20 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 0.40 g
of Serrapeptase according to Method 3, excepting addition
of 5 mL of a solution of magnesium nitrate hexahydrate in
ethanol (95) (3 in 10) instead of a solution of magnesium
nitrate hexahydrate in ethanol (95) (3 in 50), evaporating to
dryness on a water bath, then incinerating with a small
flame, and perform the test (not more than 5 ppm).

Loss on drying <2.4> Not more than 7.0% (1 g, 105°C,
4 hours).

Residue on ignition <2.4> Not more than 1.5% (1 g).

Assay (i) Sample solution: Dissolve exactly 0.100 g of
Serrapeptase in a solution of ammonium sulfate (1 in 20)
to make exactly 100 mL. Pipet 1 mL of this solution, add
borate-hydrochloric acid buffer solution, pH 9.0 to make
exactly 200 mL, and use this solution as the sample solution.

(ii) Tyrosine standard solution: Dissolve exactly 0.160 g
of Tyrosine RS, previously dried at 105°C for 3 hours, in 0.2
mol/L hydrochloric acid TS to make exactly 1000 mL. Pipet
10 mL of this solution, and add 0.2 mol/L hydrochloric acid
TS to make exactly 100 mL. Prepare before use.

(iii) Substrate solution: Previously determine the loss on
drying <2.4> (60°C, reduced pressure not exceeding 0.67
kPa, 3 hours) of milk casein, previously dried. To exactly
1.20 g of the milk casein, calculated based on the loss on dry-
ing, add 160 mL of a solution of sodium borate (19 in 1000),
and heat in a water bath to dissolve. After cooling, adjust
the pH to exactly 9.0 with 1 mol/L hydrochloric acid TS,
and add borate-hydrochloric acid buffer solution, pH 9.0 to
make exactly 200 mL. Use after warming to 37 ± 0.5°C.
Prepare before use.

(iv) Precipitation reagent: Trichloroacetic acid TS for
serrapeptase. Use after warming to 37 ± 0.5°C.

(v) Procedure: Pipet 1 mL of the sample solution,
in a glass-stoppered tube (15 × 130 mm), allow to stand at
37 ± 0.5°C for 5 minutes, add exactly 5 mL of the substrate
solution, and mix well immediately. Allow to stand at 37 ±
0.5°C for exactly 20 minutes, add exactly 5 mL of trichloroacetic
acid TS for serrapeptase, mix, allow to stand at 37 ± 0.5°C for
30 minutes, and filter through a dried filter paper. Pipet 2 mL of
the filtrate, add exactly 5 mL of a solution of anhydrous sodium carbonate (3 in 50), mix, add
exactly 1 mL of diluted Folin's TS (1 in 3), mix well, and
allow to stand at 37 ± 0.5°C for 30 minutes. Determine the
absorbance of this solution at 660 nm, A1, as directed under
Ultraviolet-visible Spectrophotometry <2.24> using water as
the blank. Separately, pipet 1 mL of the sample solution,
add exactly 5 mL of trichloroacetic acid TS for serrapeptase,
mix, add exactly 5 mL of the substrate solution, allow to
stand at 37 ± 0.5°C for 30 minutes, and proceed in the same
manner as directed above to determine the absorbance A2.
Separately, pipet 2 mL of the tyrosine standard solution, add
exactly 5 mL of a solution of anhydrous sodium carbonate (3
in 50), mix, add exactly 1 mL of diluted Folin's TS (1 in 3),
mix well, and proceed in the same manner as directed above
to determine the absorbance A1. Separately, pipet 2 mL of
0.2 mol/L hydrochloric acid TS, and proceed in the same
manner as directed above to determine the absorbance A4.

Serrapeptase Unit per mg of Serrapeptase
= (A1 - A2)/(A3 - A4) × 1/20 × 200 × 176
20: Reaction time (minute)
200: Dilution rate
176: Conversion factor (Total volume of enzyme reaction
solution/volume of filtrate taken × amount of tyro-
sine in 2 mL of tyrosine standard solution)

One serrapeptase Unit corresponds to the amount of ser-
rapeptase which produces 1 μg of tyrosine per minute from 5
mL of the substrate solution under the above conditions.

Containers and storage Containers—Tight containers.

Sesame Oil

Oleum Sesami

ゴマ油

Sesame Oil is the fixed oil obtained from the seeds of
Sesamum indicum Linné (Pedaliaceae).

Description Sesame Oil is a clear, pale yellow oil. It is
odorless or has a faint, characteristic odor, and has a bland
taste.
It is miscible with diethyl ether and with petroleum ether.
It is slightly soluble in ethanol (95).
Sevoflurane / Official Monographs

It congeals between 0°C and –5°C.
Congealing point of the fatty acids: 20 – 25°C

Identification To 1 mL of Sesame Oil add 0.1 g of sucrose and 10 mL of hydrochloric acid, and shake for 30 seconds: the acid layer becomes light red and changes to red on standing.

Specific gravity <1.13> δ(20): 0.914 – 0.921

Acid value <1.13> Not more than 0.2.

Saponification value <1.13> 187 – 194

Unsaponifiable matters <1.13> Not more than 2.0%.

Iodine value <1.13> 103 – 118

Containers and storage Containers—Tight containers.

Sevoflurane

セボフルラン

C₄H₃F₇O: 200.05
1,1,1,3,3,3-Hexafluoro-2-(fluoromethoxy)propane
[28523-86-6]

Sevoflurane contains not less than 99.0% and not more than 101.0% of C₄H₃F₇O, calculated on the anhydrous basis.

Description Sevoflurane is a clear, colorless, and mobile liquid.
It is miscible with ethanol (99.5).
It is very slightly soluble in water.
It is volatile and inflammable.
Refractive index nD²₀: 1.2745 – 1.2760
Boiling point: about 58.6°C.

Identification Transfer about 1 µL of Sevoflurane to a gas cell having light path 10 cm in length, and determine the infrared absorption spectrum as directed in the gas sampling method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Sevoflurane RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <2.50> δ(20): 1.510 – 1.530

Purity (1) Acidity or alkalinity—To 50 mL of Sevoflurane with 50 mL of freshly boiled and cooled water vigorously for 3 minutes. Separate the water layer and use this solution as the sample solution. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide VS: a red-purple color develops. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.6 mL of 0.01 mol/L hydrochloric acid VS: a yellow color is produced.

(2) Soluble fluoride—To 6 g of Sevoflurane add 12 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), and shake for 10 minutes. Transfer 4.0 mL of diluted 0.01 mol/L sodium hydroxide solution (1 in 20) layer into a Nessler tube. Add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, allow to stand for 60 minutes, and use this solution as the sample solution. Separately, transfer 0.2 mL of the fluorine standard solution and 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) into a Nessler tube, and add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1). Proceed in the same manner as directed for the preparation of the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in the same manner, as the blank: the absorbance of the sample solution is not more than that of the standard solution (not more than 1 ppm).

Fluorine standard solution: Dissolve exactly 2.21 g of sodium fluoride in water to make exactly 1000 mL. Pipet 10 mL of this solution and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of fluorine (F).

(3) Related substances—Perform the test with 2 µL of Sevoflurane as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method and calculate the amount of them by the area percentage method: the amount of the peak of hexafluoroisopropyl methyl ether, having the relative retention time of about 0.84 with respect to sevoflurane, is not more than 0.005%, the amount of each peak other than the peaks of sevoflurane and hexafluoroisopropyl methyl ether is not more than 0.0025%, and the total amount of the peaks other than the peaks of sevoflurane and hexafluoroisopropyl methyl ether is not more than 0.005%.

Operating conditions—
Detector, column, injection port temperature, detector temperature, carrier gas and split ratio: Proceed as directed in the operating conditions in the Assay.
Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 10 minutes, raise at a rate of 10°C per minute to 200°C, and maintain at a constant temperature of about 200°C.
Flow rate: Adjust the flow rate so that the retention time of sevoflurane is about 7 minutes.
Time span of measurement: About 6 times as long as the retention time of sevoflurane.

System suitability—
Test for required detectability: To 20 µL of Sevoflurane add o-xylene to make 20 mL. To 1 mL of this solution add o-xylene to make 20 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test and add o-xylene to make exactly 10 mL. Confirm that the peak area of sevoflurane obtained from 2 µL of this solution is equivalent to 7 to 13% of the peak area of sevoflurane from 2 µL of the solution for system suitability test.
System performance: When the procedure is run with 2 µL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of sevoflurane are not less than 6000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 2 µL of the solution for system suitability test under the
above operating conditions, the relative standard deviation of the peak area of Sevoflurane is not more than 5.0%.

(4) Residual solvent—Being specified separately.

(5) Residue on evaporation—Evaporate 10 mL of Sevoflurane, exactly measured, on a water bath to dryness, and dry at 105°C for 2 hours: the mass of the residue is not more than 1.0 mg.

Water <2.48> Not more than 0.2 w/v% (5 mL, volumetric titration, direct titration).

Assay Pipet 5 mL each of Sevoflurane and Sevoflurane RS (separately determine the water <2.48> using the same manner as Sevoflurane), to each add exactly 5 mL of dimethoxymethane as an internal standard, and use these solutions as the sample solution and the standard solution, respectively.

Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Qs and Qr, of the peak area of sevoflurane to that of the internal standard.

Amount (mg) of sevoflurane (C₄H₃F₇O)

\[ V_s = \frac{Q_r \times Q_s \times 1000}{V_s \times \frac{1}{5}} \times 1.521 \]

\( V_s \): Amount (mL) of Sevoflurane RS, calculated on the anhydrous basis

1.521: Specific gravity of Sevoflurane (d₂₀)

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated inside with cyanopropyl methylphenyl silicone for gas chromatography in 1.8 μm thickness.

Column temperature: 40°C.

Injection port temperature: A constant temperature of about 200°C.

Detector temperature: A constant temperature of about 225°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of Sevoflurane is about 3 minutes.


System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, sevoflurane and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sevoflurane to that of the internal standard is not more than 1.0 %.

Containers and storage Containers—Tight containers.

Purified Shellac

Purified Shellac is a resin-like substance obtained from a purified secretion of Laccifer laca Kerr (Coccidae).

Description Purified Shellac occurs as light yellow-brown to brown, lustrous, hard, brittle scutella. It has no odor or has a faint, characteristic odor.

It is freely soluble in ethanol (95) and in ethanol (99.5), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Acid value <1.13> 60 – 80 Weigh accurately about 1 g of Purified Shellac, add 40 mL of neutralized ethanol, and dissolve by warming. After cooling, titrate <2.50> with 0.1 mol/L potassium hydroxide VS (potentiometric titration).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Purified Shellac according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Purified Shellac according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 5 ppm).

(3) Ethanol-insoluble substances—Dissolve about 5 g of Purified Shellac, accurately weighed, in 50 mL of ethanol (95) on a water bath while shaking. Pour the ethanol solution into a tared extraction thimble, previously dried at 105°C for 2 hours, in a Soxhlet extractor, and extract with ethanol (95) for 3 hours. Dry the extraction thimble at 105°C for 3 hours: the mass of the residue is not more than 2.0%.

Use a cylindrical weighing bottle for taring the extraction thimble.

(4) Rosin—Dissolve 2.0 g of Purified Shellac in 10 mL of ethanol (99.5) with thorough shaking, add gradually 50 mL of petroleum ether while shaking, and filter, if necessary. Wash the solution with two 50-mL portions of water, filter the upper layer, and evaporate the filtrate on a water bath to dryness. Decompose the residue in 2 mL of a mixture of carbon tetrachloride and phenol (2:1), transfer the solution to a depression of a spot plate, and fill the neighboring depression with a mixture of carbon tetrachloride and bromine (4:1). Immediately cover both depressions with a watch glass, and allow to stand: the solution of the residue exhibits no purple or blue color within 1 minute.

(5) Wax—Dissolve 10.0 g of Purified Shellac in 150 mL of a solution of sodium carbonate decahydrate (9 in 200) with shaking on a water bath, and continue the heating for 2 hours. After cooling, collect the floating wax by filtration, wash the wax and the filter paper with water, transfer to a beaker, and dry at 65°C until the water is almost evaporated. Transfer the wax together with the filter paper to an extraction thimble in a Soxhlet extractor. Dissolve the wax remaining in the beaker with a suitable quantity of chloroform by warming. Pour the solution into the thimble, and extract with chloroform for 2 hours. Evaporate the chloroform solution to dryness, ad dry the residue at 105°C for 3 hours: the mass of the residue is not more than 20 mg.
White Shellac

White Shellac is a resin-like substance obtained from a bleached secretion of Laccifer lacca Kerr (Coccidae).

**Description** White Shellac occurs as yellowish white to light yellow, hard, brittle granules. It is odorless or has a faint, characteristic odor.

It is sparingly soluble in ethanol (95), very slightly soluble in petroleum ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

**Acid value** <1.13> 65 – 90 Weigh accurately about 0.5 g of White Shellac, add 50 mL of neutralized ethanol as a solvent, and dissolve by warming. After cooling, perform the test as directed in the Acid value under Purified Shellac.

**Purity** (1) Chloride <1.07>—Shake and dissolve 0.40 g of White Shellac in 5 mL of ethanol (95) while warming, add 40 mL of water, and cool. Add 12 mL of dilute nitric acid and water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution as follows: to 0.80 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.140%).

(2) Sulfate <1.14>—Shake and dissolve 0.40 g of White Shellac in 5 mL of ethanol (95) by warming, add 40 mL of water, and cool. Add 2 mL of dilute hydrochloric acid and water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.005 mol/L sulfuric acid VS add 2.5 mL of ethanol (95), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.110%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of White Shellac according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.13>—Prepare the test solution with 0.40 g of White Shellac according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 5 ppm).

(5) Ethanol-insoluble substances—Dissolve about 5 g of White Shellac, accurately weighed, in 50 mL of ethanol (95) on a water bath while shaking. Pour the ethanol solution into a tared extraction thimble, previously dried at 105°C for 2 hours, in a Soxhlet extractor, and extract with ethanol (95) for 3 hours. Dry the extraction thimble at 105°C for 3 hours: the mass of the residue is not more than 20 mg.

(6) Rosin—Dissolve 2.0 g of White Shellac in 10 mL of ethanol (99.5) with thorough shaking, add gradually 50 mL of petroleum ether while shaking, and filter, if necessary. Wash the solution with two 50-mL portions of water, filter the upper layer, and evaporate the filtrate on a water bath to dryness. Dissolve the residue in 2 mL of a mixture of carbon tetrachloride and phenol (2:1), transfer the solution to a depression of a spot plate, and fill the neighboring depression with a mixture of carbon tetrachloride and bromine (4:1). Immediately cover both depressions with a watch glass, and allow to stand: the solution of the residue exhibits no purple or blue color within 1 minute.

(7) Wax—Dissolve 10.0 g of White Shellac in 150 mL of a solution of sodium carbonate decahydrate (9 in 200) with shaking on a water bath, and continue the heating for 2 hours. After cooling, collect the floating wax by filtration, wash the wax and the filter paper with water, transfer to a beaker, and dry at 65°C until the water is almost evaporated. Transfer the wax together with the filter paper to an extraction thimble in a Soxhlet extractor. Dissolve the wax remaining in the beaker with a suitable quantity of chloroform by warming. Pour the solution into the thimble, and extract with chloroform for 2 hours. Evaporate the chloroform solution to dryness, and dry the residue at 105°C for 3 hours: the mass of the residue is not more than 20 mg.

**Loss on drying** Not more than 2.0%. Weigh accurately about 1 g of medium powder of Purified Shellac, and dry at 40°C for 4 hours, then for 15 hours in a desiccator (calcium chloride for drying).

**Total ash** <5.01> Not more than 1.0% (1 g).

**Containers and storage** Containers—Well-closed containers.

Storage—In a cold place.

Siccanin

Siccanin is a substance having antifungal activity produced by the growth of Helminthosporium siccans.

It contains not less than 980 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Siccanin is expressed as mass (potency) of siccanin (C_{22}H_{30}O_{3}).

**Description** Siccanin occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in acetone, soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a
solution of Siccanin in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Siccanin RS obtained in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Siccanin as directed in the potassium bromide disk method under similar intensities of absorption at the same wavelengths.

and compare the spectrum with the Reference Spectrum or the spectrum of Siccanin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \[<2.49> \quad \alpha_{D}^{20} = -165 - 175^\circ (0.1 \text{ g, ethanol (99.5), 10 mL, 100 mm)} \]

**Melting point** \[<2.60> \quad 138 - 142^\circ \text{C} \]

**Purity** (1) Heavy metals \[<1.07> \]—Proceed with 1.0 g of Siccanin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.20 g of Siccanin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-chlorobenzene dihydrogenphosphate tetrahydrate on a platinum loop. Bring the hot, transparent bead into contact with Light Anhydrous Silicic Acid RS, and compare the spots with the Reference Spectrum of Siccanin RS obtained in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Residue on ignition** \[<2.44> \quad \text{Not more than 0.5\% (1 g, reduced pressure not exceeding 0.67 kPa, 80^\circ \text{C, 3 hours)} } \]

**Assay** Weigh accurately an amount of Siccanin and Siccanin RS, equivalent to about 50 mg (potency), dissolve each in dilute hydrochloric acid.

Prepare the test solution with 10 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Liquid Chromatography. \[<2.01> \quad Q = \frac{D}{S} \times 1000 \]

D = Amount [\( \mu \text{g (potency)} \)] of \( \text{C}_{22}\text{H}_{30}\text{O}_{3} \)

\( S \) = Amount [\( g \) (potency)] of Siccanin RS

\( Q \) = Amount [\( g \) (potency)] of Siccanin RS

**Internal standard solution**—A solution of 1,4-diphenylbenzene in methanol (1 in 30,000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \text{m} \) in particle diameter)

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and phosphate buffer solution, pH 5.9 (19:6).

Flow rate: Adjust the flow rate so that the retention time of siccanin is about 17 minutes.

**System suitability**—

System performance: When the procedure is run with 10 \( \mu \text{L} \) of the standard solution under the above operating conditions, siccanin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of siccanin to that of the internal standard is not more than 1.0\%.

**Containers and storage** Containers—Tight containers.

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**Light Anhydrous Silicic Acid**

**Light Anhydrous Silicic Acid**

Light Anhydrous Silicic Acid, calculated on the in-vicinerated basis, contains not less than 98.0\% of silicon dioxide (SiO\(_2\) : 60.08).

**Description** Light Anhydrous Silicic Acid occurs as a white to bluish white, light, fine powder. It is odorless and tasteless, and smooth to the touch.

It is practically insoluble in water, in ethanol (95), and in diethyl ether.

It dissolves in hydrofluoric acid, in hot potassium hydroxide TS and in hot sodium hydroxide TS, and does not dissolve in dilute hydrochloric acid.

**Identification** (1) Dissolve 0.1 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, and add 12 mL of ammonium chloride TS: a white, gelatinous precipitate is produced. The precipitate does not dissolve in dilute hydrochloric acid.

(2) To the precipitate obtained in (1) add 10 mL of a solution of methylene blue trihydrate (1 in 10,000), and wash with water: the precipitate has a blue color.

(3) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Bring the hot, transparent bead into contact with Light Anhydrous Silicic Acid, and fuse again: an insoluble matter is perceptible in the bead. The resulting bead, upon cooling, becomes opaque and acquires a reticulated appearance.

**Purity** (1) Chloride \[<1.03> \]—Dissolve 0.5 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, cool,filter if necessary, and wash with 10 mL of water. Combine the filtrate and washings, add 18 mL of dilute nitric acid, shake, and add water to make 50 mL. Perform the test using this solution as the test solution. To 0.15 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of sodium hydroxide TS, 18 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution (not more than 0.011\%).

(2) Heavy metals \[<1.07> \]—Dissolve 0.5 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, cool, add 15 mL of acetic acid (31), shake, filter, if nec-
loss on ignition and 120°C.

(3) Iron

To 40 mg of Light Anhydrous Silicic Acid add 10 mL of dilute hydrochloric acid, and heat for 10 minutes in a water bath while shaking. After cooling, add 0.5 g of L-tartaric acid to dissolve by shaking. Prepare the test solution with this solution according to Method 2, and perform the test according to Method B. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(4) Aluminum—Dissolve 0.5 g of Light Anhydrous Silicic Acid in 40 mL of sodium hydroxide TS by boiling, cool, add sodium hydroxide TS to make 50 mL, and filter. Measure 10 mL of the filtrate, add 17 mL of acetic acid (31), shake, add 2 mL of alumon TS and water to make 50 mL, and allow to stand for 30 minutes: the color of this solution is not deeper than that of the following control solution.

Control solution: Dissolve 0.176 g of aluminum potassium sulfate dodecahydrate in water, and add make 1000 mL. To 15.5 mL of this solution add 10 mL of sodium hydroxide TS, 17 mL of acetic acid (31), 2 mL of alumon TS and water to make 50 mL.

(5) Calcium—Dissolve 1.0 g of Light Anhydrous Silicic Acid in 30 mL of sodium hydroxide TS by boiling, cool, add 20 mL of water, 1 drop of phenolphthalein TS and dilute nitric acid until the color of this solution disappears, immediately add 5 mL of dilute acetic acid, shake, add water to make 100 mL, and obtain a clear liquid by centrifugation or filtration. To 25 mL of this liquid add 1 mL of oxalic acid TS and ethanol (95) to make 50 mL, immediately shake, and allow to stand for 10 minutes: the turbidity of this solution is not deeper than that of the following control solution.

Control solution: Dissolve 0.250 g of calcium carbonate, previously dried at 180°C for 4 hours, in 3 mL of dilute hydrochloric acid, and add water to make 100 mL. To 4 mL of this solution add 5 mL of dilute acetic acid and water to make 50 mL. To 25 mL of this solution add 1 mL of oxalic acid TS and ethanol (95) to make 50 mL, and shake.

(6) Arsenic

Dissolve 0.40 g of Light Anhydrous Silicic Acid in 10 mL of sodium hydroxide TS by boiling in a porcelain crucible, cool, add 5 mL of water and 5 mL of dilute hydrochloric acid, shake, and perform the test with this solution as the test solution (not more than 5 ppm).

Loss on drying

Not more than 7.0% (1 g, 105°C, 4 hours).

Loss on ignition

Not more than 12.0% (1 g, 850 – 900°C, constant mass).

Volume test

Weigh 5.0 g of Light Anhydrous Silicic Acid, transfer gradually to a 200-mL measuring cylinder, and allow to stand: the volume is not less than 70 mL.

Assay

Weigh accurately about 1 g of Light Anhydrous Silicic Acid, add 20 mL of hydrochloric acid, and evaporate to dryness on a sand bath. Moisten the residue with hydrochloric acid, evaporate to dryness, and heat between 110°C and 120°C for 2 hours. Cool, add 5 mL of dilute hydrochloric acid, and heat. Allow to cool to room temperature, add 20 to 25 mL of hot water, filter rapidly, and wash the residue with warm water until the last washing becomes negative to the Qualitative Tests (1.09) (2) for chloride. Transfer the residue together with the filter paper to a platinum crucible, ignite to ash, and continue the ignition for 30 minutes. Cool, weigh the crucible, and designate the mass as a (g). Moisten the residue in the crucible with water, add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid, and evaporate to dryness. Heat strongly for 5 minutes, cool, weigh the crucible, and designate the mass as b (g).

\[ \text{Content (g) of silicon dioxide (SiO}_2\text{) } = a - b \]

Containers and storage

Containers—Tight containers.

Silver Nitrate

硝酸銀

AgNO₃: 169.87

Silver Nitrate, when dried, contains not less than 99.8% of AgNO₃.

Description

Silver Nitrate occurs as lustrous, colorless or white crystals.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

It gradually turns grayish black by light.

Identification

A solution of Silver Nitrate (1 in 50) responds to the Qualitative Tests (1.09) for silver salt and for nitrate.

Purity

(1) Clarity and color of solution, and acidity or alkalinity—Dissolve 1.0 g of Silver Nitrate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless. It is neutral.

(2) Bismuth, copper and lead—To 5 mL of a solution of Silver Nitrate (1 in 10) add 3 mL of ammonia TS: the solution is clear and colorless.

Loss on drying

Not more than 0.20% (2 g, silica gel, light resistant, 4 hours).

Assay

Weigh accurately about 0.7 g of Silver Nitrate, previously powdered and dried, dissolve in 50 mL of water, add 2 mL of nitric acid, and titrate (2.50) with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS = 16.99 mg of AgNO₃

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.
Silver Nitrate Ophthalmic Solution
硝酸銀点眼液

Silver Nitrate Ophthalmic Solution is an aqueous eye lotion.
It contains not less than 0.95 w/v% and not more than 1.05 w/v% of silver nitrate (AgNO₃: 169.87).

Method of preparation
Silver Nitrate 10 g
Purified Water or Purified Water in Containers a sufficient quantity
To make 1000 mL

Prepare as directed under Ophthalmic Preparations, with the above ingredients.

Description Silver Nitrate Ophthalmic Solution is a clear, colorless liquid.

Identification Silver Nitrate Ophthalmic Solution responds to the Qualitative Tests <1.09> for silver salt and for nitrate.

Assay Measure accurately 20 mL of Silver Nitrate Ophthalmic Solution, add 30 mL of water and 2 mL of nitric acid, and titrate <2.50> with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS = 16.99 mg of AgNO₃

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Silver Protein
プロテイン銀

Silver Protein is a compound of silver and proteins. It contains not less than 7.5% and not more than 8.5% of silver (Ag: 107.87).

Description Silver Protein occurs as a light yellow-brown to brown powder. It is odorless. It (1 g) dissolves slowly in 2 mL of water. It is practically insoluble in ethanol (95), in diethyl ether and in chloroform. The pH of a solution of Silver Protein (1 in 10) is between 7.0 and 8.5. It is slightly hygroscopic. It is affected by light.

Identification (1) To 1 mL of a solution of Silver Protein (1 in 100) add 2 mL of dilute hydrochloric acid, shake frequently for 5 minutes, and filter. To the filtrate add 5 mL of a solution of sodium hydroxide (1 in 10), and titrate <2.50> with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS = 10.79 mg of Ag.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Silver Protein Solution
プロテイン銀液

Silver Protein Solution contains not less than 0.22 w/v% and not more than 0.26 w/v% of silver (Ag: 107.87).

Method of preparation
Silver Protein 30 g
Glycerin 100 mL
Mentha Water a sufficient quantity
To make 1000 mL

Dissolve and mix the above ingredients.

Description Silver Protein Solution is a clear, brown liquid, having the odor of mentha oil.

Identification (1) To 1 mL of Silver Protein Solution add 10 mL of ethanol (95), mix, and add 2 mL of sodium hydroxide TS. Add immediately 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), shake, and filter: the filtrate is blue in color (glycerin).
(2) To 3 mL of Silver Protein Solution add water to make 10 mL, add 2 mL of dilute hydrochloric acid, shake frequently for 5 minutes, and filter. Add 5 mL of a solution of sodium hydroxide (1 in 10) to the filtrate, and add 2 mL of diluted copper (II) sulfate TS (2 in 25): a purple color develops (silver protein).
(3) To 5 mL of the sample solution obtained in (2) add iron (III) chloride TS dropwise: a brown precipitate is formed (silver protein).
(4) Place 3 mL of Silver Protein Solution in a crucible, heat cautiously, and evaporate almost to dryness. Then incinerate gradually by strong heating, dissolve the residue in 1 mL of nitric acid by warming, and add 10 mL of water: this solution responds to the Qualitative Tests <1.09> (1) for silver salt.
Assay  Pipet 25 mL of Silver Protein Solution into a 250-mL Kjeldahl flask, and heat cautiously until a white gas of glycerin is evolved. After cooling, add 25 mL of sulfuric acid, cover the flask with a funnel, and heat gently for 5 minutes. After cooling, drop gradually 5 mL of nitric acid, heat with occasional shaking in a water bath for 45 minutes, and cool. Add 2 mL of nitric acid, boil gently, and repeat this operation until the solution becomes colorless upon cooling. Transfer cautiously the cooled content in the flask into a 500-mL conical flask with 250 mL of water. Boil gently for 5 minutes, cool, and titrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 3 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS = 10.79 mg of Ag

Containers and storage  Containers—Tight containers.

Simple Syrup

Single Syrup

Simple Syrup is an aqueous solution of Sucrose.

Method of preparation  

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow Beeswax</td>
<td>330 g</td>
</tr>
<tr>
<td>Fixed oil</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td>Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Syrups, with the above materials.

Description  Simple Syrup is a clear, colorless to pale yellow, viscous liquid. It is odorless and has a sweet taste.

Identification (1)  Evaporate Simple Syrup on a water bath to dryness. 1 g of the residue so obtained, when ignited, melts to swell, and decomposes, emitting an odor of caramel, to bulky charcoal.

(2)  To 0.1 g of the residue obtained in (1) add 2 mL of dilute sulfuric acid, boil, add 4 mL of sodium hydroxide TS and 3 mL of Fehling’s TS, and heat to boiling: a red to dark red precipitate is produced.

Specific gravity <2.60  d$_{20}^0$: 1.310 – 1.325

Purity (1)  Artificial sweetening agents—To 100 mL of Simple Syrup add 100 mL of water, shake, acidify a 50-mL portion of the solution with dilute sulfuric acid, and make another 50-mL portion alkaline with sodium hydroxide TS. To each portion add 100 mL of diethyl ether, shake, separate the diethyl ether layer, and evaporate the combined diethyl ether extract on a water bath to dryness: the residue has no sweet taste.

(2)  Salicylic acid—To the residue obtained in (1) add 2 to 3 drops of dilute iron (III) chloride TS: no purple color develops.

Containers and storage  Containers—Tight containers.

Simvastatin

C$_{25}$H$_{38}$O$_{5}$ : 418.57 
(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-Hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate [79902-63-9]

Simvastatin contains not less than 98.0% and not more than 101.0% of C$_{25}$H$_{38}$O$_{5}$, calculated on the dried basis.

It may contain a suitable antioxidant.

Description  Simvastatin occurs as a white, crystalline powder.

It is freely soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1)  Determine the absorption spectrum of a solution of Simvastatin in acetonitrile (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Simvastatin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)  Determine the infrared absorption spectrum of Simvastatin as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Simvastatin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49  [α]$_D^0$: +285 – +300° (50 mg calculated on the dried basis, acetonitrile, 10 mL, 100 mm).

Purity (1)  Clarity and color of solution—Dissolve 1 g of
Simvastatin in 10 mL of methanol: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry (2.24). The absorbance at 440 nm is not more than 0.10.

(2) Heavy metals <2.07>—To 1.0 g of Simvastatin add 2 mL of sulfuric acid, and heat gently to carbonize. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat gently until the white fumes no more evolve, and heat to incinerate at 500 to 600°C. If the incineration is not accomplished, add 0.5 mL of nitric acid, heat in the same manner as above, and ignite at 500 to 600°C to incinerate completely. After cooling, add 2 mL of hydrochloric acid, proceed with this solution according to Method 2, and perform the test. Prepare the control solution by using the same quantities of the same reagents as directed for the preparation of the test solution, and add 2.0 mL of Standard Lead solution and water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 30 mg of Simvastatin in 20 mL of a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), and use this solution as the sample solution. Perform the test with 5 mL of the sample solution as directed under Liquid Chromatography (2.24) according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of each peak by using the area percentage method: the amounts of the peaks, having the relative retention times of about 0.45, about 0.80, about 2.42, and about 3.80 with respect to simvastatin are not more than 0.2%, respectively; the amount of the peak with a relative retention time of about 2.38 is not more than 0.3%; the amount of the peak with a relative retention time of about 0.60 is not more than 0.4%; and the amount of each peak other than simvastatin and other than the peaks mentioned above is not more than 0.1%. Furthermore, the total amount of the peaks other than simvastatin and other than the peak with relative retention time of about 0.60 with respect to simvastatin is not more than 1.0%.

Operating conditions—
Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.
Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (1:1). Mobile phase B: A solution of phosphoric acid in acetonitrile for liquid chromatography (1 in 1000).
Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 4.5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4.5 – 4.6</td>
<td>100 → 95</td>
<td>0 → 5</td>
</tr>
<tr>
<td>4.6 – 8.0</td>
<td>95 → 25</td>
<td>5 → 75</td>
</tr>
<tr>
<td>8.0 – 11.5</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>

Flow rate: 3.0 mL per minute.
Time span of measurement: About 5 times as long as the retention time of simvastatin.

System suitability—
Test for required detectability: To 0.5 mL of the sample solution, add a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), to make 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, add a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), to make exactly 10 mL. Confirm that the peak area of simvastatin obtained from 5 µL of this solution is equivalent to 16 to 24% of the peak area of simvastatin from 5 µL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 µL of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of simvastatin is not more than 1.0%.

(4) Residual solvent—Be specified separately.

Loss on drying <2.41>—Not more than 0.5% (1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44>—Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Simvastatin and Simvastatin RS (previously determine the loss on drying <2.41> under the same conditions as Simvastatin), dissolve each in a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography (2.07) according to the following conditions, and determine the peak areas, A_T and A_S, of simvastatin for each solution.

Amount (mg) of simvastatin (C_{25}H_{38}O_{5}) = M_S × A_T/A_S

M_S: Amount (mg) of Simvastatin RS, calculated on the dried basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 238 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 33 mm in length, packed with octadecylsilylized silica gel for liquid chromatography (3 µm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (1:1).
Flow rate: Adjust the flow rate so that the retention time of simvastatin is about 3 minutes.

System suitability—
System performance: Dissolve 3 mg of lovastatin in 2 mL of the standard solution. When the procedure is run with 5 µL of this solution under the above operating conditions, lovastatin and simvastatin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of simvastatin is not more than 1.0%.

Containers and storage—Containers—Tight containers.
Storage—Under nitrogen atmosphere.
Freeze-dried Smallpox Vaccine

Freeze-dried Smallpox Vaccine is a preparation for injection which is dissolved before use. It contains live vaccinia virus.

It conforms to the requirements of Freeze-dried Smallpox Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Smallpox Vaccine becomes a white to gray, turbid liquid on addition of solvent.

Freeze-dried Smallpox Vaccine Prepared in Cell Culture

Freeze-dried Smallpox Vaccine Prepared in Cell Culture is a preparation for injection which is dissolved before use. It contains live vaccinia virus.

It conforms to the requirements of Freeze-dried Smallpox Vaccine Prepared in Cell Culture in the Minimum Requirements for Biological Products.

Description Freeze-dried Smallpox Vaccine Prepared in Cell Culture becomes a reddish clear liquid on addition of solvent.

Sodium Acetate Hydrate

Sodium Acetate Hydrate, when dried, contains not less than 99.5% of sodium acetate (C₂H₃NaO₂: 82.03).

Description Sodium Acetate Hydrate occurs as colorless crystals or a white, crystalline powder. It is odorless or has a slight, acetic odor. It has a cool, saline and slightly bitter taste.

It is very soluble in water, freely soluble in acetic acid (100), soluble in ethanol (95), and practically insoluble in diethyl ether.

It is efflorescent in warm, dry air.

Identification A solution of Sodium Acetate Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for acetate and for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 2.0 g of Sodium Acetate Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 1.0 g of Sodium Acetate Hydrate in 20 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS: a red color develops. When cooled to 10°C, or 1.0 mL of 0.01 mol/L hydrochloric acid VS is added after cooling to 10°C, the red color disappears.

(3) Chloride <1.03>—Perform the test with 1.0 g of Sodium Acetate Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(4) Sulfate <1.14>—Perform the test with 1.0 g of Sodium Acetate Hydrate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.017%).

(5) Heavy metals <1.07>—Proceed with 2.0 g of Sodium Acetate Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Calcium and magnesium—Dissolve 4.0 g of Sodium Acetate Hydrate in 25 mL of water, add 6 g of ammonium chloride, 20 mL of ammonia solution (28) and 0.25 mL of a solution of sodium sulfate heptahydrate (1 in 10), and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the blue color changes to grayish blue (indicator: 0.1 g of methylythymol blue-potassium nitrate indicator): the amount of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed is not more than 0.5 mL.

(7) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sodium Acetate Hydrate, according to Method 1, and perform the test (not more than 2 ppm).

(8) Potassium permanganate-reducing substance—Dissolve 1.0 g of Sodium Acetate Hydrate in 100 mL of water, add 5 mL of dilute sulfuric acid, boil, add 0.50 mL of 0.002 mol/L potassium permanganate VS, and further boil for 5 minutes: the red color of the solution does not disappear.

Loss on drying <2.41> 39.0 - 40.5% (1 g, first at 80°C for 2 hours, and then at 130°C for 2 hours).

Assay Weigh accurately about 0.2 g of Sodium Acetate Hydrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from yellow to green (indicator: 1 mL of p-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 8.203 mg of C₂H₃NaO₂

Containers and storage Containers—Tight containers.
Sodium Aurothiomalate

金チオリン酸ナトリウム

\[
\text{C}_4\text{H}_4\text{AuNa}_2\text{O}_4\text{S} : 390.08
\]

Mixture of \( \text{C}_4\text{H}_3\text{AuNa}_2\text{O}_4\text{S} : 390.08 \) and \( \text{C}_4\text{H}_4\text{AuNa}_2\text{O}_4\text{S} : 368.09 \)

Monogold monosodium monohydrogen (2RS)-2-sulfidobutane-1,4-dioate

Monogold disodium (2RS)-2-sulfidobutane-1,4-dioate [12244-57-4, Sodium Aurothiomalate]

Sodium Aurothiomalate contains not less than 49.0\% and not more than 52.5\% of gold (Au: 196.97), calculated on the anhydrous basis and corrected by the amount of ethanol.

**Description** Sodium Aurothiomalate occurs as white to light yellow, powder or granules.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

It changes in color by light to greenish pale yellow.

**Identification** (1) To 2 mL of a solution of Sodium Aurothiomalate (1 in 10) add 1 mL of a solution of calcium nitrate tetrahydrate (1 in 10): a white precipitate is produced, and it dissolves in dilute nitric acid and reappears on the addition of ammonium acetate TS.

(2) To 2 mL of a solution of Sodium Aurothiomalate (1 in 10) add 3 mL of silver nitrate TS: a yellow precipitate is produced, and it dissolves in an excess of ammonia TS.

(3) Place 2 mL of a solution of Sodium Aurothiomalate (1 in 10) in a porcelain crucible, add 1 mL of ammonia TS and 1 mL of hydrogen peroxide (30), evaporate to dryness, and ignite. Add 20 mL of water to the residue, and filter: the residue on the filter paper occurs as a yellow or dark yellow, and ignite. Add 20 mL of water to the residue, and filter: the amount of ethanol is not more than 2 ppm.

(4) To 2 mL of a solution of Sodium Aurothiomalate (1 in 10) add 1 mL of a solution of calcium nitrate tetrahydrate (1 in 10): a white precipitate is produced, and it dissolves in dilute nitric acid and reappears on the addition of ammonium acetate TS.

(5) To 2 mL of a solution of Sodium Aurothiomalate (1 in 10) add 3 mL of silver nitrate TS: a yellow precipitate is produced, and it dissolves in an excess of ammonia TS.

**Operating conditions**—

Detector: Hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (particle diameter: 150 – 180 \( \mu \)m) (average pore size: 0.0085 \( \mu \)m; 300 – 400 \( m^2/g \)).

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 7 minutes.

**System suitability**—

System performance: When the procedure is run with 2 \( \mu \)L of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 2 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethanol to that of the internal standard is not more than 2.0\%.

**Water** 2.49 Not more than 5.0\% (0.1 g, coulometric titration). Use a water vaporizer (heating temperature: 105\°C; heating time: 30 minutes).

**Assay** Weigh accurately about 25 mg of Sodium Aurothiomalate, and dissolve in 2 mL of aqua regia by heating. After cooling, add water to make exactly 100 mL. Pipet 2 mL of the solution, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, pipet 5 mL, 10 mL and 15 mL of Standard Gold Solution for atomic absorption spectrophotometry, add water to make exactly 25 mL, and use these solutions as the standard solutions (1), (2) and (3), respectively. Perform the test with the sample solution and standard solutions (1), (2) and (3) as directed under Atomic Absorption Spectrophotometry 2.22 under the following conditions. Determine the amount of gold in the sample solution using the calibration curve obtained from the absorbances of the standard solutions.

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: Gold hollow-cathode lamp.

Wavelength: 242.8 nm.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.
Sodium Benzoate

**Description** Sodium Benzoate occurs as white granules, crystals or crystalline powder. It is odorless, and has a sweet and saline taste.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

**Identification** A solution of Sodium Benzoate (1 in 100) responds to the Qualitative Tests $<1.09>$ for benzoate and the Qualitative Tests $<1.09>$ (1) and (2) for sodium salt.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Benzoate in 5 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 2.0 g of Sodium Benzoate in 20 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid VS: the solution remains colorless. To this solution add 0.40 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Sulfate $<1.14>$—Dissolve 0.40 g of Sodium Benzoate in 40 mL of water, add slowly 3.5 mL of dilute hydrochloric acid with thorough stirring, allow to stand for 5 minutes, and filter. Discard the first 5 mL of the filtrate, take the subsequent 20 mL of the filtrate, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.120%).

(4) Heavy metals $<1.07>$—Dissolve 2.0 g of Sodium Benzoate in 44 mL of water, add gradually 6 mL of dilute hydrochloric acid with thorough stirring, and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, neutralize with ammonia TS, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(5) Arsenic $<1.17>$—Mix well 1.0 g of Sodium Benzoate with 0.40 g of calcium hydroxide, ignite, dissolve the residue in 10 mL of dilute hydrochloric acid, and perform the test using this solution as the test solution (not more than 2 ppm).

(6) Chlorinated compounds—Dissolve 1.0 g of Sodium Benzoate in 10 mL of water, add 10 mL of dilute sulfuric acid, and extract with two 20-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate the diethyl ether on a water bath. Place 0.5 g of the residue and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water, and dry. Ignite it at about 600°C, dissolve in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washing, add water to make 50 mL, and add 0.5 mL of silver nitrate TS: this solution has no more turbidity than the following control solution.

Control solution: Dissolve 0.7 g of calcium carbonate in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, add 1.2 mL of 0.01 mol/L Hydrochloric acid VS and water to make 50 mL, and add 0.5 mL of silver nitrate TS.

(7) Phthalic acid—To 0.10 g of Sodium Benzoate add 1 mL of water and 1 mL of resorcinol-sulfuric acid TS, and heat the mixture in an oil bath heated at a temperature between 120°C and 125°C to evaporate the water, then heat the residue for further 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of the solution add 10 mL of a solution of sodium hydroxide (43 in 500), shake, then examine under light at a wavelength between 470 nm and 490 nm: the green fluorescence of the solution is not more intense than that of the following control solution.

Control solution: Dissolve 61 mg of potassium hydrogen phthalate in water to make exactly 1000 mL. Pipet exactly 1 mL of the solution, add 1 mL of resorcinol-sulfuric acid TS, and proceed as directed above.

**Loss on drying** $<2.4>/$ Not more than 1.5% (2 g, 110°C, 4 hours).

**Assay** Weigh accurately about 1.5 g of Sodium Benzoate, previously dried, and transfer to a 300-mL glass-stoppered flask. Dissolve in 25 mL of water, add 75 mL of diethyl ether and 10 drops of bromophenol blue TS, and titrate $<2.5>/$ with 0.5 mol/L hydrochloric acid VS, while mixing the aqueous and diethyl ether layers by vigorous shaking, until a persistent, light green color is produced in the aqueous layer.

Each mL of 0.5 mol/L hydrochloric acid VS

$$72.05 \text{ mg of C}_7\text{H}_5\text{NaO}_2$$

**Containers and storage** Containers—Well-closed containers.

Sodium Bicarbonate

**Description** Sodium Bicarbonate occurs as white crystals or crystalline powder. It is odorless, and has a characteristic, saline taste.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

**Identification** A solution of Sodium Bicarbonate (1 in 30) responds to the Qualitative Tests $<1.09>$ for sodium salt and for bicarbonate.

**pH** $<2.5>/$ Dissolve 1.0 g of Sodium Bicarbonate in 20 mL
of water: the pH of this solution is between 7.9 and 8.4.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Bicarbonate in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.05>—To 0.40 g of Sodium Bicarbonate add 4 mL of dilute nitric acid, heat to boil, cool, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.04%).

(3) Carbonate—Dissolve 1.0 g of Sodium Bicarbonate in 20 mL of freshly boiled and cooled water with very gentle swirling at a temperature not exceeding 15°C. Add 2.0 mL of 0.1 mol/L hydrochloric acid VS and 2 mL of phenolphthalein TS: no red color develops immediately.

(4) Ammonium—Heat 1.0 g of Sodium Bicarbonate: the gas evolved does not change moistened red litmus paper to blue.

(5) Heavy metals <1.07>—Dissolve 4.0 g of Sodium Bicarbonate in 5 mL of water and 4.5 mL of hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 2 mL of dilute acetic acid, 35 mL of water and 1 drop of ammonium TS, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 4.5 mL of hydrochloric acid to dryness, and add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 5 ppm).

(6) Arsenic <1.11>—Dissolve 1.0 g of Sodium Bicarbonate in 3 mL of water and 2 mL of hydrochloric acid, and perform the test using this solution as the test solution (not more than 2 ppm).

**Assay** Weigh accurately about 2 g of Sodium Bicarbonate, dissolve in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS. When the color of the solution changes from blue to yellow-green, boil with caution, cool, and continue the titration 2.50 until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS = 84.0 mg of NaHCO₃

**Containers and storage** Containers—Tight containers.

### Sodium Bicarbonate Injection

#### 炭酸水素ナトリウム注射液

Sodium Bicarbonate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium hydrogen carbonate (NaHCO₃: 84.01).

**Method of preparation** Prepare as directed under Injections, with Sodium Bicarbonate.

**Description** Sodium Bicarbonate Injection is a clear, colorless liquid.

**Identification** To a volume of Sodium Bicarbonate Injection, equivalent to 1 g of Sodium Bicarbonate according to the labeled amount, add water to make 30 mL: the solution responds to the Qualitative Tests <1.09> for sodium salt and for bicarbonate.

**pH** <2.54> 7.0 – 8.5

**Bacterial endotoxins** <4.01> Less than 5.0 EU/mEq.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Measure exactly a volume of Sodium Bicarbonate Injection, equivalent to about 2 g of sodium hydrogen carbonate (NaHCO₃), titrate with 0.5 mol/L sulfuric acid VS, and proceed as directed in the Assay under Sodium Bicarbonate.

Each mL of 0.5 mol/L sulfuric acid VS = 84.01 mg of NaHCO₃

**Containers and storage** Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

### Sodium Bisulfite

#### 塩酸水素ナトリウム

NaHSO₃: 104.06

Sodium Bisulfite is a mixture of sodium hydrogen-sulfite and sodium pyrosulfite.

It contains not less than 64.0% and not more than 67.4% of sulfur dioxide (SO₂: 64.06).

**Description** Sodium Bisulfite occurs as white granules or powder, having the odor of sulfur dioxide.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Sodium Bisulfite (1 in 20) is acid.

It is slowly affected by air or by light.

**Identification** A solution of Sodium Bisulfite (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt and for bisulfite.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Bisulfite in 10 mL of water: the solution is clear and colorless.

(2) Thiosulfate—Dissolve 1.0 g of Sodium Bisulfite in 15 mL of water, add slowly 5 mL of dilute hydrochloric acid, shake, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Sodium Bisulfite in 10 mL of water, add 5 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 5 mL of hy-
**Sodium Borate**

The element sodium borate (Na₂B₄O₇·10H₂O) is a water-soluble salt with a molar mass of 381.37 g/mol. It is white and odorless, occurring as colorless or white crystals or a white, crystalline powder. It has a slightly characteristic, saline taste.

**Description**

Sodium borate is freely soluble in water and slightly soluble in ethanol (95). It is odorless, and has a slightly characteristic, saline taste.

**Purity (1)**

Clarity and color of solution—Dissolve 1.0 g of Sodium Borate in 20 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Sodium Borate in 10 mL of water, add 0.10 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boil, and cool: the solution is colorless.

(3) Chloride—Make a calculation from the result obtained in the Assay. Not more than 0.10 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Sodium Bromide.

(4) Sulfate C1.147—Perform the test with 2.0 g of Sodium Borate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Iodide—Dissolve 0.5 g of Sodium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform, and shake: no red-purple color develops in the chloroform layer.

(6) Bromate—Dissolve 1.0 g of Sodium Bromide in 10 mL of freshly boiled and cooled water, add 2 drops of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently, and allow to stand for 5 minutes: no blue color develops.

(7) Heavy metals C1.079—Proceed with 2.0 g of Sodium Bromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(8) Barium—Dissolve 0.5 g of Sodium Bromide in 10 mL of dilute acetic acid until the solution becomes colorless again, add 2 mL of dilute acetic acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant, preferably well-filled, and not exceeding 30°C.

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant, preferably well-filled, and not exceeding 30°C.

**Sodium Bromide**

The element sodium bromide (NaBr) is a white crystalline powder occurring as colorless or white crystals or crystalline powder. It is odorless.

**Description**

Sodium bromide is odorless. It is freely soluble in water, and soluble in ethanol (95). It is hygroscopic, but not deliquescent.

**Identification**

A solution of Sodium Bromide (1 in 10) responds to the Qualitative Tests for sodium salt and for bromide.

**Purity (1)**

Clarity and color of solution—Dissolve 1.0 g of Sodium Bromide in 3 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Sodium Bromide in 10 mL of water, add 0.10 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boil, and cool: the solution is colorless.

(3) Chloride—Make a calculation from the result obtained in the Assay. Not more than 97.9 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Sodium Bromide.

(4) Sulfate C1.147—Perform the test with 2.0 g of Sodium Bromide. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Iodide—Dissolve 0.5 g of Sodium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform, and shake: no red-purple color develops in the chloroform layer.

(6) Bromate—Dissolve 1.0 g of Sodium Bromide in 10 mL of freshly boiled and cooled water, add 2 drops of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently, and allow to stand for 5 minutes: no blue color develops.

(7) Heavy metals C1.079—Proceed with 2.0 g of Sodium Bromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(8) Barium—Dissolve 0.5 g of Sodium Bromide in 10 mL of dilute acetic acid until the solution becomes colorless again, add 2 mL of dilute acetic acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant, preferably well-filled, and not exceeding 30°C.

**Sodium Bromide**

Sodium Bromide, when dried, contains not less than 99.0% of NaBr.

**Description**

Sodium Bromide occurs as colorless or white crystals or crystalline powder. It is odorless.

**Identification**

A solution of Sodium Bromide (1 in 10) responds to the Qualitative Tests for sodium salt and for bromide.
of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

(9) Arsenic \(<1.11\)—Prepare the test solution with 1.0 g of Sodium Bromide according to Method 1, and perform the test (not more than 2 ppm).

**Loss on drying \(<2.41\)** Not more than 5.0% (1 g, 110°C, 4 hours).

**Assay** Weigh accurately about 0.4 g of Sodium Bromide, previously dried, and dissolve in 50 mL of water. Add 10 mL of dilute nitric acid and 50 mL of 0.1 mol/L silver nitrate VS, exactly measured, and titrate \(<2.50\) the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

\[
\text{Each mL of 0.1 mol/L silver nitrate VS} = 10.29 \text{ mg of NaBr}
\]

**Containers and storage** Containers—Tight containers.

### Sodium Carbonate Hydrate

炭酸ナトリウム水和物

\[
\text{Na}_2\text{CO}_3\cdot10\text{H}_2\text{O}: 286.14
\]

Sodium Carbonate Hydrate contains not less than 99.0% and not more than 103.0% of \(\text{Na}_2\text{CO}_3\cdot10\text{H}_2\text{O}\).

**Description** Sodium Carbonate Hydrate occurs as colorless or white crystals.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Sodium Carbonate Hydrate (1 in 10) is alkaline.

It effloresces in air.

It liquefies in its water of crystallization at 34°C, and becomes anhydrous at above 100°C.

**Identification** A solution of Sodium Carbonate Hydrate (1 in 20) responds to the Qualitative Tests \(<1.09\) for sodium salt and for carbonate.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Carbonate Hydrate in 5 mL of water: the solution is clear and colorless.

(2) Chloride \(<1.03\)—Dissolve 0.5 g of Sodium Carbonate Hydrate in 10 mL of water, add 7 mL of dilute nitric acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.071%).

(3) Heavy metals \(<1.07\)—Dissolve 2.0 g of Sodium Carbonate Hydrate in 10 mL of water, add 8 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 8 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 10 ppm).

(4) Arsenic \(<1.11\)—Prepare the test solution with 0.65 g of Sodium Carbonate Hydrate according to Method 1, and perform the test (not more than 3.1 ppm).

**Loss on drying \(<2.41\)** Not more than 2.0% (2 g, 105°C, 4 hours).

**Assay** Dissolve about 1.2 g of Dried Sodium Carbonate, when dried, contains not less than 99.0% of \(\text{Na}_2\text{CO}_3\).

**Description** Dried Sodium Carbonate occurs as white crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Dried Sodium Carbonate (1 in 10) is alkaline.

It is hygroscopic.

**Identification** A solution of Dried Sodium Carbonate (1 in 20) responds to the Qualitative Tests \(<1.09\) for sodium salt and for carbonate.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Dried Sodium Carbonate in 10 mL of water: the solution is clear and colorless.

(2) Chloride \(<1.03\)—Dissolve 0.5 g of Dried Sodium Carbonate in 10 mL of water, add 12 mL of dilute nitric acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.071%).

(3) Heavy metals \(<1.07\)—Dissolve 1.0 g of Dried Sodium Carbonate in 10 mL of water, add 7.5 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 7.5 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 20 ppm).

(4) Arsenic \(<1.11\)—Prepare the test solution with 0.65 g of Dried Sodium Carbonate according to Method 1, and perform the test (not more than 3.1 ppm).

**Loss on drying \(<2.41\)** Not more than 2.0% (2 g, 105°C, 4 hours).

**Assay** Dissolve about 1.2 g of Dried Sodium Carbonate,
weighed accurately, in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS until the color of the solution changes from blue to yellow-green. Then boil cautiously, cool, and further titrate \(<2.50\) until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS = 53.00 mg of Na$_2$CO$_3$

**Containers and storage**

Containers—Tight containers.

---

**Sodium Chloride**

**塩化ナトリウム**

NaCl: 58.44

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols († †).

Sodium Chloride contains not less than 99.0% and not more than 100.5% of NaCl, calculated on the dried basis.

**Description**

Sodium Chloride occurs as colorless or white, crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5%).

**Identification**

(1) A solution of Sodium Chloride (1 in 20) responds to the Qualitative Tests \(<1.09\) for sodium salt.

(2) A solution of Sodium Chloride (1 in 20) responds to the Qualitative Tests \(<1.09\) for chloride.

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of Sodium Chloride in 5 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 20.0 g of Sodium Chloride in 100.0 mL of freshly boiled and cooled water, and use this solution as the sample solution. To 20 mL of the sample solution add 0.1 mL of bromothymol blue TS and 0.5 mL of 0.01 mol/L hydrochloric acid VS: the color of the solution is yellow. Separately, to 20 mL of the sample solution add 0.1 mL of bromothymol blue TS and 0.5 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is blue.

(3) Sulfates—To 7.5 mL of the sample solution obtained in (2) add water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minutes. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: any turbidity produced does not more than that produced in the following control solution.

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as directed above using this solution instead of the sample solution.

(4) Phosphates—To 2.0 mL of the sample solution obtained in (2) add 5 mL of 2 mol/L sulfuric acid TS and water to make 100.0 mL, then add 4 mL of ammonium molybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride-hydrochloric acid TS, and allow to stand for 10 minutes: the color of the solution is not darker than the following control solution.

Control solution: To 1.0 mL of Standard Phosphoric Acid Solution add 12.5 mL of 2 mol/L sulfuric acid TS and water to make exactly 250 mL. To 100 mL of this solution add 4 mL of ammonium molybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride-hydrochloric acid TS, and allow to stand for 10 minutes.

(5) Bromides—To 0.50 mL of the sample solution obtained in (2) add 4.0 mL of water, 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10,000), and mix immediately. After allowing to stand for 2 minutes, add 0.15 mL of 0.1 mol/L sodium thiosulfate VS, mix, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, to 5.0 mL of a solution of potassium bromide (3 in 1,000,000) add 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10,000), and mix immediately. Proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\) using water as the control: the absorbance at 590 nm of the sample solution is not more than that of the standard solution.

(6) Iodides—Wet 5 g of Sodium Chloride with dropwisely added 0.15 mL of a freshly prepared mixture of starch TS, 0.5 mol/L sulfuric acid TS and sodium nitrite TS (1000:40:3), allow to stand for 5 minutes, and examine under daylight: a blue color does not appear.

(7) Ferrocyanides—Dissolve 2.0 g of Sodium Chloride in 6 mL of water, and add 0.5 mL of a mixture of a solution of iron (II) sulfate heptahydrate (1 in 100) and a solution of ammonium iron (III) sulfate dodecahydrate in diluted sulfuric acid (1 in 400) (1 in 100) (19:1): a blue color does not develop within 10 minutes.

(8) Heavy metals \(<1.07\)—Proceed with 5.0 g of Sodium Chloride according to Method 1, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 3 ppm).

(9) Iron—To 10 mL of the sample solution obtained in (2) add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercapto acetic acid, alkalize with ammonia TS, add water to make 20 mL, and allow to stand for 5 minutes: the solution has not more color than the following control solution.

Control solution: Pipet 1 mL of Standard Iron Solution, and add water to make exactly 25 mL. To 10 mL of this solution add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercapto acetic acid, and proceed in the same manner as directed for the sample solution.

(10) Barium—To 5.0 mL of the sample solution obtained in (2) add 5.0 mL of water and 2.0 mL of dilute sulfuric acid, and allow to stand for 2 hours: the solution has not more turbidity than the following control solution.

Control solution: To 5.0 mL of the sample solution obtained in (2) add 7.0 mL of water, and allow to stand for 2 hours.
(11) Magnesium and alkaline-earth materials—To 200 mL of water add 0.1 g of hydroxylammonium chloride, 10 mL of ammonium chloride buffer solution, pH 10, 1 mL of 0.1 mol/L zinc sulfate VS and 0.2 g of eriochrome black T-sodium chloride indicator, and warm to 40°C. Add 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS dropwise until the red-purple color of the solution changes to blue-purple. To this solution add a solution prepared by dissolving 10.0 g of Sodium Chloride in 100 mL of water, and add 2.5 mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS: the color of the solution is a blue-purple.

* Arsenic <1.11>—Prepare the test solution with 1.0 g of Sodium Chloride according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 50 mg of Sodium Chloride, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

![Equation]

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

*Containers and storage Containers—Tight containers.

10% Sodium Chloride Injection

10% 塩化ナトリウム注射液

10% Sodium Chloride Injection is an aqueous solution for injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of sodium chloride (NaCl: 58.44).

**Method of preparation**

<table>
<thead>
<tr>
<th>Sodium Chloride</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water for Injection or Sterile Water for Injection in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

Prepare as directed under Injections, with the above ingredients.

**Description** 10% Sodium Chloride Injection is a clear, colorless liquid. It has a slightly saline taste.

It is neutral.

**Identification** 10% Sodium Chloride Injection responds to the Qualitative Tests <1.09> for sodium salt and for chloride.

**Bacterial endotoxins** <4.01> Less than 3.6 EU/mL.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Pipet 10 mL of 10% Sodium Chloride Injection, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 30 mL of water, and titrate <2.50> with vigorous shaking, with 0.1 mol/L silver nitrate VS (indicator: 3 drops of fluorescein sodium TS).

![Equation]

Each mL 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

**Containers and storage** Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

### Isotonic Sodium Chloride Solution

0.9% Sodium Chloride Solution

Isotonic Salt Solution

Isotonic Sodium Chloride Injection

生理食塩液

Isotonic Sodium Chloride Solution is an aqueous solution for injection.

It contains not less than 0.85 w/v% and not more than 0.95 w/v% of sodium chloride (NaCl: 58.44).

**Method of preparation**

<table>
<thead>
<tr>
<th>Sodium Chloride</th>
<th>9 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water for Injection or Sterile Water for Injection in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

**Description** Isotonic Sodium Chloride Solution is a clear, colorless liquid. It has a slightly saline taste.

**Identification** Isotonic Sodium Chloride Solution responds to the Qualitative Tests <1.09> for sodium salt and for chloride.

pH <2.54> 4.5 – 8.0

**Purity** (1) Heavy metals <1.07>—Concentrate 100 mL of Isotonic Sodium Chloride Solution to about 40 mL on a water bath, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 0.3 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 20 mL of Isotonic Sodium Chloride Solution, and perform the test (not more than 0.1 ppm).

**Bacterial endotoxins** <4.01> Less than 0.50 EU/mL.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.
Sodium Citrate Hydrate

クエン酸ナトリウム水和物

\[
\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot2\text{H}_2\text{O}: 294.10
\]

Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate [6132-04-3]

Sodium Citrate Hydrate, when dried, contains not less than 99.0% and not more than 101.0% of sodium citrate (C_6H_5Na_3O_7: 258.07).

Description Sodium Citrate Hydrate occurs as colorless crystals, or a white, crystalline powder. It is odorless, and has a cooling, saline taste.

Identification A solution of Sodium Citrate Hydrate (1 in 20) responds to the Qualitative Tests for citrate and for sodium salt.

pH <2.50 Dissolve 1.0 g of Sodium Citrate Hydrate in 20 mL of water: the pH of this solution is between 7.5 and 8.5.

Purity (1) Clarity and color of solution—A solution of 1.0 g of Sodium Citrate Hydrate in 10 mL of water is clear and colorless.

(2) Chloride <1.0%—Take 0.6 g of Sodium Citrate Hydrate, and perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015%).

(3) Sulfate <1.15—To 0.5 g of Sodium Citrate Hydrate add water to make 40 mL, then add 3.0 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(4) Heavy metals <1.07—Proceed with 2.5 g of Sodium Citrate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.15—Prepare the test solution with 1.0 g of Sodium Citrate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

(6) Tartrate—To a solution of 1.0 g of Sodium Citrate Hydrate in 2 mL of water add 1 mL of potassium acetate TS and 1 mL of acetic acid (31): no crystalline precipitate is formed after the sides of the tube have been rubbed with a glass rod.

(7) Oxalate—Dissolve 1.0 g of Sodium Citrate Hydrate in a mixture of 1 mL of water and 3 mL of dilute hydrochloric acid, add 4 mL of ethanol (95) and 0.2 mL of calcium chloride TS, and allow to stand for 1 hour: the solution is clear.

(8) Readily carbonizable substances <1.15—Take 0.5 g of Sodium Citrate Hydrate, and perform the test by heating at 90°C for 1 hour: the solution has no more color than Matching Fluid K.

Loss on drying <2.47 10.0 - 13.0% (1 g, 180°C, 2 hours).

Assay Weigh accurately about 0.2 g of Sodium Citrate Hydrate, previously dried, add 30 mL of acetic acid for nonaqueous titration, warm to dissolve, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 8.602 mg of C_6H_5Na_3O_7.

Containers and storage Containers—Tight containers.

Sodium Citrate Injection for Transfusion

輸血用クエン酸ナトリウム注射液

Sodium Citrate Injection for Transfusion is an aqueous solution for injection. It contains not less than 9.5 w/v% and not more than 10.5 w/v% of sodium citrate hydrate (C_6H_5Na_3O_7.2H_2O: 294.10).

Method of preparation

Sodium Citrate Hydrate 100 g

Water for Injection or Sterile Water for Injection in Containers a sufficient quantity

To make 1000 mL

Prepare as directed under Injections, with the above ingredients. No preservatives may be added.
**Description** Sodium Citrate Injection for Transfusion is a clear, colorless liquid.

**Identification** Sodium Citrate Injection for Transfusion responds to the Qualitative Tests \(<1.09\) for sodium salt and for citrate.

\[ \text{pH} \leq 2.54 \quad 7.0 - 8.5 \]

**Bacterial endotoxins** \(<4.01\) Less than 5.6 EU/mL.

**Extractable volume** \(<6.05\) It meets the requirement.

**Foreign insoluble matter** \(<6.06\) Perform the test according to Method 1; it meets the requirement.

**Insoluble particulate matter** \(<6.07\) It meets the requirement.

**Sterility** \(<4.06\) Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Pipet 5 mL of Sodium Citrate Injection for Transfusion, and add water to make exactly 25 mL. Evaporate 10 mL of this solution, exactly measured, on a water bath to dryness, dry the residue at 180°C for 2 hours, and dissolve in 30 mL of acetic acid (100) by warming. Cool, titrate with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

\[ \text{Each mL of 0.1 mol/L perchloric acid VS} = 9.803 \text{ mg of C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot 2\text{H}_2\text{O} \]

**Containers and storage** Containers—Hermetic containers.

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**Diagnostic Sodium Citrate Solution**

診断用クエン酸ナトリウム液

Diagnostic Sodium Citrate Solution contains not less than 3.3 w/v% and not more than 4.3 w/v% of sodium citrate hydrate (C\(_6\)H\(_5\)Na\(_3\)O\(_7\)·2H\(_2\)O: 294.10).

The requirements as described for aqueous injections under Injections are applicable.

**Method of preparation**

<table>
<thead>
<tr>
<th>Sodium Citrate Hydrate</th>
<th>38 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water for Injection or Sterile Water</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

Prepare as directed under Injections, with the above ingredients. No preservative may be added.

**Description** Diagnostic Sodium Citrate Solution is a clear, colorless liquid.

**Identification** Diagnostic Sodium Citrate Solution responds to the Qualitative Tests \(<1.09\) for sodium salt and for citrate.

\[ \text{pH} \leq 2.54 \quad 7.0 - 8.5 \]

**Assay** Pipet 5 mL of Diagnostic Sodium Citrate Solution, evaporate on a water bath to dryness, dry the residue at 180°C for 2 hours, and dissolve in 30 mL of acetic acid (100) by warming. Cool, and titrate \(<2.50\) with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 9.803 mg of C\(_6\)H\(_5\)Na\(_3\)O\(_7\)·2H\(_2\)O

---

**Sodium Cromoglicate**

クロモグリク酸ナトリウム

\[ \text{C}_2\text{H}_2\text{NaO}_4\text{Na}_2\text{O}_11: \quad 512.33 \]

Disodium 5,5’-(2-hydroxypropane-1,3-diyl)bis(oxy)bis(4-oxo-4H-chromene-2-carboxylate)

\[ [15826-37-6] \]

Sodium Cromoglicate contains not less than 98.0% of C\(_2\)H\(_2\)Na\(_2\)O\(_{11}\), calculated on the dried basis.

**Description** Sodium Cromoglicate occurs as a white, crystalline powder. It is odorless and tasteless at first, and later develops a slightly bitter taste.

It is freely soluble in water, sparingly soluble in propylene glycol, very slightly soluble in ethanol (95), and practically insoluble in 2-propanol and in diethyl ether.

It is hygroscopic.

It gradually acquires a yellow color by light.

**Identification** (1) Dissolve 0.1 g of Sodium Cromoglicate in 2 mL of water, add 2 mL of sodium hydroxide TS, and boil for 1 minute: a yellow color is produced. After cooling, add 0.5 mL of concentrated diazobenzene sulfonic acid acid TS: a dark red color is produced.

(2) Determine the absorption spectrum of a solution of Sodium Cromoglicate in phosphate buffer solution, pH 7.4 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\) and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Sodium Cromoglicate responds to the Qualitative Tests \(<1.09\) for sodium salt.

**Purity** (1) Clarity and color of solution—Dissolve 0.50 g of Sodium Cromoglicate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Acidity or alkalinity—Dissolve 2.0 g of Sodium Cromoglicate in 40 mL of freshly boiled and cooled water, add 6 drops of bromothymol blue TS, and use this solution as the sample solution. To 20 mL of the sample solution add 0.25 mL of 0.1 mol/L sodium hydroxide VS: a blue color is produced. To another 20 mL of the sample solution add 0.25 mL of 0.1 mol/L hydrochloric acid VS: a yellow color is produced.

(3) Heavy metals \(<1.07\)—Proceed with 1.0 g of Sodium Cromoglicate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(4) Oxalate—Dissolve 0.25 g of Sodium Cromoglicate in water to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 49 mg of oxalic acid dihydrate, exactly weighed, in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 mL each of the sample solution and standard solution, add exactly 5 mL of iron salicylate TS to each solution, and add water to make 50 mL. Determine the absorbances of these solutions as directed under Ultraviolet-visible Spectrophotometry using water as the blank: the absorbance of the sample solution at 480 nm is not smaller than that of the standard solution.

(5) Related substances—Dissolve 0.20 g of Sodium Cromoglicate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL, pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spots 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and acetic acid (100) (9:9:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.4% Not more than 10.0% (1 g, in vacuum, 105°C, 4 hours).

Assay Weigh accurately about 0.18 g of Sodium Cromoglicate, and dissolve in a mixture of 25 mL of propylene glycol and 5 mL of 2-propanol by warming. After cooling, add 30 mL of 1,4-dioxane, and titrate with 0.1 mol/L perchloric acid-1,4-oxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS = 25.62 mg of C₁₀H₁₄N₂Na₂O₈.₂H₂O

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Disodium Edetate Hydrate

EDTA Sodium Hydrate

エデト酸ナトリウム水和物

\[
C₁₀H₁₄N₂Na₂O₈.₂H₂O: \text{372.24}
\]

Disodium dihydrogen ethylenediaminetetraacetate dihydrate [6381-92-6]

Disodium Edetate Hydrate contains not less than 99.0% of C₁₀H₁₄N₂Na₂O₈.₂H₂O.

Description Disodium Edetate Hydrate occurs as white crystals or crystalline powder. It is odorless and has a slight, acid taste.

It is soluble in water, and practically insoluble in alcohol (95) and in diethyl ether.

Identification (1) Dissolve 0.01 g of Disodium Edetate Hydrate in 5 mL of water, add 2 mL of a solution of potassium chromate (1 in 200) and 2 mL of arsenic (III) trioxide TS, and heat in a water bath for 2 minutes: a purple color develops.

(2) Dissolve 0.5 g of Disodium Edetate Hydrate in 20 mL of water, and add 1 mL of dilute hydrochloric acid: a white precipitate is produced. Collect the precipitate, wash with 50 mL of water, and dry at 105°C for 1 hour: the precipitate melts between 240°C and 244°C (with decomposition).

(3) A solution of Disodium Edetate Hydrate (1 in 20) responds to the Qualitative Tests for sodium salt.

pH <2.54 Dissolve 1 g of Disodium Edetate Hydrate in 100 mL of water: the pH of this solution is between 4.3 and 4.7.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Disodium Edetate Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Cyanide—Transfer 1.0 g of Disodium Edetate Hydrate to a round-bottomed flask, dissolve in 100 mL of water, add 10 mL of phosphoric acid, and distill. Place 15 mL of 0.5 mol/L sodium hydroxide VS in a 100-mL measuring cylinder, which is used as a receiver, and immerse the bottom end of the condenser into the solution. Distil the mixture until the distillate measures 100 mL, and use this solution as the sample solution. Transfer 20 mL of the sample solution to a glass-stoppered test tube, add 1 drop of phenolphthalein TS, neutralize with dilute acetic acid, and add 5 mL of phosphate buffer solution, pH 6.8, and 1.0 mL of diluted sodium toluenesulfonchloramide TS (1 in 5). Immediately stop the tube, mix gently, and allow to stand for a few minutes. Mix well with 5 mL of pyridine-pyrazolone TS, and allow to stand between 20°C and 30°C for 50 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, add 15 mL of 0.5 mol/L sodium hydroxide VS and water to make exactly 1000 mL, transfer 20 mL of this solution to a glass-stoppered test tube, and proceed as directed for the sample solution.

(3) Heavy metals <1.07—Proceed with 2.0 g of Disodium Edetate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Residue on ignition <2.4% 37.0 – 39.0% (1 g).

Assay Weigh accurately about 1 g of Disodium Edetate Hydrate, dissolve in 50 mL of water, add 2 mL of ammonium-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate with 0.1 mol/L zinc VS until the color of the solution changes from blue to red.
Each mL of 0.1 mol/L zinc VS = 37.22 mg of C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O

Containers and storage Containers—Well-closed containers.

**Sodium Fusidate**

フシジン酸ナトリウム

**C_{31}H_{47}NaO_{6.5} 3 8 . 6 9**

Monosodium (17Z)-ent-16α-acetoxy-3β,11β-dihydroxy-4β,8β,14α-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oate [751-94-0]

Sodium Fusidate is the sodium salt of a substance having antibacterial activity produced by the growth of *Fusidium coccineum*.

It contains not less than 935 μg (potency) and not more than 969 μg (potency) per mg, calculated on the anhydrous basis. The potency of Sodium Fusidate is expressed as mass (potency) of fusidic acid (C_{31}H_{48}O_{6} \cdot 516.71).

**Description**

Sodium Fusidate occurs as white, crystals of crystalline powder.

It is freely soluble in water, in methanol and in ethanol (99.5).

**Identification (1)** Determine the infrared absorption spectra of Sodium Fusidate as directed in the potassium bromide disk method under Infrared Spectrophotometry <\[2.25\]>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** Heavy metals <\[1.07\]>—Proceed with 2.0 g of Sodium Fusidate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Water**<\[2.48\]> Not more than 2.0% (1 g, volumetric titration, direct titration).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <\[4.02\]> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Diethanolamine Fusidate RS, equivalent to about 20 mg (potency), dissolve in 2 mL of ethanol (95), add water to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Sodium Fusidate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature 2 to 8°C.

**Purified Sodium Hyaluronate**

精製ヒアルロン酸ナトリウム

\((C_{14}H_{20}NNaO_{11})_n\)

[Purified Sodium Hyaluronate is the sodium salt of glycosaminoglycans composed of disaccharide units of \(D\)-glucuronic acid and \(N\)-acetyl-\(D\)-glucosamine obtained from cockscomb or microorganisms.

It contains not less than 90.0% and not more than 105.5% of sodium hyaluronate (C_{14}H_{30}NNaO_{11}n), calculated on the dried basis.

It is composed of an average molecular mass of the sodium salt of hyaluronic acid between 500,000 and 1,200,000 or between 1,500,000 and 3,900,000.

The average molecular mass of Purified Sodium Hyaluronate should be labeled.

**Description** Purified Sodium Hyaluronate occurs as white powder, granules or fibrous masses.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

**Identification (1)** Determine the infrared absorption spectrum of Purified Sodium Hyaluronate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <\[2.25\]>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
(2) A solution of Purified Sodium Hyaluronate (1 in 1000) responds to the Qualitative Tests \(<1.09\) (1) for sodium salt.

**Viscosity** \(<2.5\alpha\) Weigh accurately an amount of Purified Sodium Hyaluronate so that the downflowing time of its solution in 100 mL of 0.2 mol/L sodium chloride TS is 2.0 to 2.4 times longer than that of 0.2 mol/L sodium chloride TS, dissolve in 0.2 mol/L sodium chloride TS to make exactly 100 mL, and use this solution as the sample solution (1). Pipet 16 mL, 12 mL and 8 mL of the sample solution (1), to each add 0.2 mol/L sodium chloride TS to make exactly 20 mL, and use these solutions as the sample solutions (2), (3) and (4), respectively. Perform the test with the sample solutions (1), (2), (3) and (4) as directed under Method 1 at 30 ± 0.1°C using a Ubbelohde-type viscometer in which the downflowing time for 0.2 mol/L sodium chloride TS is 200 to 300 seconds: the intrinsic viscosity calculated on the dried basis is between 10.0 and 19.5 dL/g or between 25.0 and 55.0 dL/g.

**Purity** (1) Clarity and color of solution—Dissolve 0.10 g of Purified Sodium Hyaluronate in 10 mL of water: the solution is clear and colorless.

(2) Chloride \(<1.05\alpha\) Dissolve 0.20 g of Purified Sodium Hyaluronate in 15 mL of water, add 6 mL of dilute nitric acid, and heat on a water bath for 30 minutes. After cooling, add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.124%).

(3) Heavy metals \(<1.07\) Proceed with 1.0 g of Purified Sodium Hyaluronate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Residual solvent—Being specified separately.

(5) Protein—Weigh accurately about 20 mg of Purified Sodium Hyaluronate, calculated on the dried basis, dissolve in 1.0 mL of dilute sodium hydroxide TS, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of bovine serum albumin, dissolve in dilute sodium hydroxide TS to make exactly 1000 mL, and use this solution as the standard solution. To 1.0 mL each of the sample solution and standard solution add 5.0 mL of alkaline copper TS (2), immediately stir, allow to stand at room temperature for 10 minutes, add 0.5 mL of diluted Folin’s TS (1 in 2), immediately stir, and allow to stand at room temperature for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry \(<2.2\alpha\), using a solution, prepared with 1.0 mL of dilute sodium hydrochloride in the same manner, as the blank: the absorbance of the sample solution at 750 nm does not exceed the absorbance of the standard solution (not more than 0.05%).

(6) Nucleic acid—Determine the absorbance of a solution of 0.10 g Purified Sodium Hyaluronate in 50 mL of water as directed under Ultraviolet-visible Spectrophotometry \(<2.2\alpha\), using water as the blank: the absorbance at 260 nm is not more than 0.02.

(7) Other acidic mucopolysaccharides—In the case of chicken-derived samples) Dissolve 0.25 g of Purified Sodium Hyaluronate in 100 mL of water, and use this solution as the sample solution. Immerse a cellulose acetate membrane 6 cm in length in 0.2 mol/L pyridine-formic acid buffer solution, pH 3.0. Take out the membrane and remove excessive buffer solution using a filter paper. Place the membrane in an electrophoresis vessel saturated with 0.2 mol/L pyridine-formic acid buffer solution, pH 3.0, and run at 0.5 mA/cm for 1 minute. Apply 2 μL of the sample solution to the membrane in an area 1 cm in width at 1.5 cm from the anode. Carry out electrophoresis at 0.5 mA/cm for 1 hour. After the electrophoresis, stain the membrane by immersing it in alcian blue staining solution for 10 to 20 minutes. After staining, decolorize sufficiently with diluted acetic acid (100) (3 in 100): no bands other than the principal band appears.

(8) Hemolytic streptococci—(In the case of microorganism-derived samples) Dissolve 0.5 g of Purified Sodium Hyaluronate in sterile isotonic sodium chloride solution to make exactly 100 mL. Take 0.5 mL of this solution, apply to 2 blood agar plates, respectively, using a Conradi stick, and incubate at 37°C for 48 hours: no hemolytic colonies appear, or if any, no streptococci are observed in the colony under a microscope.

(9) Hemolysis—(In the case of microorganism-derived samples) Dissolve 0.40 g of Purified Sodium Hyaluronate in sterile isotonic sodium chloride solution to make exactly 100 mL. To 0.5 mL of this solution add 0.5 mL of 1% blood suspension, mix, allow to stand at 37°C for 2 hours, and, if necessary, centrifuge at 3000 revolutions per minute for 10 minutes: the erythrocytes precipitate and the supernatant liquid is clear as in a blank determination performed in the same manner using 0.5 mL of sterile isotonic sodium chloride solution as the blank and 0.5 mL of sterile purified water as the positive control.

**Loss on drying** \(<2.4\alpha\) Not more than 15.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 60°C, 5 hours).

**Microbial limit** \(<4.0\alpha\) The acceptance criteria of TAMC and TYMC are \(10^2\) CFU/g and \(10^5\) CFU/g.

**Average molecular mass** (1) In the case of the labeled average molecular mass of between 500,000 and 1,200,000. Calculate the average molecular mass of Purified Sodium Hyaluronate according to the following equation: it is between 500,000 and 1,200,000. For \([\eta]\), use the maximum viscosity under Viscosity.

\[
\text{Average molecular mass} = \left( \frac{[\eta] \times 10^5}{36} \right)^{1/2}
\]

(2) In the case of the labeled average molecular mass of between 1,500,000 and 3,900,000. Calculate the average molecular mass of Purified Sodium Hyaluronate according to the following equation: it is between 1,500,000 and 3,900,000. For \([\eta]\), use the maximum viscosity under Viscosity.

\[
\text{Average molecular mass} = \left( \frac{[\eta] \times 10^5}{22.8} \right)^{1/2}
\]

**Assay** Weigh accurately about 50 mg of Purified Sodium Hyaluronate, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of D-Glucuronolactone RS, previously dried (under reduced pressure not exceeding 0.67 kPa, silica gel, 24 hours), and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make...
Sodium Hydroxide

水酸化ナトリウム

NaOH: 40.00

Sodium Hydroxide contains not less than 95.0% of NaOH.

Description Sodium Hydroxide occurs as white fused masses, in small pellets, in flakes, in sticks, and in other forms. It is hard and brittle, and shows a crystalline fracture.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It rapidly absorbs carbon dioxide in air.

It deliquesces in moist air.

Identification (1) A solution of Sodium Hydroxide (1 in 250) is alkaline.

A solution of Sodium Hydroxide (1 in 25) responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Hydroxide in 20 mL of water: the solution is clear and colorless.

Chloride <1.07>—Dissolve 2.0 g of Sodium Hydroxide in water, and add water to make 100 mL. To 25 mL of the solution add 10 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.7 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.050%).

Heavy metals <1.07>—Dissolve 1.0 g of Sodium Hydroxide in 5 mL of water, add 11 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, add 2 mL of dilute acetic acid and 1 drop of ammonia TS, add water to make 50 mL, and perform the test using this solution as the test solution. Evaporate 11 mL of dilute hydrochloric acid on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, add water to make 50 mL, and use this solution as the control solution (not more than 30 ppm).

Potassium—Dissolve 0.10 g of Sodium Hydroxide in water and dilute with water to make 40 mL. Add 1.0 mL of dilute acetic acid to 4.0 mL of this solution, and shake. Add 5.0 mL of a solution of sodium tetraphenylboron (1 in 30), shake immediately, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water, and dilute with water to make 1000 mL. Add 1.0 mL of dilute acetic acid to 4.0 mL of this solution, shake, and proceed as directed above.

C. Sodium carbonate—The amount of sodium carbonate (Na2CO3: 105.99) is not more than 2.0%, when calculated by the following equation using B (mL) which is obtained in the Assay.

Amount (mg) of sodium carbonate = 105.99 × B

Mercury—Dissolve 2.0 g of Sodium Hydroxide in 1 mL of a solution of potassium permanganate (3 in 50) and 30 mL of water, neutralize gradually with purified hydrochloric acid, and add 5 mL of dilute sulfuric acid (1 in 2). To this solution add a solution of hydroxylammonium chloride (1 in 5) until the precipitate of manganese dioxide disappears, add water to make exactly 100 mL, and use this solution as the sample solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.2> (Cold vapor type) with the sample solution. Place the sample solution in the sample bottle of an atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the atomic absorption spectrophotometer, and circulate air. Read the absorbance of the sample solution, and record the amount, A of the sample solution when the indication of the recorder rises rapidly and becomes constant at the wavelength of 253.7 nm. On the other hand, to 2.0 mL of Standard Mercury Solution add 1 mL of a solution of potassium permanganate (3 in 50), 30 mL of water and a volume of purified hydrochloric acid equal to that used in the preparation of the sample solution, and read the absorbance of the solution obtained by the same procedure as used for the sample solution: A is smaller than A.

Assay Weigh accurately about 1.5 g of Sodium Hydroxide, and dissolve in 40 mL of freshly boiled and cooled water. Cool the solution to 15°C, add 2 drops of phenolphthalein TS, and titrate <2.50> with 0.5 mol/L sulfuric acid VS until the red color of the solution disappears. Record the amount, A (mL), of 0.5 mol/L sulfuric acid VS consumed. Then add 2 drops of methyl orange TS to the solution, and further titrate <2.50> with 0.5 mol/L sulfuric acid VS until the solution shows a persistent light red color. Record the amount, B (mL), of 0.5 mol/L sulfuric acid VS consumed. Calculate the amount of NaOH from the difference, A (mL) − B (mL).

Each mL of 0.5 mol/L sulfuric acid VS = 40.00 mg of NaOH

Containers and storage Containers—Tight containers.
Sodium Iodide

ヨウ化ナトリウム

Sodium Iodide, when dried, contains not less than 99.0% of NaI.

Description Sodium Iodide occurs as colorless crystals or a white, crystalline powder. It is odorless.

Identification A solution of Sodium Iodide (1 in 20) responds to the Qualitative Tests 1.09 for sodium salt and for iodide.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Iodide in 2 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Sodium Iodide in 10 mL of freshly boiled and cooled water, and add 1.0 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS: no color is produced.

(3) Chloride, bromide and thiosulfate—Dissolve 0.20 g of Sodium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for a few minutes, and filter. To 10 mL of the filtrate add 15 mL of dilute nitric acid: no brown color appears. The solution has no more turbidity than the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid: no green color develops.

(4) Nitrate, nitrite and ammonium—Place 1.0 g of Sodium Iodide in a 40-mL test tube, and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert a pledget of absorbent cotton in the mouth of the test tube, and place a piece of moistened red litmus paper on the cotton. Heat the test tube on a water bath for 15 minutes: the evolved gas does not turn moistened red litmus paper to blue.

(5) Cyanide—Dissolve 0.5 g of Sodium Iodide in 10 mL of water. To 5 mL of this solution add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm, and add 4 mL of hydrochloric acid: no green color develops.

(6) Iodate—Dissolve 0.5 g of Sodium Iodide in 10 mL of freshly boiled and cooled water, and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color develops immediately.

(7) Heavy metals 1.07—Proceed with 2.0 g of Sodium Iodide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(8) Barium—Dissolve 0.5 g of Sodium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 5 minutes: no turbidity is produced.

(9) Potassium—Dissolve 1.0 g of Sodium Iodide in water, and add water to make 100 mL. To 4.0 mL of this solution add 1.0 mL of dilute acetic acid, shake, add 5.0 mL of a solution of sodium tetraphenylboron (1 in 30), immediately shake, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water, and add water to make 1000 mL. To 4.0 mL of this solution add 1.0 mL of dilute acetic acid, shake, and then proceed as directed above.

(10) Arsenic 1.1D—Prepare the test solution with 0.40 g of Sodium Iodide according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <2.4—Not more than 5.0% (2 g, 120°C, 2 hours).

Assay Weigh accurately about 0.4 g of Sodium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform, and titrate 2.50 with 0.05 mol/L potassium iodate VS while shaking vigorously until the red-purple color of the chloroform layer disappears. The end point is attained when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS = 14.99 mg of NaI

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Sodium Iodide (123I) Capsules

ヨウ化ナトリウム (123I) カプセル

Sodium Iodide (123I) Capsules contain iodine-123 in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (123I) Capsules in the Minimum Requirements for Radiopharmaceuticals.

Sodium Iodide (131I) Capsules

ヨウ化ナトリウム (131I) カプセル

Sodium Iodide (131I) Capsules contain iodine-131 in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (131I) Capsules in the Minimum Requirements for Radiopharmaceuticals.

Sodium Iodide (131I) Solution

ヨウ化ナトリウム (131I) 液

Sodium Iodide (131I) Solution contains iodine-131 (131I) in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (131I) Solution in the Minimum Requirements for Radiopharmaceuticals.

Description Sodium Iodide (131I) Solution is a clear, colorless liquid. It is odorless, or has an odor due to the preservatives or stabilizers.
Sodium Iodohippurate (\(^{131}\text{I}\)) Injection

ヨウ化ヒプル酸ナトリウム (\(^{131}\text{I}\) 注射液)

Sodium Iodohippurate (\(^{131}\text{I}\)) Injection is an aqueous solution for injection containing iodine-131 (\(^{131}\text{I}\)) in the form of sodium o-iodohippurate.

It conforms to the requirements of Sodium Iodohippurate (\(^{131}\text{I}\)) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

**Description** Sodium Iodohippurate (\(^{131}\text{I}\)) Injection is a clear, colorless liquid. It is odorless or has an odor of the preservatives or stabilizers.

Sodium Iotalamate Injection

イオタラム酸ナトリウム注射液

Sodium Iotalamate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of iotalamic acid (\(\text{C}_{11}\text{H}_{9}\text{I}_{3}\text{N}_{2}\text{O}_{4}: 613.91\)).

**Method of preparation**

1. Iotalamic Acid 645.0 g
2. Sodium Hydroxide 42.0 g
3. Water for Injection or Sterile Water for Injection in Containers a sufficient quantity

To make 1000 mL

Prepare as directed under Injections, with the above ingredients (1) or (2).

**Description** Sodium Iotalamate Injection is a clear, colorless or pale yellow, slightly viscous liquid.

It is gradually colored by light.

**Identification** (1) To a volume of Sodium Iotalamate Injection, equivalent to 1 g of Iotalamic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with thorough stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash the precipitate with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate as directed in the Identification (2) under Iotalamic Acid.

(2) Sodium Iotalamate Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> 6.5 – 7.7

**Purity** (1) Primary aromatic amines—To a volume of Sodium Iotalamate Injection, equivalent to 0.20 g of Iotalamic Acid according to the labeled amount, add 15 mL of water, shake, add 4 mL of a solution of sodium nitrite (1 in 100) under ice-cooling, and proceed as directed in the Purity (2) under Iotalamic Acid: the absorbance is not more than 0.17.

(2) Iodine and iodide—To a volume of Sodium Iotalamate Injection, equivalent to 1.5 g of Iotalamic Acid according to the labeled amount, add 20 mL of water and 5 mL of dilute sulfuric acid, shake well, and filter the precipitate by suction through a glass filter (G4). To the filtrate add 5 mL of toluene, and shake vigorously: the toluene layer is colorless. Then add 2 mL of a solution of sodium nitrite (1 in 100), and shake vigorously: the toluene layer has no more color than the following control solution.

Control solution: Dissolve 0.25 g of potassium iodide in water to make 1000 mL. To 2.0 mL of this solution add 20 mL of water, 5 mL of dilute sulfuric acid, 5 mL of toluene and 2 mL of a solution of sodium nitrite (1 in 100), and shake vigorously.

**Bacterial endotoxins** <4.01> Less than 3.4 EU/mL.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Direct inoculation method: it meets the requirement.

**Assay** Pipet a volume of Sodium Iotalamate Injection, equivalent to about 4 g of iotalamic acid (\(\text{C}_{11}\text{H}_{9}\text{I}_{3}\text{N}_{2}\text{O}_{4}\)), add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of iotalamic acid for assay, previously dried at 105°C for 4 hours, dissolve in 100 mL of water and 1 mL of sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of iotalamic acid to that of the internal standard.

\[
M_5 = \text{Amount (mg) of iotalamic acid (C}_{11}\text{H}_{9}\text{I}_{3}\text{N}_{2}\text{O}_{4}) = M_S \times \frac{Q_T}{Q_S}
\]

**Internal standard solution**—A solution of L-tryptophan in the mobile phase (3 in 2500).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Sodium L-Lactate Solution

Sodium L-Lactate Solution is an aqueous solution of sodium salt of L-lactic acid. It contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium L-lactate (C₃H₅NaO₃).

The label states the content amount of sodium L-lactate.

Description
Sodium L-Lactate Solution occurs as a clear and colorless viscous liquid. It has no odor or has a slight like nor lactic acid like odor is produced.

It is miscible with water or with ethanol (99.5).

Identification
To an amount of Sodium L-Lactate Solution, equivalent to 1 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add water to make 50 mL. This solution responds to the Qualitative Tests 1.00 for sodium salt and for lactate.

Optical rotation
To a solution of Sodium L-Lactate Solution, equivalent to 2.5 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add 30 mL of water and 5.0 g of hexaammonium heptamolybdate tetrahydrate, then add water to make exactly 50 mL, and determine using a 100-mm cell.

pH
To an amount of Sodium L-Lactate Solution, equivalent to 5 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add water to make 50 mL: the pH of this solution is between 6.5 and 7.5.

Purity
Chloride
Perform the test with an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.01%).

Sulfate
To an amount of Sodium L-Lactate Solution, equivalent to 2.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add 7 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.01%).

Heavy metals
To an amount of Sodium L-Lactate Solution, equivalent to 2.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add 5 mL of dilute hydrochloric acid, 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

Iron
Prepare the test solution with an amount of Sodium L-Lactate Solution, equivalent to 2.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 5 ppm).

Arsenic
To an amount of Sodium L-Lactate Solution, equivalent to 2.5 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, and add water to make 10 mL. Perform the test using 2 mL of this solution as the test solution (not more than 4 ppm).

Sugars
To an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add 10 mL of water and 10 mL of Fehling’s TS, and boil for 5 minutes: no red precipitate is produced.

Citric, oxalic, phosphoric and L-tartaric acids
To an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add 1 mL of water and 1 mL of dilute hydrochloric acid, then add 40 mL of calcium hydroxide TS, and boil for 2 minutes: the solution is not changed.

Volatile fatty acids
To an amount of Sodium L-Lactate Solution, equivalent to 3.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add 2 mL of dilute sulfuric acid, and heat on a water bath: no acetic acid like nor lactic acid like odor is produced.

Cyanide
Transfer an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, then add dropwise a solution of sodium hydroxide (1 in 10) while shaking until a pale red color appears. Add further 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. After cooling, add dropwise dilute hydrochloric acid until a red color of the solution disappears, then add 1 drop of acetic acid (31), 10 mL of phosphate buffer solution, pH 6.8, and 0.25 mL of sodium toluenesulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. Add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the color of the solution is not more intense than that of the following control solution.

Control solution: To 1.0 mL of Standard Cyanide Solution add water to make 20 mL. Transfer 1.0 mL of this solution...
Sodium Lauryl Sulfate

ラウリル硫酸ナトリウム

Sodium Lauryl Sulfate is a mixture of sodium alkyl sulfate consisting chiefly of sodium lauryl sulfate (C12H25NaO4S: 288.38).

Description Sodium Lauryl Sulfate occurs as white to light yellow crystals or powder. It has a slightly characteristic odor.

It is sparingly soluble in methanol and in ethanol (95). A solution of Sodium Lauryl Sulfate (1 in 10) is a clear or an opalescent solution, which foams on agitation.

Identification (1) To 0.2 g of the residue obtained in Total alcohol content add 4 mL of bromine-cyclohexane TS with vigorous shaking, add 0.3 g of N-bromosuccinimide, and heat in a water bath at 80°C for 5 minutes: a red color develops.

(2) A solution of Sodium Lauryl Sulfate (1 in 10) responds to the Qualitative Tests 1.09> (1) for sodium salt.

(3) To a solution of Sodium Lauryl Sulfate (1 in 10) add dilute hydrochloric acid to make acid, boil gently, and cool: the solution responds to the Qualitative Tests 1.09> for sulfate.

Purity (1) Alkalinity—Dissolve 1.0 g of Sodium Lauryl Sulfate in 100 mL of water, add 2 drops of phenol red TS and 0.60 mL of 0.1 mol/L hydrochloric acid VS: the solution remains yellow.

(2) Sodium chloride—Dissolve about 5 g of Sodium Lauryl Sulfate, accurately weighed, in 50 mL of water, neutralize the solution with dilute nitric acid, if necessary, add exactly 5 mL of 0.1 mol/L sodium chloride TS, and titrate 2.50> with 0.1 mol/L silver nitrate VS (indicator: 2 drops of fluorescein sodium TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

The combined content of sodium chloride (NaCl: 58.44) and sodium sulfate (Na2SO4: 142.04) obtained in the next paragraph (3) is not more than 8.0%.

(3) Sodium sulfate—Dissolve about 1 g of Sodium Lauryl Sulfate, accurately weighed, in 10 mL of water, add 100 mL of ethanol (95), and heat at a temperature just below the boiling point for 2 hours. Filter through a glass filter (G4) while hot, and wash with 100 mL of boiling ethanol (95). Dissolve the precipitate by washing with 150 mL of water, collecting the washings in a beaker. Add 10 mL of hydrochloric acid, heat to boiling, add 25 mL of barium chloride TS, and allow to stand overnight. Collect the precipitate, and wash with water until the last washing shows no opalescence with silver nitrate TS. Dry the precipitate, ignite to a constant mass between 500°C and 600°C by raising the temperature gradually, and weigh as barium sulfate (BaSO4: 233.39).

Amount (mg) of sodium sulfate (Na2SO4) = amount (mg) of barium sulfate (BaSO4) × 0.609

(4) Unsulfated alcohols—Dissolve about 10 g of Sodium Lauryl Sulfate, accurately weighed, in 100 mL of water, add...
Sodium Pertechnetate (99mTc) Injection

Description

Sodium Pertechnetate (99mTc) Injection is an aqueous solution for injection. It contains technetium-99m (99mTc) in the form of sodium pertechnetate.

It conforms to the requirements of Sodium Pertechnetate (99mTc) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Dibasic Sodium Phosphate Hydrate

Description

Dibasic Sodium Phosphate Hydrate is a clear, colorless liquid.
Sodium Picosulfate Hydrate

ピコスルファートナトリウム水和物

C_{18}H_{13}NNa_{2}O_{8}S_{2}·H_{2}O: 499.42
Disodium 4,4'-(pyridin-2-ylmethylene)bis(phenyl sulfate) monohydrate
[10040-45-6, anhydride]

Sodium Picosulfate Hydrate contains not less than 98.5% of sodium picosulfate (C_{18}H_{13}NNa_{2}O_{8}S_{2}: 481.41), calculated on the anhydrous basis.

**Description** Sodium Picosulfate Hydrate occurs as a white, crystalline powder. It is odorless and tasteless.

It is very soluble in water, soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

It is gradually colored by light.

The pH of a solution of Sodium Picosulfate Hydrate (1 in 20) is between 7.4 and 9.4.

**Identification** (1) Mix 5 mg of Sodium Picosulfate Hydrate with 0.01 g of 1-chloro-2,4-dinitrobenzene, and melt by gentle heating for 5 to 6 seconds. After cooling, add 4 mL of potassium hydroxide-ethanol TS: an orange-red color develops.

(2) To 0.2 g of Sodium Picosulfate Hydrate add 5 mL of dilute hydrochloric acid, boil for 5 minutes, cool, and add 1 mL of barium chloride TS: a white precipitate is formed.

(3) Determine the absorption spectrum of a solution of Sodium Picosulfate Hydrate (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Sodium Picosulfate Hydrate, previously dried at 105° C in vacuum for 4 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Sodium Picosulfate Hydrate (1 in 10) responds to the Qualitative Tests 1.07 for sodium salt.

**Absorbance** 2.24 E_{1%}^{1cm} (263 nm): 120 – 130 (calculated on the anhydrous basis, 4 mg, water, 100 mL).

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Picosulfate Hydrate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride 1.03—Perform the test with 0.5 g of Sodium Picosulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Sulfate 1.14—Perform the test with 0.40 g of Sodium Picosulfate Hydrate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.042%).

(4) Heavy metals 1.07—Proceed with 2.0 g of Sodium Picosulfate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic 1.11—Prepare the test solution with 2.0 g of Sodium Picosulfate Hydrate according to Method 3, and perform the test (not more than 1 ppm).

(6) Related substances—Dissolve 0.25 g of Sodium Picosulfate Hydrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 500 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.63. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (74:20:19) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Water** 2.48 3.0 – 4.5% (0.5 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 0.4 g of Sodium Picosulfate Hydrate, dissolve in 50 mL of methanol, add 7 mL of acetic acid (100), and titrate 2.50 with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 48.14 mg of C_{18}H_{13}NNa_{2}O_{8}S_{2}

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Sodium Polystyrene Sulfonate**

ポリスチレンスルホン酸ナトリウム

Sodium Polystyrene Sulfonate is a cation exchange resin prepared as the sodium form of the sulfonated styrene divinylbenzene copolymer.

It contains not less than 9.4% and not more than 11.0% of sodium (Na: 22.99), calculated on the anhydrous basis.

Each g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, exchanges with not less than 0.110 g and not more than 0.135 g of potassium (K: 39.10).

**Description** Sodium Polystyrene Sulfonate occurs as a yellow-brown powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95), in acetone and in diethyl ether.

**Identification** (1) Determine the infrared absorption spectrum of Sodium Polystyrene Sulfonate as directed in the potassium bromide disk method under the Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities
of absorption at the same wave numbers.

(2) To 1 g of Sodium Polystyrene Sulfonate add 10 mL of dilute hydrochloric acid, stir, and filter. Add ammonia TS to the filtrate to neutralize: the solution responds to the Qualitative Tests \( < 1.0 \) for sodium salt.

**Purity** (1) Ammonium—Place 1.0 g of Sodium Polystyrene Sulfonate in a flask, add 5 mL of sodium hydroxide TS, cover the flask with a watch glass having a moistened strip of red litmus paper on the underside, and boil for 15 minutes: the gas evolved does not change the red litmus paper to blue.

(2) Heavy metals \( < 0.07 \)—Proceed with 2.0 g of Sodium Polystyrene Sulfonate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic \( < 1.1 \)—Prepare the test solution with 2.0 g of Sodium Polystyrene Sulfonate according to Method 3, and perform the test (not more than 1 ppm).

(4) Styrene—To 10.0 g of Sodium Polystyrene Sulfonate add 10 mL of acetone, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of styrene in acetone to make exactly 100 mL. Pipet 1 mL of this solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L of each of the sample solution and standard solution as directed under Liquid Chromatography \( < 2.01 \) according to the following conditions, and determine peak areas, \( A_1 \) and \( A_2 \), of styrene in each solution: \( A_1 \) is not larger than \( A_2 \).

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of styrene is about 8 minutes.

**System suitability**

System performance: Dissolve 20 mg each of styrene and butyl parahydroxybenzoate in 100 mL of acetone. To 5 mL of this solution add acetone to make 100 mL. When the procedure is run with 20 \( \mu \)L of this solution under the above operating conditions, butyl parahydroxybenzoate and styrene are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of styrene is not more than 2.0%.

**Water** \( < 2.4 \) Not more than 10.0% (0.2 g, volumetric titration, direct titration).

**Assay** (1) Sodium—Weigh accurately about 1 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, in a glass-stoppered flask, add exactly 50 mL of 3 mol/L hydrochloric acid TS, shake for 60 minutes, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 5 mL of the filtrate, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add water to make exactly 1000 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Sodium Stock Solution, dilute exactly with water so that each mL of the solution contains 1 to 3 \( \mu \)g of sodium (Na: 22.99), and use these solutions as the standard solutions. Perform the test with the sample solution and the standard solutions as directed under Atomic Absorption Spectrophotometry \( < 2.23 \) according to the following conditions, and calculate the amount of sodium in the sample solution using the calibration curve obtained from the standard solutions.

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: A sodium hollow-cathode lamp.

Wavelength: 589.0 nm.

(2) Potassium exchange capacity—Weigh accurately about 1.5 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, in a glass-stoppered flask, add exactly 100 mL of Standard Potassium Stock Solution, shake for 15 minutes, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 10 mL of the filtrate, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 1000 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Potassium Stock Solution, dilute with water so that each mL of the solution contains an exact amount of 1 to 5 \( \mu \)g of potassium (K: 39.10), and use these solutions as the standard solutions. Perform the test with these solutions as directed under Atomic Absorption Spectrophotometry \( < 2.23 \) according to the following conditions, and calculate the amount \( Y \) (mg) of potassium in 1000 mL of the sample solution using the calibration curve obtained from the standard solution. The quantity of potassium absorbed on each g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, is calculated from the following equation: it is between 0.110 g and 0.135 g.

**Quantity** (mg) of potassium (K) absorbed on 1 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis

\[
Y = \frac{(X - 100Y)}{M}
\]

\( X \): Amount (mg) of potassium in 100 mL of the Standard Potassium Stock Solution before exchange

\( M \): Mass (g) of Sodium Polystyrene Sulfonate taken, calculated on the anhydrous basis

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: A potassium hollow-cathode lamp.

Wavelength: 765.5 nm.

**Containers and storage** Containers—Tight containers.
Sodium Prasterone Sulfate Hydrate

プラステロン硫酸エステルナトリウム水和物

C_{19}H_{27}NaO_{5}S.2H_{2}O: 426.50
Monosodium 17-oxoandrost-5-en-3β-yl sulfate dihydrate [1099-87-2, anhydride]

Sodium Prasterone Sulfate Hydrate contains not less than 98.0% of sodium prasterone sulfate (C_{19}H_{27}NaO_{5}S: 390.47), calculated on the dried basis.

**Description** Sodium Prasterone Sulfate Hydrate occurs as white crystals or crystalline powder. It is odorless.

It is soluble in methanol, sparingly soluble in water and in ethanol (95), and practically insoluble in acetone and in diethyl ether.

The pH of a solution of Sodium Prasterone Sulfate Hydrate (1 in 200) is between 4.5 and 6.5.

Melting point: about 160°C (with decomposition, after drying).

**Identification**

1. Dissolve 0.01 g of Sodium Prasterone Sulfate Hydrate in 4 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of a solution of sodium hydroxide (1 in 8); a red-purple color develops, and gradually changes to brown.

2. To 10 mL of a solution of Sodium Prasterone Sulfate Hydrate (1 in 200) add 0.5 mL of bromine TS: the color of bromine TS immediately disappears.

3. Determine the infrared absorption spectrum of Sodium Prasterone Sulfate Hydrate as directed in the potash.

4. A solution of Sodium Prasterone Sulfate Hydrate (1 in 200) responds to the Qualitative Tests (**1.09**) for sodium salt.

**Optical rotation** &lt;2.49° [α]_D +10.7 – +12.1° (0.73 g, calculated on the dried basis, methanol, 20 mL, 100 mm).

**Purity**

1. Clarity and color of solution—Dissolve 0.25 g of Sodium Prasterone Sulfate Hydrate in 50 mL of water: the solution is clear and colorless.

2. Chloride &lt;1.05%—Dissolve 1.0 g of Sodium Prasterone Sulfate Hydrate in 20 mL of acetic acid and 20 mL of water, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L sulfuric acid VS add 20 mL of acetic acid, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011%).

3. Sulfate &lt;1.14%—To 1.2 g of Sodium Prasterone Sulfate Hydrate add 20 mL of water, shake vigorously for 5 minutes, and filter. To 10 mL of the filtrate add 20 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 20 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.032%).

4. Heavy metals &lt;1.07%—Proceed with 2.0 g of Sodium Prasterone Sulfate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

5. Related substances—Dissolve 0.10 g of Sodium Prasterone Sulfate Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography &lt;2.03%. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and water (75:22:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of sulfuric acid and ethanol (95) (1:1) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** &lt;2.41% 8.0 – 9.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

**Assay** Weigh accurately about 0.25 g of Sodium Prasterone Sulfate Hydrate, dissolve in 30 mL of water. Apply this solution to a chromatographic column 10 mm in inside diameter, previously prepared by pouring 5 mL of strongly acidic ion-exchange resin (H type) for column chromatography, and elute at the rate of 4 mL per minute. Wash the chromatographic column with 100 mL of water, combine the washings with above effluent solution, and titrate &lt;2.50% with 0.05 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L sodium hydroxide VS = 19.52 mg of C_{19}H_{27}NaO_{5}S

**Containers and storage** Containers—Tight containers.

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**Sodium Pyrosulfite**

**Sodium Metabisulfite**

ピロ亜硫酸ナトリウム

Na_{2}S_{2}O_{5}: 190.11

Sodium Pyrosulfite contains not less than 95.0% of Na_{2}S_{2}O_{5}.

**Description** Sodium Pyrosulfite occurs as white crystals or crystalline powder. It has the odor of sulfur dioxide.

It is freely soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Sodium Pyrosulfite (1 in 20) is acid.

It is hygroscopic. It decomposes slowly on exposure to air.
Identification A solution of Sodium Pyrosulfite (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt and for bisulfite.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Pyrosulfite in 10 mL of water: the solution is clear and colorless.

(2) Thiouate—Dissolve 1.0 g of Sodium Pyrosulfite in 15 mL of water, add slowly 5 mL of dilute hydrochloric acid, shake, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Sodium Pyrosulfite in 10 mL of water, and evaporate with 5 mL of hydrochloric acid on a water bath to dryness. Dissolve the residue in 10 mL of water, add 1 drop of phenolphthalein TS, and add ammonia TS until the solution becomes slightly red. Add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution.

Prepare the control solution as follows: evaporate 5 mL of hydrochloric acid on a water bath to dryness, and to the residue add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(4) Iron <1.10>—Prepare the test solution with 1.0 g of Sodium Pyrosulfite according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Dissolve 0.5 g of Sodium Pyrosulfite in 10 mL of water, heat with 1 mL of sulfuric acid on a sand bath until white fumes are evolved, and add water to make 5 mL. Perform the test with this solution as the test solution (not more than 4 ppm).

Assay Weigh accurately about 0.15 g of Sodium Pyrosulfite, and transfer to an iodine flask containing an exactly measured 50 mL of 0.05 mol/L iodine VS. Stopper tightly, shake well, and allow to stand for 5 minutes in a dark place. Add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution.

Prepare the control solution as follows: evaporate 5 mL of hydrochloric acid on a water bath to dryness, and to the residue add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

Each mL of 0.05 mol/L iodine VS = 4.753 mg of Na₂S₂O₅

Containers and storage Contain—Light-resistant, preferably well-filled, and not exceeding 30°C.

Sodium Risedronate Hydrate

リセドロン酸ナトリウム水和物

C₇H₁₀NNaO₇P₂.2H₂O: 350.13
Monosodium trihydrogen 1-hydroxy-2-(pyridin-3-yl)ethane-1,1-diylidiphosphonate hemipentahydrate

[S29003-65-8]

Sodium Risedronate Hydrate contains not less than 98.0% and not more than 102.0% of sodium risedronate (C₁₀H₁₀NNaO₇P₂: 305.09), calculated on the anhydrous basis.

Description Sodium Risedronate Hydrate occurs as a white crystalline powder.

It is soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in diluted dilute sodium hydroxide TS (1 in 20).

Identification (1) Determine the absorption spectrum of a solution of Sodium Risedronate Hydrate in diluted dilute sodium hydroxide TS (1 in 20) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.25>, and compare the spectrum with that of the reference spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sodium Risedronate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the reference spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Sodium Risedronate Hydrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Purity (1) Heavy metals—To 0.50 g of Sodium Risedronate Hydrate in a quartz crucible add 0.50 g of magnesium oxide, mix, heat until the content becomes a light gray while mixing occasionally with a glass rod, then incinerate at 800°C. After cooling, dissolve the residue with 3 mL of hydrochloric acid, and add 3 mL of water. Adjust this solution to pH 8.5 with ammonium TS, then adjust to pH 4 with acetic acid (100), and adjust the pH to 3.4 with dilute hydrochloric acid. Filter the solution into a Nessler tube using a filter paper, rinse the crucible and filter with water, add the rinsings to the Nessler tube, then add water to make 50 mL, and use this solution as the test solution. Separately, to 1.0 mL of Standard Lead Solution add 0.50 g of magnesium oxide, dryness at 110°C, and proceed with the residue in the same manner as for the test solution, and use the solution so obtained as the control solution. To the test and control solutions add 1 drop each of sodium sulfate TS, mix, and allow to stand for 5 minutes, and compare the colors of both solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution by dissolving 1.0 g of Sodium Risedronate Hydrate in 5 mL of sodium hydroxide (1 in 5), and perform the test (not more than 2 ppm).

(3) Related substance—Dissolve 50 mg of Sodium Risedronate Hydrate in 1.5 mL of 0.2 mol/L sodium hydroxide TS, add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2.0 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risedronic acid obtained from the sample solution is not larger than the peak area of risedronic acid from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and
flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of risedronic acid, beginning after the solvent peak.

System suitabity—

System performance: When the procedure is run with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risedronic acid are not less than 4500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 5.0%.

(4) Related substance 2—Dissolve 0.10 g of Sodium Risedronate Hydrate in 3 mL of 0.2 mL/L sodium hydroxide TS, add the diluting solution below to make 50 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add the diluting solution to make exactly 50 mL. Pipet 2 mL of this solution, add the diluting solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risedronic acid obtained from the sample solution is not larger than the peak area of risedronic acid from the standard solution.

Diluting solution: Dissolve 0.11 g of disodium dihydrogen ethylenediamine tetracetate dihydrate and 2.47 g of tetradecyl trimethylammonium bromide in 1000 mL of water, and adjust to pH 6.5 with 0.2 mL/L sodium hydroxide TS. To 700 mL of this solution add 300 mL of acetonitrile.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \text{m} \) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.14 g of disodium dihydrogen ethylenediamine tetracetate dihydrate, 3.16 g of tetradecyl trimethylammonium bromide, 4.81 g of ammonium dihydrogen phosphate and 2.93 g of diammonium hydrogen phosphate in 1280 mL of water, and add 720 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of risedronic acid is about 5 minutes.

Time span of measurement: About 10 times as long as the retention time of risedronic acid, beginning after the solvent peak.

System suitabity—

System performance: When the procedure is run with 50 \( \mu \text{L} \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risedronic acid are not less than 5000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 50 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 2.0%.

(5) Residual solvent Being specified separately.

Water <2.48> 11.9 – 13.9% (40 mg, volumetric titration, direct titration. Use a mixture of formamide for Karl Fischer method and methanol for Karl Fisher method (1:1) instead of methanol for Karl Fisher method).

Assay Weigh accurately about 50 mg of Sodium Risedronate Hydrate, dissolve in 1.5 mL of 0.2 mL/L sodium hydroxide TS, and add the mobile phase to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Risedronic Acid RS (separately determine the water <2.48> using 80 mg, in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, and add the mobile phase to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 20 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of risedronic acid to that of the internal standard.

Amount (mg) of sodium risedronate (\( \text{C}_{7}\text{H}_{10}\text{NNaO}_{7}\text{P}_{2} \))

\[
M_{5} = \frac{Q_{1}}{Q_{2}} \times 1.078
\]

\( M_{5} \): Amount (mg) of Risedronic Acid RS, calculated on the anhydrous basis

Internal standard solution—A solution of sodium benzoate in the mobile phase (1 in 125).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A polyether ether ketone column 4 mm in inside diameter and 25 cm in length, packed with quaternary alkylaminated styrene-divinylbenzene copolymer for liquid chromatography (10 \( \mu \text{m} \) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.8 g of disodium dihydrogen ethylenediamine tetracetate dihydrate in 1000 mL of water, and adjust to pH 9.5 with 0.2 mL/L sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of risedronic acid is about 14 minutes.

System suitabity—

System performance: When the procedure is run with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the internal standard and risedronic acid are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 1.0%.

Containers and storage Containers—Well-closed containers.
Sodium Risedronate Tablets

リセドロン酸ナトリウム錠

Sodium Risedronate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sodium risedronate (C\textsubscript{7}H\textsubscript{10}NNaO\textsubscript{7}P\textsubscript{2}: 305.09).

**Method of preparation**  Prepare as directed under Tablets, with Sodium Risedronate Hydrate.

**Identification**  Powder Sodium Risedronate Tablets. To a portion of the powder, equivalent to 2.5 mg of sodium risedronate (C\textsubscript{7}H\textsubscript{10}NNaO\textsubscript{7}P\textsubscript{2}) according to the labeled amount, add 50 mL of diluted dilute sodium hydroxide TS (1 in 20), shake, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.2 \( \mu \)m. Discard the first 2 mL of the filtrate, and determine the absorption spectrum of the subsequent filtrate as directed under Ultraviolet-visible Spectrophotometry \( \leq 2.24 \); it exhibits a maximum between 260 nm and 264 nm.

**Uniformity of dosage units**  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sodium Risedronate Tablets add exactly 10 mL of the mobile phase, shake, and allow to stand for 10 minutes. Disperse the particles for 10 minutes with the aid of ultrasonic waves with occasional shaking, then centrifuge, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.2 \( \mu \)m. Discard the first 1 mL of the filtrate, pipet exactly 5 mL of the subsequent filtrate, equivalent to about 1.75 mg of sodium risedronate (C\textsubscript{7}H\textsubscript{10}NNaO\textsubscript{7}P\textsubscript{2}), add exactly 1 mL of the internal standard solution and the mobile phase to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of Risedronic Acid RS (separately determine the water \( \leq 2.48 \) using 80 mg in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( \leq 2.01 \) according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of risedronic acid to that of the internal standard.

\[
\text{Amount (mg) of sodium risedronate} \ (\text{C}_7\text{H}_{10}\text{NNaO}_7\text{P}_2) = M_S \times Q_2/Q_1 \times 1/\sqrt{1/4} \times 1.078
\]

\[M_S: \text{Amount (mg) of Risedronic Acid RS, calculated on the anhydrous basis}
\]

**Internal standard solution**—A solution of sodium benzoate in the mobile phase (7 in 2000).

**Operating conditions**—

Proceed as directed in the operating conditions in the Assay under Sodium Risedronate Hydrate.

**System suitability**—

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risedronic acid are not less than 5000.
and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 200 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 2.0%.

**Assay** Weigh accurately the mass of not less than 20 Sodium Risedronate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of sodium risedronate (C7H10NNaO7P2), add exactly 10 mL of the internal standard solution, add 190 mL of the mobile phase, shake, and allow to stand for 10 minutes. Disperse the particles with the aid of ultrasonic waves with occasional shaking, then centrifuge, and filter through a membrane filter with a pore size not exceeding 0.2 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Risedronic Acid RS (separately determine the water <2.48> using 80 mg, in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Sodium Risedronate Hydrate.

Amount (mg) of sodium risedronate (C7H10NNaO7P2)

\[ M_r = M_s \times \frac{Q_r}{Q_s} \times 1.078 \]

**Internal standard solution**—A solution of sodium benzoate in the mobile phase (1 in 100).

**Containers and storage** Containers—Well-closed containers.

### Sodium Salicylate

サリチル酸ナトリウム

C7H5NaO3: 160.10

Monosodium 2-hydroxybenzoate

[Sodium Starch Glycolate](#)

Sodium Salicylate, when dried, contains not less than 99.5% of C7H5NaO3.

**Description** Sodium Salicylate occurs as white, crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and soluble in ethanol (95).

It is gradually colored by light.

**Identification** (1) Determine the infrared absorption spectrum of Sodium Salicylate, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Sodium Salicylate (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt.

**pH <2.54** The pH of a solution of 2.0 g of Sodium Salicylate in 20 mL of water is between 6.0 and 8.0.

**Purity** (1) Clarity of solution—Dissolve 1.0 g of Sodium Salicylate in 10 mL of water: the solution is clear, and its absorbance at 420 nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.02.

(2) Chloride <1.07>—Dissolve 0.5 g of Sodium Salicylate in 15 mL of water, add 6 mL of dilute nitric acid and ethanol (95) to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 28 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(3) Sulfate—Dissolve 0.25 g of Sodium Salicylate in 5 mL of water, and add 0.5 mL of barium chloride TS: the solution shows no change.

(4) Sulfite and thiosulfate—Dissolve 1.0 g of Sodium Salicylate in 20 mL of water, add 1 mL of hydrochloric acid, and filter. Add 0.15 mL of 0.05 mol/L iodine VS to the filtrate: a yellow color develops.

(5) Heavy metals <1.07>—Proceed with 1.0 g of Sodium Salicylate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—To 1.0 g of Sodium Salicylate in a decomposition flask add 5 mL of nitric acid and 2 mL of sulfuric acid, and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat. After cooling, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution is colorless to pale yellow. Repeat the procedure of adding nitric acid and hydrogen peroxide (30) and heating, if necessary. After cooling, add 2 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution (not more than 2 ppm).

**Loss on drying <2.41** Not more than 0.5% (1 g, 105°C, 2 hours).

**Assay** Weigh accurately about 0.3 g of Sodium Salicylate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

\[ = 16.01 \text{ mg of C}_7\text{H}_5\text{NaO}_3 \]

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

### Sodium Starch Glycolate

デンプングリコール酸ナトリウム

[9063-38-1]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (*).
boxymethyl ether of starch or of a cross-linked carboxymethyl ether of starch.

There are two neutralization types of Sodium Starch Glycolate, Type A and Type B, and their insoluble matter in a mixture of ethanol (99.5) and water (8:2), when dried, contains not less than 2.8% and not more than 4.2%, and not less than 2.0% and not more than 3.4% of sodium (Na: 22.99), respectively.

*The label states the type of neutralization.

**Description** Sodium Starch Glycolate occurs as a white powder, and has a characteristic salty taste.

It practically insoluble in ethanol (99.5). It swells with water, and becomes viscous, pasty liquid. It is hygroscopic.

**Identification** (1) Acidify 5 mL of a solution of Sodium Starch Glycolate (1 in 500) with dilute hydrochloric acid, then add one drop of iodine TS, and stir: a blue to violet color is produced.

(2) Determine the infrared absorption spectrum of Sodium Starch Glycolate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) The sample solution obtained in the Purity (2) responds to the Qualitative Tests <1.09> (2) for sodium salt. Perform the test using 2 mL of the sample solution and 4 mL of potassium hexahydroxoantimonate (V) TS.

**Purity** *(1)* Heavy metals <1.07>—Proceed with 1.0 g of Sodium Starch Glycolate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Iron

(i) Sample solution Take 2.5 g of Sodium Starch Glycolate in a silica or platinum crucible, add 2 mL of 5 mol/L sulfuric acid TS. Heat on a water bath, then ignite cautiously with a gas burner or preferably in an electric furnace at 600 ± 25°C, and incinerate the residue completely. Allow to cool, add a few drops of 1 mol/L sulfuric acid TS, and heat and ignite as above. Allow to cool, add a few drops of ammonium carbonate TS, evaporate to dryness on a water bath, and heat and ignite as above. After cooling, dissolve the residue by adding 50 mL of water.

(ii) Standard solution Weigh accurately 863.4 mg of ammonium iron (III) sulfate dodecahydrate, dissolve in water, and add 25 mL of 1 mol/L sulfuric acid TS, and add water to make exactly 500 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Each mL of this solution contains 1.0 µg of iron (Fe).

(iii) Procedure Pipet 10 mL each of the sample solution and standard solution, and to each solution add 2 mL of citric acid solution (1 in 5) and 0.1 mL of thioglycolic acid. Then add ammonia solution (28) dropwise to render the solution alkaline, using litmus paper as an indicator. Add water to make 20 mL, and use these solutions as the test solution and the control solution, respectively. Allow these solutions to stand for 5 minutes, and compare the color of the solutions using white background: the color of the test solution is not deeper than that of the control solution (not more than 20 ppm).

(3) Sodium glycolate—Conduct this procedure without exposure to light, using light-resistant vessels.

(i) Sample solution Weigh accurately 0.200 g of Sodium Starch Glycolate in a beaker, add 4 mL of 6 mol/L acetic acid TS and 5 mL of water, and stir to dissolve. Add 50 mL of acetone and 1 g of sodium chloride, stir, and filter through a filter paper previously soaked with acetone. Rinse the beaker and the filter paper with acetone, combine the filtrate and washings, and add acetone to make exactly 100 mL. Allow to stand for 24 hours, and use the supernatant liquid as the sample solution.

(ii) Standard solution To exactly 0.310 g of glycolic acid, previously dried in a desiccator (silica gel) for 18 hours, add water to dissolve to make exactly 500 mL. Pipet 5 mL of this solution, add 4 mL of 6 mol/L acetic acid TS, and allow to stand for 30 minutes. Add 50 mL of acetone and 1 g of sodium chloride, proceed as (i) above, and use the supernatant liquid as the standard solution.

(iii) Procedure Pipet 2.0 mL each of the sample solution and standard solution into 25-mL stopped test tubes, and heat on a water bath for 20 minutes to remove acetone. After cooling, add 20.0 mL of 2,7-dihydroxynaphthalene TS to the residue, stopper the test tube, and heat on a water bath for 20 minutes. Cool under running water, and transfer whole quantity of the content to a 25-mL volumetric flask. Maintain the flask under running water, and add sulfuric acid to make 25 mL. Within 10 minutes, determine the absorbance of these solutions at 540 nm using water as the blank as directed under Ultraviolet-visible Spectrophotometry <2.24>; the absorbance of the sample solution is not larger than that of the standard solution (not more than 2.0%).

(4) Sodium chloride—Weigh accurately about 0.5 g of Sodium Starch Glycolate in a beaker, disperse in 100 mL of water, and add 1 mL of nitric acid. Titrate <2.5D> with 0.1 mol/L silver nitrate VS (potentiometric titration): the amount of sodium chloride (NaCl: 58.44) is not more than 7.0%.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

**Loss on drying** <2.4I> Not more than 10.0% (1 g, 130°C, 90 minutes).

**Assay** To about 1 g of Sodium Starch Glycolate add 20 mL of a mixture of ethanol (99.5) and water (8:2), stir for 10 minutes, and filter. Repeat this procedure until no more turbidity is produced by adding silver nitrate TS, and dry the residue on the filter paper at 105°C to constant mass. Weigh accurately 0.7 g of the mass, add 80 mL of acetic acid (100), and heat the mixture under a reflux condenser on a water bath for 2 hours. After cooling, titrate <2.5D> with 0.1 mol/L perchloric acid VS (potentiometric titration).

Content (%) of sodium (Na) = \((V \times 2.299 \times 100)/M\)

V: Consumed amount (mL) of 0.1 mol/L perchloric acid VS
M: Mass (mg) of the dried residue

*Containers and storage* Containers—Tight containers.
Dried Sodium Sulfite
乾燥亜硫酸ナトリウム

Na₂SO₃: 126.04

Dried Sodium Sulfite contains not less than 97.0% of Na₂SO₃.

Description Dried Sodium Sulfite is white crystals or powder. It is odorless.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Dried Sodium Sulfite (1 in 10) is about 10.

It gradually changes in moist air.

Identification An aqueous solution of Dried Sodium Sulfite (1 in 20) responds to the Qualitative Tests for sodium salt and sulfite.

Purity (1) Thiosulfate—Dissolve 1.0 g of Dried Sodium Sulfite in 15 mL of water, add gradually 5 mL of hydrochloric acid, shake, and allow to stand for 5 minutes: no turbidity is produced.

(2) Heavy metals <1.07>—Dissolve 1.0 g of Dried Sodium Sulfite in 5 mL of water, add 2 mL of hydrochloric acid gradually, and evaporate the mixture on a water bath to dryness. Add 3 mL of boiling water and 1 mL of hydrochloric acid to the residue, and again evaporate to dryness on a water bath. Dissolve the residue in 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the control solution.

(3) Calcium—Dissolve 1.0 g of Sodium Thiosulfate in 10 mL of water, add 2 mL of ammonium oxalate TS, and allow to stand for 4 minutes: no turbidity is produced.

(4) Arsenic <1.11>—To 0.40 g of Sodium Thiosulfate add 3 mL of nitric acid and 5 mL of water, evaporate on a water bath to dryness, and perform the test with the residue. Prepare the test solution according to Method 2, and perform the test (not more than 20 ppm).

Assay Weigh accurately about 0.2 g of Dried Sodium Sulfite, transfer immediately to an iodine flask containing exactly 50 mL of 0.05 mol/L iodine VS, stopper, shake, and allow to stand for 5 minutes in a dark place. Add 1 mL of hydrochloric acid, and titrate with the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 6.302 mg of Na₂SO₃

Containers and storage Containers—Tight containers.

Sodium Thiosulfate Hydrate
チオ硫酸ナトリウム水和物

Na₂S₂O₃·5H₂O: 248.18

Sodium Thiosulfate Hydrate, when dried, contains not less than 99.0% and not more than 101.0% of sodium thiosulfate (Na₂S₂O₃, 158.11).

Description Sodium Thiosulfate Hydrate occurs as colorless, crystals or crystalline powder. It is odorless.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It effloresces in dry air, and is deliquescent in moist air.

Identification (1) A solution of Sodium Thiosulfate Hydrate (1 in 10) responds to the Qualitative Tests for thiosulfate.

(2) A solution of Sodium Thiosulfate Hydrate (1 in 10) responds to the Qualitative Tests for sodium salt.

pH <2.54>—Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water, add slowly 5 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Add 15 mL of water to the residue, boil gently for 2 minutes, and filter. Heat the filtrate to boil, and add bromine TS to the hot filtrate to produce a clear solution and provide a slight excess of bromine. Boil the solution to expel the bromine. Cool, add 1 drop of phenolphthalein TS, and add dropwise sodium hydroxide TS until a slight red color is produced. Add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) Calcium—Dissolve 1.0 g of Sodium Thiosulfate in 10 mL of water, add 2 mL of ammonium oxalate TS, and allow to stand for 4 minutes: no turbidity is produced.

(4) Arsenic <1.11>—To 0.40 g of Sodium Thiosulfate add 3 mL of nitric acid and 5 mL of water, evaporate on a water bath to dryness, and perform the test with the residue. Prepare the test solution according to Method 2, and perform the test (not more than 5 ppm).

Loss on drying <2.41> 32.0 – 37.0% (1 g, in vacuum, 40 – 45°C, 16 hours).

Assay Weigh accurately about 0.4 g of Sodium Thiosulfate, previously dried, dissolve in 30 mL of water, and titrate with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS = 15.81 mg of Na₂S₂O₃

Containers and storage Containers—Tight containers.

Sodium Thiosulfate Injection
チオ硫酸ナトリウム注射液

Sodium Thiosulfate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium thiosulfate hydrate (Na₂S₂O₃·5H₂O: 248.18).
Method of preparation  Prepare as directed under Injections, with Sodium Thiosulfate Hydrate.

Description  Sodium Thiosulfate Injection is a clear, colorless liquid.

Identification  Sodium Thiosulfate Injection responds to the Qualitative Tests <1.09> for sodium salt and for thiosulfate.

Bacterial endotoxins  <4.01>  Less than 0.01 EU/mg.

Extractable volume  <6.05>  It meets the requirement.

Foreign insoluble matter  <6.06>  Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter  <6.07>  It meets the requirement.

Sterility  <4.06>  Perform the test according to the Membrane filtration method: it meets the requirement.

Assay  Measure exactly a volume of Sodium Thiosulfate Injection, equivalent to about 0.5 g of sodium thiosulfate hydrate (Na₂S₂O₃·5H₂O), add water to make 30 mL, and titrate with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS = 24.82 mg of Na₂S₂O₃·5H₂O

Containers and storage  Containers—Hermetic containers.

Sodium Valproate

パルプロ酸ナトリウム

C₇H₁₅NaO₂: 166.19

Monosodium 2-propylpentanoate

[1069-66-5]

Sodium Valproate, when dried, contains not less than 98.5% and not more than 101.0% of C₇H₁₅NaO₂.

Description  Sodium Valproate occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in ethanol (99.5) and in acetic acid (100).

It is hygroscopic.

Identification  (1)  To 5 mL of a solution of Sodium Valproate (1 in 20) add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

(2)  Dissolve 0.5 g of Sodium Valproate in 5 mL of water, add 5 mL of diethyl ether and 1 mL of 2 mol/L hydrochloric acid TS, and shake vigorously for 1 minute. Separate the diethyl ether layer, dehydrate with anhydrous sodium sulfate, and filter. Evaporate the solvent of the filtrate, determine the infrared spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3)  A solution of Sodium Valproate (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

pH  <2.5>  Dissolve 1.0 g of Sodium Valproate in 20 mL of water: the pH of this solution is between 7.0 and 8.5.

Purity  (1)  Heavy metals  <1.07>—Dissolve 2.0 g of Sodium Valproate in 44 mL of water, shake with 6 mL of dilute hydrochloric acid, allow to stand for 5 minutes, and filter. Discard the first 5 mL of the filtrate, neutralize the subsequent filtrate 25 mL with ammonia TS, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2)  Related substances—Dissolve 0.10 g of Sodium Valproate in 10 mL of a mixture of formic acid and methyl acetate (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of formic acid and methyl acetate (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total area of the peaks other than the peak of valproic acid from the sample solution is not larger than the peak area of valproic acid from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm in particle diameter) coated with diethylene glycol adipate ester for gas chromatography and phosphoric acid at the ratios of 5% and 1%, respectively.

Column temperature: A constant temperature of about 145°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of valproic acid is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of valproic acid, beginning after the solvent peak.

System suitability—

System performance: To 2 mL of the sample solution and 8 μL of n-valerianic acid, add a mixture of formic acid and methyl acetate (1:1) to make 10 mL. When the procedure is run with 2 μL of this solution under the above operating conditions, n-valerianic acid and valproic acid are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: Pipet 2 mL of the standard solution and add a mixture of formic acid and methyl acetate (1:1) to make exactly 10 mL. When the test is repeated 6 times with 2 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of valproic acid is not more than 5.0%.

Loss on drying  <2.41>  Not more than 1.0% (1 g, 105°C, 3 hours).

Assay  Weigh accurately about 0.2 g of Sodium Valproate, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potenti-
Sodium Valproate Syrup
パルプロ酸ナトリウムシロップ

Sodium Valproate Syrup contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium valproate (C8H15NaO2: 166.19).

**Method of preparation** Prepare as directed under Syrups, with Sodium Valproate.

**Identification** To a volume of Sodium Valproate Syrup, equivalent to 50 mg of Sodium Valproate according to the labeled amount, add water to make 10 mL. To 5 mL of this solution add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

**Microbial limit** The acceptance criteria of TAMC and TYMC are 10^2 CFU/mL and 10^1 CFU/mL, respectively. *Escherichia coli* is not observed.

**Assay** Pipet a volume of Sodium Valproate Syrup, equivalent to about 0.1 g of sodium valproate (C8H15NaO2) and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of sodium valproate (C8H15NaO2), and centrifuge. To 5 mL of the supernatant liquid add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

**Uniformity of dosage units** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sodium Valproate Tablets add 7V/10 mL of the mobile phase, shake vigorously, add the mobile phase to make exactly V mL so that each mL contains about 1 mg of sodium valproate (C8H15NaO2), and centrifuge. Filter the supernatant liquid, pipet 20 mL of the filtrate, add exactly 5 mL of the internal standard solution, shake vigorously, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sodium valproate (C8H15NaO2) = MS × Q1/Qs × 2

M5: Amount (mg) of sodium valproate for assay

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50,000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length packed with octadecylsilsanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogenphosphate TS, pH 3.0 and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of valproic acid is about 6 minutes.

**System suitability**—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and valproic acid are eluted with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of valproic acid to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Sodium Valproate Tablets
パルプロ酸ナトリウム錠

Sodium Valproate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sodium valproate (C8H15NaO2: 166.19).

**Method of preparation** Prepare as directed under Tablets, with Sodium Valproate.

**Identification** To a quantity of powdered Sodium Valproate Tablets, equivalent to 0.5 g of Sodium Valproate according to the labeled amount, add 10 mL of water, shake well, and centrifuge. To 5 mL of the supernatant liquid add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

**Uniformity of dosage units** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sodium Valproate Tablets add 7V/10 mL of the mobile phase, shake vigorously, add the mobile phase to make exactly V mL so that each mL contains about 1 mg of sodium valproate (C8H15NaO2), and centrifuge. Filter the supernatant liquid, pipet 20 mL of the filtrate, add exactly 5 mL of the internal standard solution, shake vigorously, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sodium valproate (C8H15NaO2) = MS × Q1/Qs × V/100

M5: Amount (mg) of sodium valproate for assay

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50,000).

**Dissolution** When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Sodium Valproate Tablets is not less than 85%. Start the test with 1 tablet of Sodium Valproate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 0.11 mg of sodium valproate (C8H15NaO2) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of sodium valproate for assay, previously dried at 105°C for
3 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL of the solution, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, \( A_1 \) and \( A_3 \), of the valproic acid in each solution.

Dissolution rate (\%) with respect to the labeled amount of sodium valproate (C\textsubscript{8}H\textsubscript{15}NaO\textsubscript{2})
\[
\text{Dissolution rate} = \frac{M_2 \times A_1 / A_3}{V'/V \times 1/C \times 180}
\]

\( M_2 \): Amount (mg) of sodium valproate for assay
\( C \): Labeled amount (mg) of sodium valproate (C\textsubscript{8}H\textsubscript{15}NaO\textsubscript{2}) in 1 tablet

Operating conditions— Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of valproic acid are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of valproic acid is not more than 1.5%.

Assay

Weigh accurately the mass of not less than 20 Sodium Valproate Tablets, and powder. Weigh accurately a tablet for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of sodium valproate for assay, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_3 \), of the peak area of valproic acid to that of the internal standard.

\[
\text{Amount (mg) of sodium valproate (C\textsubscript{8}H\textsubscript{15}NaO\textsubscript{2})} = M_2 \times \frac{Q_1}{Q_3} \times 2
\]

\( M_2 \): Amount (mg) of sodium valproate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS, pH 3.0 and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of valproic acid is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and valproic acid are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of valproic acid to that of the internal standard is not more than 1.0%.

Containers and storage

Containers—Tight containers.

**Sorbitan Sesquioleate**

ソルビタンセスキオレイン酸エステル

Sorbitan Sesquioleate is a mixture of monoester and diester of sorbitol anhydride, partially esterified with oleic acid.

Description

Sorbitan Sesquioleate is a pale yellow to light yellow-brown, viscous oily liquid. It has a faint, characteristic odor and a slightly bitter taste.

It is freely soluble in diethyl ether, slightly soluble in ethanol (95%), and very slightly soluble in methanol.

It is dispersed as fine oily drops in water.

Identification

1. To 0.5 g of Sorbitan Sesquioleate add 5 mL of ethanol (95%) and 5 mL of dilute sulfuric acid, and heat on a water bath for 30 minutes. Cool, shake with 5 mL of petroleum ether, and allow to stand, and separate the upper layer and the lower layer. Shake 2 mL of the lower layer with 2 mL of freshly prepared catechol solution (1 in 10), then with 5 mL of sulfuric acid: a red to red-brown color develops.

2. Heat the upper layer obtained in (1) on a water bath, and evaporate petroleum ether. To the residue add 2 mL of dilute nitric acid (1 in 2), and then add 0.5 g of potassium nitrite between 30°C and 35°C with stirring: the solution develops an opalescence, and, when cooled, crystals are formed.

Specific gravity

\( <1.13 \left\{ d_{25}^{\circ} \right. \text{; 0.960 – 1.020} \)

Saponification value

\( <1.13 \text{; 150 – 168} \)

Purity

1. Acidity—To 2.0 g of Sorbitan Sesquioleate add 50 mL of neutralized ethanol, and heat on a water bath nearly to boiling with stirring once or twice. Cool, add 4.3 mL of 0.1 mol/L sodium hydroxide VS and 5 drops of phenolphthalein TS: a red color develops.

2. Heavy metals

Proceed with 1.0 g of Sorbitan Sesquioleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

3. Arsenic

Prepare the test solution with 1.0 g of Sorbitan Sesquioleate according to Method 2, and perform the test (not more than 2 ppm).

Water

\( <2.48 \text{; Not more than 3.0% (1 g, volumetric titra-} \)

JP XVI
tion, direct titration, stir for 30 minutes).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Containers and storage Containers—Tight containers.

D-Sorbitol

D-ソルビトール

C₆H₁₂O₆: 182.17
D-Glucitol [50-70-4]

D-Sorbitol, when dried, contains not less than 97.0% of C₆H₁₂O₆.

Description D-Sorbitol occurs as white granules, powder, or crystalline masses. It is odorless, and has a sweet taste with a cold sensation.

It is very soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) To 1 mL of a solution of D-Sorbitol (7 in 10) add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue-green color develops, but no turbidity is produced.

(2) Shake thoroughly 1 mL of a solution of D-Sorbitol (1 in 10) with 1 mL of a freshly prepared solution of catechol (1 in 10), add rapidly 2 mL of sulfuric acid, and shake: a reddish purple to red-purple color immediately develops.

(3) Boil 0.5 g of D-Sorbitol with 10 mL of acetic anhydride and 1 mL of pyridine under a reflux condenser for 10 minutes, cool, shake with 25 mL of water, and allow to stand in a cold place. Transfer the solution to a separator, extract with 30 mL of chloroform, and evaporate the extract on a water bath. Add 80 mL of water to the oily residue, heat for 10 minutes on a water bath, then filter the hot mixture. After cooling, collect the precipitated through a glass filter (G3), wash with water, recrystallize once from ethanol (95), and dry in a desiccator (in vacuum, silica gel) for 4 hours: the precipitate melts between 97°C and 101°C.

Purity (1) Clarity and color of solution, and acidity or alkalinity—Dissolve 5 g of D-Sorbitol in 20 mL of water by warming with shaking: the solution is clear, colorless, and not more than 5 ppm.

(2) Chloride <1.07>—Perform the test with 2.0 g of D-Sorbitol. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate <1.14>—Perform the test with 4.0 g of D-Sorbitol. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Heavy metals <1.07>—Proceed with 5.0 g of D-Sorbitol according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(5) Nickel—Dissolve 0.5 g of D-Sorbitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color develops.

(6) Arsenic <1.17>—Prepare the test solution with 1.5 g of D-Sorbitol according to Method 1, and perform the test (not more than 1.3 ppm).

(7) Glucose—Dissolve 20.0 g of D-Sorbitol in 25 mL of water, and boil gently with 40 mL of Fehling's TS for 3 minutes. After cooling, filter the supernatant liquid cautiously through a glass filter (G4), leaving the precipitate in the flask as much as possible, wash the precipitate with hot water until the last washings no longer show an alkali reaction, and filter the washings through the glass filter. Dissolve the precipitate in the flask in 20 mL of iron (III) sulfate TS, filter through the glass filter, and wash with water. Combine the filtrate and the washings, heat at 80°C, and titrate <2.50> with 0.02 mol/L potassium permanganate VS: not more than 6.3 mL of volume for titration consumed or consumption is required.

(8) Sugars—Dissolve 20.0 g of D-Sorbitol in 25 mL of water, and heat with 8 mL of dilute hydrochloric acid under a reflux condenser in a water bath for 3 hours. After cooling, add 2 drops of methyl orange TS, followed by sodium hydroxide TS until an orange color develops, and add water to make 100 mL. Boil gently 10 mL of this solution with 10 mL of water and 40 mL of Fehling's TS for 3 minutes and proceed as directed in (7).

Loss on drying <2.41> Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.02% (5 g).

Assay Weigh accurately about 0.2 g of D-Sorbitol, previously dried, dissolve in water and add water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. Cool, add 2.5 g of potassium iodide, immediately stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS = 1.822 mg of C₆H₁₂O₆

Containers and storage Containers—Tight containers.

D-Sorbitol Solution

D-ソルビトール液

D-Sorbitol Solution contains not less than 97.0% and not more than 103.0% of the labeled amount of D-sorbitol (C₆H₁₂O₆: 182.17).

Description D-Sorbitol Solution is a clear, colorless liquid. It is odorless, and has a sweet taste. It is miscible with water, with ethanol (95), with glycerin and with propylene glycol. It sometimes separates crystalline masses.

Identification (1) To a volume of D-Sorbitol Solution, equivalent to 0.7 g of D-Sorbitol according to the labeled amount, add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue-green color develops, but no turbidity is produced.
Sorbitol Solution, equivalent to 5 g of D-Sorbitol according to the labeled amount, add water to make exactly 250 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. Cool, add 2.5 g of potassium iodide, immediately stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS = 1.822 mg of C6H12O6

Containers and storage Containers—Tight containers.

Soybean Oil

Oleum Sojae

ダイズ油

Soybean Oil is the fixed oil obtained from the seeds of Glycine max Merrill (Leguminosae).

Description Soybean Oil is a clear, pale yellow oil. It is odorless or has a slight odor, and has a bland taste. It is miscible with diethyl ether and with petroleum ether. It is slightly soluble in ethanol (95), and practically insoluble in water.

It congeals between −10°C and −17°C.

Congealing point of the fatty acids: 22 – 27°C

Specific gravity 1.13 d30: 0.916 – 0.922

Acid value 1.13 Not more than 0.2.

Saponification value 1.13 188 – 195

Unsaponifiable matter 1.13 Not more than 1.0%.

Iodine value 1.13 126 – 140

Containers and storage Containers—Tight containers.
Spectinomycin Hydrochloride Hydrate
スペクチノマイシン塩酸塩水和物

C_{14}H_{24}N_{2}O_{7}.2HCl.5H_{2}O: 495.35  
(2R,4aR,5aR,6S,7S,8R,9S,9aR,10aS)-
4a,7,9-Trihydroxy-2-methyl-6,8-bis(methylamino)-
2,3,4a,5a,6,7,8,9,9a,10a-decahydro-
4H-pyran[2,3-b][1,4]benzodioxin-4-one
dihydrochloride pentahydrate
[22189-32-8]

Spectinomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces spectabilis*.

It contains not less than 603 μg (potency) and not more than 713 μg (potency) per mg. The potency of Spectinomycin Hydrochloride Hydrate is expressed as mass (potency) of spectinomycin (C_{14}H_{24}N_{2}O_{7}: 332.35).

Description Spectinomycin Hydrochloride Hydrate occurs as a white to light yellowish white crystalline powder. It is freely soluble in water, and practically insoluble in ethanol (95).

Identification (1) To 5 mL of a solution of Spectinomycin Hydrochloride Hydrate (1 in 100) add gently anthrone TS: a blue to blue-green color is produced at the zone of contact.

(2) Determine the infrared absorption spectra of Spectinomycin Hydrochloride Hydrate and Spectinomycin Hydrochloride RS as directed in the paste method under the Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 3 mL of a solution of Spectinomycin Hydrochloride Hydrate (1 in 150) add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation <2.49> [α]_{D}^{20}: +15 - +21° (2.1 g calculated on the anhydrous basis, water, 25 mL, 200 mm).

pH <2.52> Dissolve 0.10 g of Spectinomycin Hydrochloride Hydrate in 10 mL of water: the pH of the solution is between 4.0 and 5.6.

Water <2.45> Not less than 16.0% and not more than 20.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Klebsiella pneumoniae* ATCC 10031

(ii) Culture medium—Use the medium i in 3) under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Spectinomycin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C and use within 10 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Spectinomycin Hydrochloride Hydrate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.
**Spiramycin Acetate**


(Spiramycin II Acetate (Spiramycin I Acetate))

(3R,4R,5S,6R,8R,9R,10E,12E,15R)-3-Acetoxy-5-{4-O-acetyl-2,6-dideoxy-3-C-methyl-α-L-ribo-hexopyranosyl(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosylxyloxy-9(2,3,4,6-tetraoxy-4-dimethylamino-β-D-erythro-hexopyranosylxyloxy)-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

(Spiramycin III Acetate)

(3R,4R,5S,6R,8R,9R,10E,12E,15R)-5-{4-O-Acetyl-2,6-dideoxy-3-C-methyl-α-L-ribo-hexopyranosyl(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosylxyloxy-9(2,3,4,6-tetraoxy-4-dimethylamino-β-D-erythro-hexopyranosylxyloxy)-6-formylmethyl-9-hydroxy-4-methoxy-8-methyl-3-propanoyloxyhexadeca-10,12-dien-15-olide

(Spiramycin IV Acetate)

(Spiramycin V Acetate)

(Spiramycin VI Acetate)

(Spiramycin VII Acetate)

Spiramycin Acetate is a derivative of a mixture of macrolide substances having antibacterial activity produced by the growth of *Streptomyces ambiofaciens*.

It contains not less than 900 μg (potency) and not more than 1450 μg (potency) per mg, calculated on the dried basis. The potency of Spiramycin Acetate is expressed as mass (potency) of spiramycin II acetate (C_{47}H_{78}N_{2}O_{16}, 927.13). One mg (potency) of Spiramycin Acetate is equivalent to 0.7225 mg of spiramycin II acetate (C_{47}H_{78}N_{2}O_{16}).

**Description**

Spiramycin Acetate occurs as a white to light yellowish white powder.

It is very soluble in acetonitrile and in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

**Identification**

(1) Determine the absorption spectrum of a solution of Spiramycin Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Spiramycin Acetate as directed in the potassium bromide

**Assay**

Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

**Purity**

(1) Heavy metals <1.07>—Proceed with 1 g of Spiramycin Acetate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Spiramycin Acetate according to Method 3, and perform the test (not more than 1 ppm).

**Loss on drying** <2.41>—Not more than 3.0% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

**Residue on ignition** <2.44>—Not more than 0.5% (1 g).

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 231 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile, 0.02 mol/L potassium dihydrogen phosphate TS and a solution of dipotassium hydrogen phosphate (87 in 25,000) (26:7:7).

Flow rate: Adjust the flow rate so that the retention time of spiramycin II acetate is about 10 minutes.

**System suitability**

System performance: Dissolve 25 mg of Spiramycin II Acetate RS in the mobile phase to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of spiramycin II acetate are not less than 14,500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak areas of spiramycin II acetate is not more than 2.0%.

**Content ratio of the active principle**

Dissolve 25 mg of Spiramycin Acetate in 25 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas, A_{III}, A_{IV}, A_{V}, A_{VI} and A_{VII}, of the peaks of spiramycin II acetate, spiramycin III acetate, spiramycin IV acetate, spiramycin V acetate, spiramycin VI acetate and spiramycin VII acetate, respectively, by the automatic integration method, and calculate the ratios of the amounts of A_{II}, A_{IV} and the total of A_{III} and A_{V} to the total amount of all these peaks: the amount of A_{II} is 30 – 45%, A_{IV} is 30 – 45%, and the total of A_{III} and A_{V} is not more than 25%. The relative retention times of spiramycin III acetate, spiramycin IV acetate, spiramycin V acetate, spiramycin VI acetate and spiramycin VII acetate with respect to spiramycin II acetate are 1.3, 1.7, 2.3, 0.85 and 1.4, respectively.

**Heavy metals**

Purity (1)

**Residue on ignition**

Dissolve 25 mg of Spiramycin Acetate according to Method 2, and perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Spiramycin II Acetate RS, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5 °C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Spiramycin II Acetate, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, and add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

Containers and storage Containers—Tight containers.

**Spironolactone**

スピロノラクトン

C₉₂₆₅₂₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆₁₈₂₈₂₃₂₁₆1

Spironolactone, when dried, contains not less than 97.0% and not more than 103.0% of C₂₄H₃₂O₄S.

**Description** Spironolactone occurs as a white to light yellow-brown, fine powder.

It is freely soluble in chloroform, soluble in ethanol (95), slightly soluble in methanol, and practically insoluble in water.

Melting point: 198 – 207 °C (Insert the capillary tube into a bath at about 125 °C, and continue the heating so that the temperature rises at a rate of about 10 °C per minute until the temperature is near the expected melting range, then cool to room temperature, and heat at a rate of about 3 °C per minute.)

**Identification** (1) Determine the absorption spectrum of a solution of Spironolactone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.42>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Spironolactone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Spironolactone, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Spironolactone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Spironolactone and Spironolactone RS in methanol, respectively, then evaporate methanol to dryness, and repeat the test on the residues.

**Optical rotation** <2.49> [α]D < -33 – -37 ° (after drying, 0.25 g, chloroform, 25 mL, 200 mm).

**Purity** (1) Mercapto compounds—Shake 2.0 g of Spironolactone with 20 mL of water, and filter. To 10 mL of the filtrate add 1 mL of starch TS and 0.05 mL of 0.01 mol/L iodine VS, and mix: a blue color develops.

(2) Related substances—Dissolve 0.20 g of Spironolactone in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with n-butyl acetate to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of sulfuric acid in methanol (1 in 10) on the plate, and heat the plate at 105 °C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.47> Not more than 0.5% (1 g, 105 °C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 50 mg each of Spironolactone and Spironolactone RS, previously dried at 105 °C for 2 hours, dissolve in methanol to make exactly 250 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.42>, and determine the absorbances, A₁ and A₅, of the sample solution and standard solution at 238 nm.

Amount (mg) of C₂₄H₃₂O₄S

\[ M_S = \frac{A_1}{A_5} \times M_s \]

Mₕ: amount (mg) of Spironolactone RS

Containers and storage Containers—Tight containers.

**Spironolactone Tablets**

スピロノラクトン錠

Spironolactone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of spironolactone (C₂₄H₃₂O₄S: 416.57).

**Method of preparation** Prepare as directed under Tablets,
with Spironolactone.

**Identification** To an amount of powdered Spironolactone Tablets, equivalent to 10 mg of Spironolactone according to the labeled amount, add 100 mL of methanol, shake vigorously, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 236 nm and 240 nm.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Spironolactone Tablets add a mixture of water and acetonitrile (1:1) to make exactly V mL so that each mL contains about 0.5 mg of spironolactone (C_{24}H_{32}O_{4}S). After stirring for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of spironolactone (C_{24}H_{32}O_{4}S) = M_{S} \times A_{T}/A_{S} \times V/50

M_{S}: Amount (mg) of Spironolactone RS

**Dissolution** <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution prepared by dissolving 1 g of polysorbate 80 in water to make 500 mL as the dissolution medium, the dissolution rate in 30 minutes of a 25-mg tablet and a 50-mg tablet are not less than 80% and not less than 70%, respectively.

Start the test with 1 tablet of Spironolactone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 14 μg of spironolactone (C_{24}H_{32}O_{4}S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Spironolactone RS, previously dried at 105°C for 2 hours, dissolve in 20 mL of ethanol (95), and add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_{T} and A_{S}, at 243 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of spironolactone (C_{24}H_{32}O_{4}S) = M_{S} \times A_{T}/A_{S} \times V/V \times 1/C \times 45

M_{S}: Amount (mg) of Spironolactone RS

C: Labeled amount (mg) of spironolactone (C_{24}H_{32}O_{4}S) in 1 tablet

**Assay** Weigh accurately the mass of not less than 10 Spironolactone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of spironolactone (C_{24}H_{32}O_{4}S), add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. After stirring this solution for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of Spironolactone RS, previously dried at 105°C for 2 hours, dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{T} and A_{S}, of spironolactone from each solution.

Amount (mg) of spironolactone (C_{24}H_{32}O_{4}S) = M_{S} \times A_{T}/A_{S} \times 2

M_{S}: Amount (mg) of Spironolactone RS

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of methanol and water (3:2).
Flow rate: Adjust the flow rate so that the retention time of spironolactone is about 11 minutes.

**System suitability**—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of spironolactone are not less than 4000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of spironolactone is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

**Stearic Acid**

ステアリン酸

Stearic Acid is solid fatty acids obtained from fats, and it consists chiefly of stearic acid (C_{18}H_{36}O_{2}: 284.48) and palmitic acid (C_{16}H_{32}O_{2}: 256.42).

**Description** Stearic Acid occurs as white, unctuous or crystalline masses or powder. It has a faint, fatty odor.

It is freely soluble in diethyl ether, soluble in ethanol (95), and practically insoluble in water.

Melting point: 56 – 72°C

**Acid value** <1.13> 194 – 210

**Iodine value** <1.13> Not more than 4.0.

**Purity** (1) Mineral acid—Melt 5 g of Stearic Acid by warming, shake with 5 mL of boiling water for 2 minutes, filter after cooling, and add 1 drop of methyl orange TS to the filtrate: no red color develops.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Stearic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Fat and paraffin—Boil 1.0 g of Stearic Acid with
0.5 g of anhydrous sodium carbonate and 30 mL of water: the solution, while hot, is clear or not more turbid than the following control solution.

Control solution: To 0.70 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 30 mL, and add 1 mL of silver nitrate TS.

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Containers and storage** Containers—Well-closed containers.

### Stearyl Alcohol

ステアリルアルコール

Stearyl Alcohol is a mixture of solid alcohols, and consists chiefly of stearyl alcohol (C\(_{18}\)H\(_{38}\)O: 270.49).

**Description** Stearyl Alcohol occurs as a white, unctuous matter. It has a faint, characteristic odor. It is tasteless. It is freely soluble in ethanol (95), in ethanol (99.5), in diethyl ether, and practically insoluble in water.

**Melting point** <1.13> 56 – 62°C under Melting Point Determination. Prepare the sample according to Method 2 under Melting Point Determination, then attach tightly a capillary tube to the bottom of the thermometer by means of a rubber band or by any suitable means, and make the bottom of the capillary tube equal in position to the lower end of the thermometer. Insert this thermometer into a test tube 17 mm in inside diameter and about 170 mm in height, fasten the thermometer with cork stopper so that the lower end of the thermometer is about 25 mm distant from the bottom of the test tube. Suspend the test tube in a beaker containing water, and heat the beaker with constant stirring until the temperature rises to 5°C below the expected melting point. Then regulate the rate of increase to 1°C per minute. The temperature at which the sample is transparent and no turbidity is produced is taken as the melting point.

**Acid value** <1.13> Not more than 1.0.

**Ester value** <1.13> Not more than 3.0.

**Hydroxyl value** <1.13> 200 – 220

**Iodine value** <1.13> Not more than 2.0.

**Purity** (1) Clarity of solution—Dissolve 3.0 g of Stearyl Alcohol in 25 mL of ethanol (99.5) by warming: the solution is clear.

(2) Alkalinity—To the solution obtained in (1) add 2 drops of phenolphthalein TS: no red color develops.

**Residue on ignition** <2.44> Not more than 0.05% (2 g).

**Containers and storage** Containers—Well-closed containers.

### Streptomycin Sulfate

ストレプトマイシン硫酸塩

Streptomycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces griseus*.

It contains not less than 740 μg (potency) and not more than 820 μg (potency) per mg, calculated on the dried basis. The potency of Streptomycin Sulfate is expressed as mass (potency) of streptomycin (C\(_{21}\)H\(_{39}\)N\(_{7}\)O\(_{12}\): 581.57).

**Description** Streptomycin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and very slightly soluble in ethanol (95).

**Identification** (1) Dissolve 50 mg of Streptomycin Sulfate in 5 mL of water, add 1 mL of ninhydrin TS and 0.5 mL of pyridine, and heat for 10 minutes: a purple color is developed.

(2) Dissolve 10 mg each of Streptomycin Sulfate and Streptomycin Sulfate RS in 10 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (7 in 100) to a distance of about 12 cm, and air-dry the plate. Spray evenly a mixture of a solution of 1,3-dihydroxynaphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1:1) on the plate, and heat at about 150°C for about 5 minutes: the principal spots from the sample solution and the standard solution show the same in color tone and R\(_f\) value.

(3) A solution of Streptomycin Sulfate (1 in 5) responds to the Qualitative Tests <1.09> for sulfate.
Optical rotation <2.49> [\alpha]_D^20: \text{–}79 \text{ to } \text{–}88^\circ \text{ (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).}

pH <2.54> The pH of a solution obtained by dissolving 2.0 g of Streptomycin Sulfate in 10 mL of water is between 4.5 and 7.0.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Streptomycin Sulfate in 5 mL of water is clear, and colorless or pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Streptomycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.17>—Prepare the test solution with 2.0 g of Streptomycin Sulfate according to Method 3 and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve exactly 0.20 g of Streptomycin Sulfate in a mixture of methanol and sulfuric acid (97:3) to make 5 mL, and heat under a reflux condenser for 1 hour. After cooling, wash the inside of the condenser with a suitable amount of a mixture of methanol and sulfuric acid (97:3), add a mixture of methanol and sulfuric acid (97:3) to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve exactly 36 mg of D(+)-mannose in a mixture of methanol and sulfuric acid (97:3) to make 5 mL, and heat under a reflux condenser for 1 hour. After cooling, wash the inside of the condenser with a suitable amount of a mixture of methanol and sulfuric acid (97:3), and add a mixture of methanol and sulfuric acid (97:3) to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of methanol and sulfuric acid (97:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \mu L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of toluene, methanol and acetic acid (100) (2:1:1) to a distance of 13 to 15 cm, and air-dry the plate. Spray evenly a mixture of a solution of 1,3-dihydroxynaphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1:1) on the plate, and heat at 110°C for 5 minutes: the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer, having pH 7.8 \text{ to } 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Streptomycin Sulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5°C and 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 \mu g (potency) and 2 \mu g (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Streptomycin Sulfate, equivalent to about 20 mg (potency), dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 \mu g (potency) and 2 \mu g (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Streptomycin Sulfate for Injection

注射用ストレプトマイシン硫酸塩

Streptomycin Sulfate for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of streptomycin (C_{12}H_{29}N,O_{12}: 581.57).

Method of preparation Prepare as directed under Injections, with Streptomycin Sulfate.

Description Streptomycin Sulfate for Injection occurs as a white or light yellowish white masses or powder.

Identification Perform the test as directed in the Identification (2) under Streptomycin Sulfate.

Osmotic pressure ratio Being specified separately.

pH <2.54> The pH of a solution prepared by dissolving an amount of Streptomycin Sulfate for Injection, equivalent to 2.0 g (potency) of Streptomycin Sulfate according to the labeled amount, in 10 mL of water is 5.0 to 7.0.

Purity Clarity and color of solution—Dissolve an amount of Streptomycin Sulfate for Injection, equivalent to 1.0 g (potency) of Streptomycin Sulfate according to the labeled amount, in 3 mL of water: The solution is clear, and the absorbance of this solution at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry <2.2>, is not more than 0.50.

Loss on drying <2.41> Not more than 4.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 0.10 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate
Sucralfate Hydrate

Aluminum Sucrose Sulfate Ester

Sucralfate Hydrate contains not less than 17.0% and not more than 21.0% of aluminum (Al: 26.98) and not less than 34.0% and not more than 43.0% of sucrose octasulfate ester (C_{12}H_{22}O_{35}S_{8}: 982.80), calculated on the dried basis.

**Description**  Sucralfate Hydrate occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in hot water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sulfuric acid-

**Identification**

(1) To 0.05 g of Sucralfate Hydrate in a small test tube add 0.05 g of fresh pieces of sodium, and melt by careful heating. Immers the test tube immediately in 100 mL of water, break the test tube, shake well, and filter. To 5 mL of the filtrate add 1 drop of sodium pentacyanoferrate (III) TS: a red-purple color develops.

(2) Dissolve 40 mg of Sucralfate Hydrate in 2 mL of dilute sulfuric acid, and add gently 2 mL of anthrone TS to make 2 layers: a blue color develops at the zone of contact, and gradually changes to blue-green.

(3) Dissolve 0.5 g of Sucralfate Hydrate in 10 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for aluminum.

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of Sucralfate Hydrate in 10 mL of dilute sulfuric acid: the solution is clear and colorless.

(2) Chloride <1.07>—Dissolve 0.5 g of Sucralfate Hydrate in 30 mL of dilute nitric acid, and heat gently to boiling. After cooling, add water to make 100 mL, and to 10 mL of this solution add 3 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.50%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Sucralfate Hydrate in 20 mL of a solution of sodium chloride (1 in 5) and 1 mL of dilute hydrochloric acid, and to this solution add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 1 mL of dilute hydrochloric acid on a water bath to dryness, and add 20 mL of a solution of sodium chloride (1 in 5), 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(4) Arsenic <1.11>—Dissolve 1.0 g of Sucralfate Hydrate in 5 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 2 ppm).

(5) Free aluminum—To 3.0 g of Sucralfate Hydrate add 50 mL of water, heat in a water bath for 5 minutes, cool, and filter. Wash the residue with four 5-mL portions of water, combine the filtrate with the washings, add 2 mL of dilute hydrochloric acid, and heat in a water bath for 30 minutes. After cooling, neutralize the solution with sodium hydroxide TS, add water to make exactly 100 mL, and use this solution as the sample solution. Pipet 50 mL of the sample solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.5, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.05 mol/L zinc acetate VS until the color of the solution changes from green-purple through purple to red (indicator: 3 mL of dithizone TS). Perform a blank determination (not more than 0.2%).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.349 mg of Al

(6) Related substances—Proceed with 50 μL of the sample solution obtained in the Assay (2) Sucrose octasulfate ester as directed in the Assay (2) Sucrose octasulfate ester, and perform the test as directed under Liquid Chromatography <2.07>. Determine the peak area of sucrose octasulfate ester from the sample solution and that of a related substance with the relative retention time about 0.7 to the peak of sucrose octasulfate ester by the automatic integration method, and calculate the ratio of the peak area of the related substance to that of sucrose octasulfate ester: it is not more than 0.1.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of sucrose octasulfate ester from 50 μL of the standard solution obtained in the Assay (2) Sucrose octasulfate ester composes 60% to 100% of the full scale.

**Loss on drying** <2.41>  Not more than 14.0% (1 g, 105°C, 3 hours).

**Acid-consuming capacity**  Weigh accurately about 0.25 g of Sucralfate Hydrate, previously dried, place in a 200-mL glass-stoppered conical flask, add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper the flask tightly, and shake at 37 ± 2°C for exactly 1 hour (150 shakings per
minute, amplitude: 20 mm). After cooling in water for 5 minutes, pipet 10 mL of the supernatant liquid, and titrate 2.50 ml of the excess acid with 0.1 mol/L sodium hydroxide VS until the pH becomes 3.5. Perform a blank determination. The amount of 0.1 mol/L hydrochloric acid VS consumed per g of Sucralfate is not less than 130 mL.

Assay (1) Aluminum—Weigh accurately about 1 g of Sucralfate Hydrate, dissolve in 10 mL of dilute hydrochloric acid by warming on a water bath, cool, and add water to make exactly 250 mL. Pipet 25 mL of this solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.5, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate 2.50 ml of the excess disodium dihydrogen ethylenediamine tetraacetate with 0.05 mol/L zinc acetate VS until the color of the solution changes from green-purple through purple to red (indicator: 3 mL of dithizone TS). Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.349 mg of Al

(2) Sucrose octasulfate ester—Weigh accurately about 0.55 g of Sucralfate Hydrate, add exactly 10 mL of sulfuric acid-sodium hydroxide TS, shake vigorously, and dissolve with ultrasonic wave at below 30°C for 5 minutes. To this solution add 0.1 mol/L sodium hydroxide VS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of Potassium Sucrose Octasulfate RS, add the mobile phase to make exactly 25 mL, and use this solution as the mobile solution. Prepare rapidly the sample solution and the standard solution, and perform the test immediately. Pipet 50 mL each of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography 2.07 according to the following conditions. Determine the peak areas, $A_2$ and $A_3$, of sucrose octasulfate ester from each solution.

Amount (mg) of sucrose octasulfate ester ($C_{12}H_{22}O_{35}S_8$) = $M_S \times A_2/A_3 \times 0.763$

$M_S$: Amount (mg) of Potassium Sucrose Octasulfate RS, calculated on the anhydrous basis

Operating conditions—
Detector: A differential refractometer.
Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with amino-propylsilanized silica gel for liquid chromatography (about 8 μm in particle diameter).
Column temperature: Room temperature.
Mobile phase: Dissolve a suitable amount (26 to 132 g) of ammonium sulfate in 1000 mL of water, and adjust with phosphoric acid to a pH of 3.5. Allow a solution of Potassium Sucrose Octasulfate RS in dilute hydrochloric acid (1 in 100) to stand at 60°C for 10 minutes, cool, and perform the test immediately. Adjust the amount of ammonium sulfate in the mobile phase so that the peak of a related substance with the relative retention time being about 0.7 to that of sucrose octasulfate ester almost returns to the base line, and the peak of sucrose octasulfate ester elutes most rapidly.
Flow rate: Adjust the flow rate so that the retention time of sucrose octasulfate ester is between 6 and 11 minutes.

Selection of column: Allow a solution of Potassium Sucrose Octasulfate RS in dilute hydrochloric acid (1 in 100) to stand at 60°C for 10 minutes, cool, and proceed immediately with 50 μL of this solution under the above operating conditions. Use a column with a resolution being not less than 1.5 between sucrose octasulfate ester and a related substance with the relative retention time being about 0.7 to sucrose octasulfate ester.

System repeatability: Repeat the test 6 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of sucrose octasulfate ester is not more than 2.0%.

Containers and storage Containers—Tight containers.

Sucrose  
精製白糖

C$_{12}$H$_{22}$O$_{11}$: 342.30  
β-D-Fructofuranosyl α-D-glucopyranoside  
[57-50-1]

Sucrose contains no additives. For Sucrose used for preparation of the large volume infusions, the label states the purpose.

Description Sucrose is a white crystalline powder, or lustrous colorless or white crystals.
It is very soluble in water, and slightly soluble in ethanol (95).

Identification (1) To 10 mg each of Sucrose and white soft sugar add diluted methanol (3 in 5) to make 20 mL each, and use these solutions as the sample solution and the standard solution (a), respectively. Separately, to 10 mg each of glucose, lactose monohydrate, fructose and white soft sugar add methanol (3 in 5) to make 20 mL, and use this solution as the standard solution (b). Perform the test with these solutions as directed under Thin-layer Chromatography 2.07. Spot 2 μL each of the sample solution and standard solutions (a) and (b) on a plate of silica gel for thin-layer chromatography, and dry the plate completely. Develop the plate with a mixture of 1,2-dichloroethane, acetic acid (100), methanol and water (10:5:3:2) to a distance of about 15 cm, and dry the plate with a hot air. And immediately repeat the development with replaced developing mixture, and dry the plate in the same way. Spray evenly a solution of 0.5 g of thymol in 100 mL of a mixture of ethanol (95) and sulfuric acid (19:1), heat at 130°C for 10 minutes: the principal spot from the sample solution is the same with the principal spot from the standard solution (a) in the $R_f$ value, color and size, and...
four spots from the standard solution (b) are apparently distinguishable.

(2) Dissolve 50.0 g of Sucrose in recently boiled and cooled water to make 100 mL, and use this solution as the sample solution. To 1 mL of the sample solution add water to make 100 mL, then to 5 mL of this solution add 0.15 mL of freshly prepared copper (II) sulfate TS and 2 mL of freshly prepared 2 mol/L sodium hydroxide TS: the solution is clear and blue, and not changes on boiling. Then to this solution add 4 mL of dilute hydrochloric acid, boil, and add 4 mL of 2 mol/L sodium hydroxide TS: orange precipitates are immediately produced.

Optical rotation <2.49> $\alpha$<sup>20</sup>: +66.3 - +67.0° (26 g, water, 100 mL, 100 mm).

Purity (1) Clarity and color of solution—The sample solution obtained in the Identification (2) is clear, and has no more color than the following control solution.

Control solution: To exactly 2.4 mL of Iron (III) Chloride CS and exactly 0.6 mL of Cobalt (II) Chloride CS add 7.0 mL of dilute hydrochloric acid (7 in 250). To 5.0 mL of this solution add 95.0 mL of dilute hydrochloric acid (7 in 250).

(2) Acidity or alkalinity—To 10 mL of the sample solution obtained in the Identification (2) add 0.3 mL of phenolphthalein TS: the solution is colorless, and develops a red color on addition of 0.3 mL of 0.01 mol/L sodium hydroxide TS.

(3) Sulfite—Dissolve 5.0 g of Sucrose in 40 mL of water, add 2.0 mL of dilute sodium hydroxide TS and water to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 76 mg of sodium disulfite in water to make exactly 50 mL, then pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Immediately, pipet 10 mL of each of the sample solution and the standard solution, add 1.0 mL of 3 mol/L hydrochloric acid TS, 2.0 mL of decolorized fuchsin TS and 2.0 mL of formaldehyde solution TS, and allow to stand for 30 minutes. Determine the absorbance at 583 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using the control solution obtained by proceeding with 10.0 mL of water in the same manner as above: the absorbance of the sample solution is not larger than that of the standard solution (not more than 15 ppm as SO₂). When the standard solution does not show a red-purple to blue-purple color, result of the test is invalid.

(4) Lead—Put exactly 50 mg of Sucrose in a polytetrafluoroethylene decomposition-vessel, add 0.5 mL of nitric acid to dissolve, seal up the vessel, and heat at 150°C for 5 hours. After cooling, add water to make exactly 5 mL, and use this solution as the sample solution. Perform the test with more than 3 parts of the sample solution as directed in the standard addition method under Atomic Absorption Spectrophotometry <2.23> (electrothermal type) according to the following conditions. The standard solution is prepared by adding water to a suitable volume of Standard Lead Solution exactly volumed, and perform a blank determination with a solution prepared by adding water to 10.0 mL of nitric acid to make exactly 100 mL, and make any necessary correction (not more than 0.5 ppm).

Operating conditions—
Lamp: A hollow cathode lamp.
Wavelength: 283.3 mm.

Temperature for drying: 110°C.
Temperature for incineration: 600°C.
Temperature for atomization: 2100°C.

(5) Invert sugar—Transfer 5 mL of the sample solution obtained in the Identification (2) to a test-tube about 150 mm long and about 16 mm in diameter, add 5 mL of water, 1.0 mL of 1 mol/L sodium hydroxide VS and 1.0 mL of methylene blue TS, mix, and place in a water bath. After exactly 2 minutes, take the tube out of the bath, and examine the solution immediately: the blue color does not disappear completely (0.04%). Ignore any blue color at the air and solution interface.

Conductivity
(i) Potassium chloride conductivity calibration standard solution—Dissolve powdered potassium chloride, previously dried at 500 – 600°C for 4 hours, in newly distilled water having less conductivity than 2 µS · cm<sup>-1</sup> to get three kinds of the standard solution containing 0.7455 g, 0.0746 g and 0.0149 g of potassium chloride in 1000.0 g, respectively. The conductivities of these solutions at 20°C are shown in the following table.

<table>
<thead>
<tr>
<th>Standard solution (g/1000.0 g)</th>
<th>Conductivity (µS · cm&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Resistivity (Ω · cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7455</td>
<td>1330</td>
<td>752</td>
</tr>
<tr>
<td>0.0746</td>
<td>133.0</td>
<td>7519</td>
</tr>
<tr>
<td>0.0149</td>
<td>26.6</td>
<td>37594</td>
</tr>
</tbody>
</table>

(ii) Apparatus—Use an appropriate conductivity meter. The conductivity is determined to measure the electrical resistance of the column of liquid between the electrodes of the immersed measuring device (conductivity cell). The apparatus is supplied with alternative current to avoid the effects of electrode polarization. It is usually equipped with a temperature compensation device. The conductivity cell contains of two parallel platinum electrodes coated with platinum black, and both electrodes are generally protected by a glass tube which allows good exchange between the solution and the electrodes. Use a cell giving the cell constant of 0.01 to 1 cm<sup>-1</sup>.

(iii) Procedure—Use the suitable potassium chloride conductivity calibration standard solution to the measurement. After washing the well with water, rinse 2 to 3 times with the calibration standard solution, fill up the cell with the calibration standard solution, and determine the conductivity of the calibration standard solution kept at 20 ± 0.1°C. Repeat the determination, and measure the conductivity of the calibration standard solution, $G_{58}$ (µS), after a stable reading of ± 3% is obtained. The cell constant, $J$, is calculated by the following:

$$J = \frac{Z_{KC\text{Cl}}}{G_{58}}$$

$J$: Cell constant (cm<sup>-1</sup>)
$Z_{KC\text{Cl}}$: Conductivity constant of the potassium chloride conductivity calibration standard solution (µS·cm<sup>-1</sup>) (20°C)
$G_{58}$: Conductivity measured (µS)

Dissolve 31.3 g of Sucrose in newly distilled water to make exactly 100 mL, and use this solution as the sample so-
solution. After washing well the cell with water, rinse the cell with the sample solution 2 to 3 times, fill up with the sample solution, and determine the conductivity of the sample solution, $G_T (\mu S)$, kept at 20 ± 0.1°C, while stirring. Determine the conductivity of the water used for preparation of the sample solution, $G_0 (\mu S)$, in the same manner as above, and calculate the conductivity, $\chi_T (\mu S \cdot \text{cm}^{-1})$ and $\chi_0 (\mu S \cdot \text{cm}^{-1})$, by the following expressions:

$$\chi_T (\mu S \cdot \text{cm}^{-1}) = G_T$$
$$\chi_0 (\mu S \cdot \text{cm}^{-1}) = G_0$$

Determine the corrected conductivity, $\chi_C$, of the sample solution by the following expression: not more than 35 $\mu S \cdot \text{cm}^{-1}$.

$$\chi_C (\mu S \cdot \text{cm}^{-1}) = \chi_T - 0.35 \chi_0$$

Loss on drying $<$2.41$>$ Not more than 0.1% (2 g, 105°C, 3 hours).

Dextrins For Sucrose used to prepare large volume infusions, to 2 mL of the sample solution obtained in the Identification (2) add 8 mL of water, 0.05 mL of dilute hydrochloric acid and 0.05 mL of iodine TS: the solution remains yellow.

Bacterial endotoxins $<$4.01$>$ Less than 0.25 EU/mg, for Sucrose exclusively to be used to prepare Injections for intravenous infusion of larger volume.

Containers and storage Containers—Well-closed containers.

White Soft Sugar

白糖

C₁₂H₂₂O₁₁: 342.30
β-D-Fructofuranosyl α-D-glucopyranoside
[57-50-1]

Description White Soft Sugar is colorless or white crystals or crystalline powder. It is odorless and has a sweet taste.

It is very soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of White Soft Sugar (1 in 10) is neutral.

Identification (1) When 1 g of White Soft Sugar is ignited, it melts and swells, and decomposes, emitting an odor of caramel, to bulky charcoal.

(2) To 0.1 g of White Soft Sugar add 2 mL of dilute sulfuric acid, boil, add 4 mL of sodium hydroxide TS and 3 mL of Fehling’s TS, and heat to boiling: a red to dark red precipitate is produced.

Optical rotation $<$2.44$>$ $[\alpha]_D^{20}$: $+65.0$ to $+67.0^\circ$ (after drying, 13 g, water, 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 100 g of White Soft Sugar in 100 mL of water, take 50 mL of this solution in a Nessler tube, and view transversely the Nessler tube against a white background: the solution is colorless or only slightly yellow and has no blue color. Fill the solution in the Nessler tube, stopper, and allow to stand for 2 days: no precipitate is produced.

(2) Chloride $<$1.07$>$—To 10.0 g of White Soft Sugar add water to make 100 mL, and use this solution as the sample solution. To 20 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate $<$1.14$>$—To 40 mL of the sample solution obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Calcium—To 10 mL of the sample solution obtained in (2) add 1 mL of ammonium oxalate TS: this solution shows immediately no change.

(5) Heavy metals $<$1.17$>$—Proceed with 5.0 g of White Sugar according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(6) Arsenic $<$1.11$>$—Prepare the test solution with 1.0 g of White Sugar according to Method 1, and perform the test (not more than 2 ppm).

(7) Invert sugar—Dissolve 5.0 g of White Sugar in water to make 100 mL, filter if necessary, and use this solution as the sample solution. Separately place 100 mL of alkaline copper (II) sulfate solution in a 300-mL beaker, cover the beaker with a watch glass, and boil. Immediately add 50.0 mL of the sample solution, boil the mixture exactly for 5 minutes, add at once 50 mL of freshly boiled and cooled water, dip it in a water bath of a temperature below 10°C for 5 minutes, and collect the precipitate in a tared glass filter (G4). Wash the residue on the filter with water until the last washing is neutral, then wash with 10 mL of ethanol (95), add 10 mL of diethyl ether, and dry at 105°C for 30 minutes: the mass of the residual precipitate is not more than 0.120 g.

Loss on drying $<$2.41$>$ Not more than 1.30% (15 g, 105°C, 2 hours).

Residue on ignition $<$2.44$>$ Not more than 0.1% (2 g).

Containers and storage Containers—Well-closed containers.
**Sulbactam Sodium**

スルバクタムナトリウム

C₈H₁₀NNaO₅S: 255.22
Monosodium (2S,5R)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide

Sulbactam Sodium contains not less than 875 µg (potency) and not more than 941 µg (potency) per mg, calculated on the anhydrous basis. The potency of Sulbactam Sodium is expressed as mass (potency) of sulbactam (C₈H₁₁NO₅S: 233.24).

**Description**
Sulbactam Sodium occurs as a white to yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

**Identification**

1. Determine the infrared absorption spectrum of Sulbactam Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry §2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

2. Sulbactam Sodium responds to the Qualitative Tests §1.09 (1) for sodium salt.

**Optical rotation**

\[ [\alpha]_D^2: +219 \sim +233^\circ (1 \text{ g}, \text{ water}, 100 \text{ mL}, 100 \text{ mm}) \]

**pH**

\[ \text{pH} < 2.54 \]

Dissolve 1.0 g of Sulbactam Sodium in 20 mL of water: the pH of the solution is between 5.2 and 7.2.

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Sulbactam Sodium in 20 mL of water: the solution is clear, and colorless to pale yellow.

2. Heavy metals §1.07—Proceed with 1.0 g of Sulbactam Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 2 ppm).

3. Arsenic §1.11—Proceed with the test solution with 1.0 g of Sulbactam Sodium as directed in Method 3, and perform the test (not more than 2 ppm).

4. Sulbactam penicillamine—Weigh accurately about 0.2 g of Sulbactam Sodium, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of sulbactam sodium for sulbactam penicillamine, dissolve in 2 mL of water, add 0.5 mL of sodium hydroxide TS, allow to stand for 10 minutes at a room temperature, and add 0.5 mL of 1 mol/L hydrochloric acid TS, then add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography §2.01 according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of sulbactam penicillamine by the automatic integration method: the amount of sulbactam penicillamine is not more than 1.0%.

\[
\text{Amount (％) of sulbactam penicillamine} = \frac{M_S}{M_T} \times \frac{A_T}{A_S} \times 5
\]

\( M_S \): Amount (mg) of sulbactam sodium for sulbactam penicillamine

\( M_T \): Amount (mg) of the sample

**Operating conditions**

1. Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.


**System suitability**

1. System performance: Proceed as directed in the system suitability in the Assay.

2. System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sulbactam penicillamine is not more than 2.0%.

**Water**

\[ \text{Water} < 2.48 \]

Not more than 1.0% (0.5 g, volumetric titration, direct titration).

**Assay**

Weigh accurately an amount of Sulbactam Sodium and Sulbactam RS, equivalent to about 0.1 g (potency), dissolve each in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography §2.01 according to the following conditions, and calculate the ratios, \( Q_T / Q_S \), of the peak area of sulbactam to that of the internal standard.

\[ \text{Amount (µg (potency)) of sulbactam (C₈H₁₁NO₅S)} = \frac{M_S}{M_T} \times \frac{Q_T}{Q_S} \times 1000 \]

\( M_S \): Amount [mg (potency)] of Sulbactam RS

**Internal standard solution**

A solution of ethyl parahydroxybenzoate in the mobile phase (7 in 1000).

**Operating conditions**

1. Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 µm in particle diameter).

2. Temperature: A constant temperature of about 35°C.

3. Mobile phase: To 750 mL of 0.005 mol/L tetrabutylammonium hydroxide TS add 250 mL of acetonitrile for liquid chromatography.

4. Flow rate: Adjust the flow rate so that the retention time of sulbactam is about 6 minutes.

**System suitability**

1. System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, sulbactam and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

2. System repeatability: When the test is repeated 6 times...
with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sulbactam is not more than 1.0%.

Containers and storage Containers—Tight containers.

Sulbenicillin Sodium

スルベニシリンナトリウム

C₁₆H₁₆N₂Na₂O₇S₂: 458.42
Disodium (2S,5R,6R)-3,3-dimethyl-7-oxo-6-(2R)-phenyl-2-sulfonatoacetlylamin]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [28002-18-8]

Sulbenicillin Sodium contains not less than 900 μg (potency) and not more than 970 μg (potency) per mg, calculated on the anhydrous basis. The potency of Sulbenicillin Sodium is expressed as mass (potency) of sulbenicillin (C₁₆H₁₆N₂O₇S₂: 414.45).

Description Sulbenicillin Sodium occurs as white to light yellowish white powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Sulbenicillin Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Sulbenicillin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sulbenicillin Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> [α]D²⁰: +167° to +182° (1 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.20 g of Sulbenicillin Sodium in 10 mL of water is between 4.5 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 2.5 g of Sulbenicillin Sodium in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Sulbenicillin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sulbenicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Sulbenicillin Sodium in 15 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of these peaks by the area percentage method: the amount of the each peak other than the two peaks of sulbenicillin is not more than 2.0%, and the total amount of the peaks other than the two peaks of sulbenicillin is not more than 5.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecysilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 10 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.0 ± 0.1 with sodium hydroxide TS, and add water to make 1000 mL. To 940 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of the lately eluted peak of sulbenicillin is about 18 minutes.

Time span of measurement: About 1.5 times as long as the retention time of the lately eluted peak of sulbenicillin beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the total area of the two peaks of sulbenicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the resolution between the two peaks of sulbenicillin is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the total areas of the two peaks of sulbenicillin is not more than 5.0%.

Water <2.48> Not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.4 to 6.6 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Sulbenicillin Sodium RS, equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution in a freezer, and use within 4 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 40 μg (potency) and 10 μg (potency), and use these solutions as
the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Sulbenicillin Sodium, equivalent to about 50 mg (potency), and dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 40 μg (potency) and 10 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Sulfadiazine Silver

Sulfadiazine Silver occurs as a white to pale yellow, crystalline powder. It is odorless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in ammonia TS.

It is gradually colored by light.

Melting point: about 275°C (with decomposition).

Identification Determine the infrared absorption spectrum of Sulfadiazine Silver, previously dried, as directed in the past method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Sulfadiazine Silver RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Nitrile—To 250 mL of water add 1.0 g of Sulfadiazine Silver, shake well for 50 minutes, filter, and use this filtrate as the sample solution. Separately, weigh accurately 0.25 g of potassium nitrate, and dissolve in water to make exactly 2000 mL. Pipet 5 mL of this solution and add water to make exactly 200 mL, and use this solution as the standard solution. Pipet 2.0 mL each of the sample solution and the standard solution, and add 5 mL of a solution of chromotropic acid in sulfuric acid (1 in 10,000) and sulfuric acid to make exactly 10 mL. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with exactly 2.0 mL of water in the same manner, as the blank: $A_T$ is not larger than $A_S$ (not more than 0.05%).

(2) Related substances—Dissolve 50 mg of Sulfadiazine Silver in 5 mL of a mixture of ethanol (95) and ammonia solution (28) (3:2), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of ethanol (95) and ammonia solution (28) (3:2) to make exactly 20 mL. Pipet 2 mL of this solution, add a mixture of ethanol (95) and ammonia solution (28) (2:2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (10:5:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.47> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80%, 4 hours).

Residue on ignition <2.44> 41–45% (1 g).

Silver content Weigh accurately about 50 mg of Sulfadiazine Silver, previously dried, dissolve in 2 mL of nitric acid, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the sample solution. Measure accurately a suitable quantity of Standard Silver Solution for Atomic Absorption Spectrophotometry, dilute with water to make solutions containing 1.0 to 2.0 μg of silver (Ag:107.87) per mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <2.27> according to the following conditions, and calculate the silver content of the sample solution from the calibration curve obtained from the absorbances of the standard solutions: it contains not less than 28.7% and not more than 30.8% of silver.

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: A silver hollow cathode lamp.

Wavelength: 328.1 nm.

Assay Weigh accurately about 0.1 g each of Sulfadiazine Silver and Sulfadiazine Silver RS, each previously dried, and add ammonia TS to make exactly 100 mL, respectively. Pipet 1 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 255 nm, as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with exactly 1 mL of ammonia TS and a sufficient water to make exactly 100 mL, as the blank.

$$M_S = \frac{M_A}{A_T/A_S}$$

$M_S$: Amount (mg) of Sulfadiazine Silver RS

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.
Sulfamethizole

スルファメチゾール

C₉H₁₀N₄O₂S₂: 270.33
4-Amino-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide
[144-82-1]

Sulfamethizole, when dried, contains not less than 99.0% of C₉H₁₀N₄O₂S₂.

Description Sulfamethizole occurs as white to yellowish white crystals or crystalline powder. It is odorless.

It is slightly soluble in ethanol (95), and in acetic acid (100) and practical insoluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamethizole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 208 – 211°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Sulfamethizole in 3 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Sulfamethizole add 50 mL of water, warm at 70°C for 5 minutes, allow to stand in ice water for 1 hour, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Sulfamethizole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.17>—Prepare the test solution with 1.0 g of Sulfamethizole according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Sulfamethizole in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 50 mL, then pipet 5 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.63>.

Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100):20:1:1 to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.47> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Sulfamethizole, previously dried, dissolve in 5 mL of hydrochloric acid and 50 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool below 15°C, and titrate <2.50> with 0.1 mol/L sodium nitrite VS according to the potentiometric titration method or the amperometric titration method.

Each mL of 0.1 mol/L sodium nitrite VS = 27.03 mg of C₉H₁₀N₄O₂S₂

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Sulfamethoxazole

Sulfisomezole

スルファメトキサゾール

C₁₀H₁₁N₃O₃S: 253.28
4-Amino-N-(5-methylisoxazol-3-yl)benzenesulfonamide
[723-46-6]

Sulfamethoxazole, when dried, contains not less than 99.0% of C₁₀H₁₁N₃O₃S.

Description Sulfamethoxazole occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in N,N-dimethylformamide, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamethoxazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 169 – 172°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sulfamethoxazole in 5 mL of sodium hydroxide TS, and add 20 mL of water: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Sulfamethoxazole add 50 mL of water, heat at 70°C for 5 minutes, allow to stand in ice water for 1 hour, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Sul-
famethoxazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11> — Prepare the test solution with 1.0 g of Sulfamethoxazole according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Sulfamethoxazole in 10 mL of a solution of ammonia solution (28) in methanol (1 in 50), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of ammonia solution (28) in methanol (1 in 50) to make exactly 10 mL. Pipet 1 mL of this solution, add a solution of ammonia solution (28) in methanol (1 in 50) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetonitrile and diluted ammonia solution (28) (7 in 100) (10:8:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Sulfamethoxazole, previously dried, dissolve in 30 mL of N,N-dimethylformamide, add 10 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until a light blue color is produced (indicator: 0.5 mL of thymolphthalein TS). Separately, perform a blank determination in the same manner with a mixture of 30 mL of N,N-dimethylformamide and 26 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 25.33 mg of C10H11N3O3S

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

**Sulfamonomethoxine Hydrate**

スルファモノメトキシン水和物

C11H12N4O3S·H2O: 298.32

4-Amino-N-(6-methoxy pyrimidin-4-yl) benzenesulfonamide Monohydrate
[220-83-3, anhydride]

Sulfamonomethoxine Hydrate, when dried, contains not less than 99.0% of sulfamonomethoxine (C11H12N4O3S: 280.31).

**Description** Sulfamonomethoxine Hydrate occurs as white to pale yellow crystals, granules or crystalline powder. It is odorless.

It is soluble in acetone, slightly soluble in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

It is gradually colored by light.

**Identification** Determine the infrared absorption spectrum of Sulfamonomethoxine Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.60> 204 – 206°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Sulfamonomethoxine Hydrate in 5 mL of sodium hydroxide TS and 20 mL of water; the solution is clear and colorless to pale yellow. Dissolve 0.5 g of Sulfamonomethoxine Hydrate in 5 mL of sodium hydroxide TS, and heat: no turbidity is produced. After cooling, add 5 mL of acetone: the solution is clear.

(2) Heavy metals <1.07> — Proceed with 1.0 g of Sulfamonomethoxine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11> — Prepare the test solution with 1.0 g of Sulfamonomethoxine Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.02 g of Sulfamonomethoxine Hydrate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of l-butanol and ammonia solution (28) (4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not larger and not more intense than the spot from the standard solution.

**Loss on drying** <2.41> 4.5 – 6.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.10% (1 g).

**Assay** Weigh accurately about 0.5 g of Sulfamonomethoxine Hydrate, previously dried, dissolve in 5 mL of hydrochloric acid and 50 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool below 15°C, and titrate <2.50> with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS = 28.03 mg of C10H11N3O3S

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.
Sulfisoxazole

Sulfisoxazole occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in pyridine and in n-butylamine, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in acetic acid (100), and very slightly soluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid, in sodium hydroxide TS and in ammonia TS.

Identification

(1) Dissolve 0.01 g of Sulfisoxazole in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Dissolve 0.02 g of Sulfisoxazole in 5 mL of water and 1 mL of n-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. Add 5 mL of chloroform, shake, and allow to stand: a blue-green color develops in the chloroform layer.

(3) Dissolve 0.01 g of Sulfisoxazole in 1 mL of pyridine, add 2 drops of copper (II) sulfate TS, and shake. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a light yellow-brown color develops in the chloroform layer.

(4) To 0.5 g of Sulfisoxazole add 2 mL of acetic acid (100), dissolve by heating under a reflux condenser, add 1 mL of acetic anhydride, and boil for 10 minutes. Add 10 mL of water, cool, and alkalize with about 7 mL of a solution of sodium hydroxide (3 in 10). Filter, if necessary, immediately acidify by adding acetic acid (100) dropwise, collect the produced precipitate, recrystallize from methanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 208°C and 210°C.

Melting point <2.60> 192 – 196°C (with decomposition).

Purity

(1) Clarity and color of solution—Dissolve 1.0 g of Sulfisoxazole in 5 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless to pale yellow.

(2) Acidity—To 1.0 g of Sulfisoxazole add 50 mL of water, warm at 70°C for 5 minutes, allow to stand in an ice bath for 1 hour, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Sulfisoxazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.47> Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay

Weigh accurately about 1 g of Sulfisoxazole, previously dried, dissolve in 50 mL of methanol by warming, cool and titrate <2.50> with 0.2 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination using a mixture of 50 mL of methanol and 18 mL of water, and make any necessary correction.

Each mL of 0.2 mol/L sodium hydroxide VS = 53.46 mg of C_{11}H_{13}N_{3}O_{3}S.

Containers and storage

Containers—Well-closed containers.

Storage—Light-resistant.

Sulfobromophthalein Sodium

Sulfobromophthalein Sodium occurs as a white, crystalline powder. It is odorless.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification

(1) Dissolve 0.02 g of Sulfobromophthalein Sodium in 5 mL of water and 1 mL of n-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a blue-green color develops in the chloroform layer.

(2) Dissolve 0.02 g of Sulfobromophthalein Sodium in 5 mL of water and 1 mL of n-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a blue-green color develops in the chloroform layer.

(3) Dissolve 0.01 g of Sulfobromophthalein Sodium in 1 mL of pyridine, add 2 drops of copper (II) sulfate TS, and shake. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a light yellow-brown color develops in the chloroform layer.

(4) To 0.5 g of Sulfobromophthalein Sodium add 2 mL of acetic acid (100), dissolve by heating under a reflux condenser, add 1 mL of acetic anhydride, and boil for 10 minutes. Add 10 mL of water, cool, and alkalize with about 7 mL of a solution of sodium hydroxide (3 in 10). Filter, if necessary, immediately acidify by adding acetic acid (100) dropwise, collect the produced precipitate, recrystallize from methanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 208°C and 210°C.

Melting point <2.60> 192 – 196°C (with decomposition).

Purity

(1) Clarity and color of solution—Dissolve 1.0 g of Sulfobromophthalein Sodium in 5 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless to pale yellow.

(2) Acidity—To 1.0 g of Sulfobromophthalein Sodium add 50 mL of water, warm at 70°C for 5 minutes, allow to stand in an ice bath for 1 hour, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Sulfobromophthalein Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.47> Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay

Weigh accurately about 1 g of Sulfobromophthalein Sodium, previously dried, dissolve in 50 mL of methanol by warming, cool and titrate <2.50> with 0.2 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination using a mixture of 50 mL of methanol and 18 mL of water, and make any necessary correction.

Each mL of 0.2 mol/L sodium hydroxide VS = 53.46 mg of C_{20}H_{8}Br_{4}Na_{2}O_{10}S_{2}.

Containers and storage

Containers—Well-closed containers.

Storage—Light-resistant.
Determine the absorbance \(A\) of a solution of 1.0 g of Sulfobromophthalein Sodium in 20 mL of water is between 4.0 and 5.5.

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Sulfobromophthalein Sodium in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride \(<1.03\>—Perform the test with 2.0 g of Sulfobromophthalein Sodium. Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.002%).

(3) Sulfate—To 10 mL of a solution of Sulfobromophthalein Sodium (1 in 500) add 5 drops of dilute hydrochloric acid, heat to boil, and add 1 mL of hot barium chloride TS: the solution is clear when observed 1 minute after the addition of the barium chloride TS.

(4) Calcium—Weigh accurately about 5 g of Sulfobromophthalein Sodium, transfer to a porcelain dish, heat gently to char, and heat strongly between 700–750°C until the residue is incinerated. After cooling, add 10 mL of dilute hydrochloric acid, and heat for 5 minutes on a water bath. Transfer the contents to a flask with 50 mL of water, and add 5 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator. Titrate \(<2.50\> with 0.01 mol/L disodium dihydrogen ethylenediamine tetracacetate VS until the red-purple color of the solution changes to blue.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetracacetate VS

\[= 0.4008 \text{ mg of Ca} \]

The content of calcium (Ca: 40.08) is not more than 0.05%.

(5) Heavy metals \(<0.07\>—Proceed with 1.0 g of Sulfobromophthalein Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic \(<1.11\>—Transfer 0.65 g of Sulfobromophthalein Sodium to a crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), fire to burn, then heat gently until the residue is incinerated. If any carbon remains, moisten the residue with a small amount of nitric acid, and incinerate again by ignition. After cooling, add 10 mL of dilute sulfuric acid, and heat until white fumes are evolved. After cooling, add 5 mL of water to the residue, and perform the test with this solution as the test solution (not more than 3.1 ppm).

**Loss on drying** \(<2.41\> Not more than 5.0% (0.5 g, 105°C, 3 hours).

**Residue on ignition** \(<2.44\> 14 – 19% (after drying, 0.5 g, 700 – 750°C).

**Assay** Dissolve about 0.1 g of Sulfobromophthalein Sodium, previously dried and accurately weighed, in water to make exactly 500 mL. Pipet 5 mL of this solution, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>.

Determine the absorbance \(A\) of this solution at the wavelength of maximum absorption at about 580 nm, using water as the blank.

Amount (mg) of \(\text{C}_{20}\text{H}_{35}\text{Br}_{4}\text{Na}_{2}\text{O}_{10}\text{S}_{2}\)

\[= A/881 \times 200,000 \]

**Containers and storage** Containers—Tight containers.

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**Sulfobromophthalein Sodium Injection**

スルホプロモフタレインナトリウム注射液

Sulfobromophthalein Sodium Injection is an aqueous solution for Injection.

It contains not less than 94.0% and not more than 106.0% of the labeled amount of sulfobromophthalein sodium \((\text{C}_{20}\text{H}_{35}\text{Br}_{4}\text{Na}_{2}\text{O}_{10}\text{S}_{2}: 838.00)\).

**Method of preparation** Prepare as directed under Injections, with Sulfobromophthalein Sodium.

**Description** Sulfobromophthalein Sodium Injection is a clear and colorless or pale yellow liquid.

**pH** 5.0 – 6.0

**Identification** (1) Measure a volume of Sulfobromophthalein Sodium Injection, equivalent to 0.02 g of Sulfobromophthalein Sodium according to the labeled amount, and proceed as directed in the Identification (1) under Sulfobromophthalein Sodium.

(2) Measure a volume of Sulfobromophthalein Sodium Injection, equivalent to 0.1 g of Sulfobromophthalein Sodium according to the labeled amount, add 0.5 g of anhydrous sodium carbonate, and evaporate on a water bath to dryness. Ignite the residue until it is charred. Proceed as directed in the Identification (2) under Sulfobromophthalein Sodium.

**Pyrogen** \(<4.04\> Add isotonic sodium chloride solution to Sulfobromophthalein Sodium Injection to make a 0.5 w/v% solution of Sulfobromophthalein Sodium according to the labeled amount. Inject into each of the rabbits 5 mL of this solution per kg of body mass: it meets the requirement.

**Extractable volume** \(<6.05\> It meets the requirement.

**Assay** Measure exactly a volume of Sulfobromophthalein Sodium Injection, equivalent to about 0.1 g of sulfobromophthalein sodium \((\text{C}_{20}\text{H}_{35}\text{Br}_{4}\text{Na}_{2}\text{O}_{10}\text{S}_{2})\), add water to make exactly 500 mL, and proceed as directed in the Assay under Sulfobromophthalein Sodium.

Amount (mg) of sulfobromophthalein sodium

\[(\text{C}_{20}\text{H}_{35}\text{Br}_{4}\text{Na}_{2}\text{O}_{10}\text{S}_{2}) = A/881 \times 200,000 \]

**Containers and storage** Containers—Hermetic containers. Storage—Light-resistant.
**Sulfur**

イオウ

S: 32.07

Sulfur, when dried, contains not less than 99.5% of S.

**Description**  Sulfur occurs as a light yellow to yellow powder. It is odorless and tasteless. It is freely soluble in carbon disulfide, and practically insoluble in water, in ethanol (95) and in diethyl ether.

**Identification (1)**  Ignite Sulfur: it burns with a blue flame and gives a pungent odor of sulfur dioxide.

(2)  Dissolve 5 mg of Sulfur in 5 mL of sodium hydroxide TS by heating in a water bath, cool, and add 1 drop of sodium pentacyanonitrosylferrate (III) TS: a blue-purple color develops.

(3)  Boil 1 mg of sulfur with 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS: a blue color develops.

**Purity (1)**  Clarity of solution—Dissolve 1.0 g of Sulfur in a mixture of 20 mL of a solution of sulfur dioxide (1 in 6) and 2 mL of ethanol (95) by boiling: the solution is clear. Dissolve 2.0 g of Sulfur in 10 mL of carbon disulfide: the solution is almost clear or slightly opalescent.

(2)  Acidity or alkalinity—Shake 2.0 g of Sulfur with 50 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS: no red color develops. Further add 1.0 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3)  Arsenic <1.11>—Prepare the test solution with 0.20 g of Sulfur according to Method 3, and perform the test (not more than 10 ppm).

**Loss on drying <2.47>**  Not more than 1.0% (1 g, in vacuum, not more than 0.67 kPa, silica gel, 4 hours).

**Residue on ignition <2.44>**  Not more than 0.2% (1 g).

**Assay**  Weigh accurately about 0.4 g of Sulfur, previously dried, dissolve in 20 mL of potassium hydroxide-ethanol TS and 10 mL of water by boiling, cool, and add water to make exactly 100 mL. Transfer exactly 25 mL of the solution to a 400-mL beaker, add 50 mL of hydrogen peroxide TS, and heat on a water bath for 1 hour. Acidify the solution with dilute hydrochloric acid, add 200 mL of water, heat to boil, add hot barium chloride TS dropwise until no more precipitate is formed, and heat on a water bath for 1 hour. Collect the precipitate, and wash with water until the last washing shows no opalescence with silver nitrate TS. Dry the precipitate, heat strongly to constant mass, and weigh as barium sulfate (BaSO₄: 233.39). Perform a blank determination, and make any necessary correction.

Amount (mg) of S  = amount (mg) of barium sulfate (BaSO₄) × 0.13739

**Containers and storage**  Containers—Well-closed containers.

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**Sulfur and Camphor Lotion**

イオウ・カンフルローション

**Method of preparation**

<table>
<thead>
<tr>
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<th>Amount (mg)</th>
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<tbody>
<tr>
<td>Sulfur</td>
<td>60 g</td>
</tr>
<tr>
<td>dl-Camphor or dl-Camphor</td>
<td>5 g</td>
</tr>
<tr>
<td>Hydroxypropylcellulose</td>
<td>4 g</td>
</tr>
<tr>
<td>Calcium Hydroxide</td>
<td>1 g</td>
</tr>
<tr>
<td>Ethanol</td>
<td>4 mL</td>
</tr>
<tr>
<td>Water, Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Dissolve Hydroxypropylcellulose in 200 mL of Water, Purified Water or Purified Water in Containers. Add this solution in small portions to the triturate of Sulfur with the Ethanol solution of dl-Camphor or dl-Camphor, and triturate again the mixture. Separately, dissolve Calcium Hydroxide in 300 mL of Water, Purified Water or Purified Water in Containers, stopper tightly, shake, and allow to stand. Add 300 mL of this supernatant liquid to the above mixture, then add Water, Purified Water or Purified Water in Containers to make 1000 mL, and shake thoroughly.

**Description**  Sulfur and Camphor Lotion is a light yellow suspension.

A part of the components separates out on standing.

**Identification (1)**  To 5 mL of well shaken Sulfur and Camphor Lotion add 25 mL of water, and centrifuge [use this supernatant liquid for test (3)]. To 0.02 g of the precipitate add 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS, and boil: a blue color develops (sulfur).

(2)  To 10 mL of well shaken Sulfur and Camphor Lotion add 5 mL of diethyl ether, and mix. Separate the diethyl ether layer, and filter through a pledge of cotton. Wash the cotton with a small portion of diethyl ether, combine the washings with the filtrate, and distil cautiously on a water bath to remove the diethyl ether. Dissolve the residue in 1 mL of methanol, add 1 mL of 2,4-dinitrophenylhydrazine TS, and heat for about 2 minutes on a water bath. Cool, dilute with water to make about 5 mL, and allow to stand. Filter the produced precipitate through a glass filter (G4), and wash the residue on the filter with water until the last washing is colorless. Dissolve the residue in 10 mL of ethanol (95), add 5 mL of sodium hydroxide TS, and allow to stand for 2 minutes: a red color develops (dl-camphor or dl-camphor).

(3)  The supernatant liquid obtained in (1) responds to the Qualitative Tests <1.69> (2) and (3) for calcium salt.

**Containers and storage**  Containers—Tight containers.
Sulfur, Salicylic Acid and Thianthol Ointment

スリンダク

**Identification (1)** Stir well 0.5 g of Sulfur, Salicylic Acid and Thianthol Ointment with 10 mL of water while heating, cool, and filter. To 1 mL of the filtrate add 5 mL of iron (III) nitrate TS: a purple color is produced (salicylic acid).

(2) Shake 1 g of Sulfur, Salicylic Acid and Thianthol Ointment with 20 mL of diethyl ether, remove the supernatant liquid and floating materials. Wash the residue with 10 mL of diethyl ether, and remove the diethyl ether by suction. To the residue add 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS, and boil: a light blue to blue color is produced (sulfur).

(3) To 1 g of Sulfur, Salicylic Acid and Thianthol Ointment add 15 mL of ethanol (95), stir well while warming on a water bath, cool, and filter. Use the filtrate as the sample solution. Dissolve 0.01 g each of salicylic acid and thianthol in water bath, cool, and filter. Use the filtrate as the sample solution add 15 mL of ethanol (95), stir well while warming on a water bath, cool, and filter.

**Identification (2)** Determine the infrared absorption spectrum of Sulindac as directed in the potassium bromide disc method under Infrared Spectrophotometry 2.2.5 and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)** Heavy metals 

Sulindac, when dried, contains not less than 99.0% and not more than 101.0% of C_{20}H_{17}FO_{3}S.

**Description** Sulindac occurs as a yellow, crystalline powder.

It is sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

A solution of Sulindac in methanol (1 in 100) shows no optical rotation.

Melting point: about 184°C (with decomposition).

**Identification (1)** Dissolve 15 mg of Sulindac in 1000 mL of a solution of hydrochloric acid in methanol (1 in 120). Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry 2.2.4 and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Related substances**—Dissolve 0.25 g of Sulindac in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample solution and add methanol to make exactly 100 mL. Pipet 5 mL, 4 mL and 2 mL of this solution, to each add methanol to make exactly 10 mL, and use these solutions as the standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thin-layer Chromatography 2.2.9. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the total intensity of spots other than the principal spot from the sample solution is not more than 1.0% calculated.
on the basis of intensities of the spots from the standard solution (1), (2) and (3).

4. Residual solvent Being specified separately.

Loss on drying 2.41 Not more than 0.5% (1 g, in vacuum not exceeding 0.7 kPa, 100°C, 2 hours).

Residue on ignition 2.44 Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.3 g of Sulindac, previously dried, dissolve in 50 mL of methanol and titrate 2.50 with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 35.64 mg of C₉H₁₅O₄S

Containers and storage Containers—Tight containers.

Sulpiride

スルピリド

C₁₅H₂₃N₃O₄S: 341.43
N-(1-Ethylpyrolidin-2-ylmethyl)-2-methoxy-5-sulfamoylbenzamide

Sulpiride, when dried, contains not less than 98.5% and not more than 101.0% of C₁₅H₂₃N₃O₄S.

Description Sulpiride is a white, crystalline powder.

It is freely soluble in acetic acid (100) and in dilute acetic acid, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

It is soluble in 0.05 mol/L sulfuric acid TS.

A solution of Sulpiride in methanol (1 in 100) shows no optical rotation.

Melting point: about 178°C (with decomposition).

Identification (1) Dissolve 0.1 g of Sulpiride in 0.05 mol/L sulfuric acid TS to make 100 mL. Dilute 5 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry 2.24, using water as the blank, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

2. Determine the infrared absorption spectrum of Sulpiride as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity of solution—Dissolve 2.0 g of Sulpiride in 7 mL of dilute acetic acid, and add water to make 20 mL: the solution is clear. Perform the test with the solution as directed under Ultraviolet-visible Spectrophotometry 2.24, using water as the blank: the absorbance at a wavelength of 450 nm does not exceed 0.020.

2. Heavy metals 1.07—Proceed with 2.0 g of Sulpiride as directed under Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Sulpiride in 10 mL of methanol, and use this solution as the sample solution. Dilute 1 mL of the sample solution, accurately measured, with methanol to make exactly 100 mL. Dilute 2 mL of this solution, accurately measured, with methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 20 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spots other than the principal spot from the sample solution is not more than 2, and they have no more intense than the spot from the standard solution. When the plate is exposed to iodine vapor for 30 minutes, the number of the spots other than the principal spot from the sample solution is not more than 2, and they have no more intense than the spot from the standard solution.

Loss on drying 2.41 Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition 2.44 Not more than 0.1% (1 g).

Assay Dissolve about 0.4 g of Sulpiride, previously dried and accurately weighed, in 80 mL of acetic acid (100), and titrate 2.50 with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS) until the color of the solution changes from violet through blue to bluish green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 34.14 mg of C₁₅H₂₃N₃O₄S

Containers and storage Containers—Well-closed containers.

Sulpiride Capsules

スルピリドカプセル

Sulpiride Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of sulpiride (C₁₅H₂₃N₃O₄S: 341.43).

Method of preparation Prepare as directed under Capsules, with Sulpiride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry 2.24, using water as the blank: it exhibits a maximum between 289 nm and 293 nm.

Uniformity of dosage units 6.02 Perform the test according to the following method: it meets the requirement of the
Content uniformity test.

To 1 capsule of Sulpiride Capsules add 30 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL of the solution contains about 1 mg of sulpiride (C₁₅H₂₃N₃O₄S), and filter the solution. Discard the first 20 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of sulpiride (C₁₅H₂₃N₃O₄S)} = M_S \times \frac{A_T}{A_S} \times \frac{V}{50}
\]

M₅: Amount (mg) of sulpiride for assay

Dissolution

Cut the capsule of not less than 20 Sulpiride Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of sulpiride (C₁₅H₂₃N₃O₄S), add 70 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, and add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of sulpiride (C₁₅H₂₃N₃O₄S)} = M_S \times \frac{A_T}{A_S} \times 2
\]

M₅: Amount (mg) of sulpiride for assay

Containers and storage

Containers—Tight containers.

Sulpiride Tablets

スルピリド錠

Sulpiride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sulpiride (C₁₅H₂₃N₃O₄S: 341.43).

Method of preparation

Prepare as directed under Tablets, with Sulpiride.

Identification

Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: it exhibits a maximum between 289 nm and 293 nm.

Uniformity of dosage units

Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sulpiride Tablets add 30 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL of the solution contains about 1 mg of sulpiride (C₁₅H₂₃N₃O₄S), and filter the solution. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of sulpiride (C₁₅H₂₃N₃O₄S)} = M_S \times \frac{A_T}{A_S} \times V/50
\]

M₅: Amount (mg) of sulpiride for assay

Dissolution

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate of a 50-mg tablet in 30 minutes is not less than 80%, and that of a 100-mg tablet in 45 minutes is not less than 75%, and that of a 200-mg tablet in 45 minutes is not less than 70%.

Start the test with 1 tablet of Sulpiride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 56 μg of sulpiride (C₁₅H₂₃N₃O₄S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mL sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, Aₕ and Aₘ, of the sample solution and standard solution at 291 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

\[
\text{Dissolution rate (%, with respect to labeled amount of sulpiride (C₁₅H₂₃N₃O₄S)} = M_S \times \frac{A_T}{A_S} \times \frac{V}{V/V \times 1/C \times 180}
\]

M₅: Amount (mg) of sulpiride for assay

C: Labeled amount (mg) of sulpiride (C₁₅H₂₃N₃O₄S) in 1 tablet

Assay

Weigh accurately, and powder not less than 20 Sulpiride Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of sulpiride (C₁₅H₂₃N₃O₄S), add 70 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, and add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mL sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, Aₕ and Aₘ, of the sample solution and standard solution at 291 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

\[
\text{Amount (mg) of sulpiride (C₁₅H₂₃N₃O₄S)} = M_S \times \frac{A_T}{A_S} \times 2
\]

M₅: Amount (mg) of sulpiride for assay

Containers and storage

Containers—Tight containers.
Sulpyrine Hydrate

スルピリン水和物

C_{13}H_{16}N_3NaO_4S.H_2O: 351.35
Monosodium [(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)(methyl)amino]methanesulfonate monohydrate [5907-38-0]

Sulpyrine Hydrate contains not less than 98.5% of sulpyrine (C_{13}H_{16}N_3NaO_4S: 333.34), calculated on the dried basis.

Description Sulpyrine Hydrate occurs as white to light yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is colored by light.

Identification (1) Add 2 drops of dilute sulfuric acid and 1 mL of chlorinated lime TS to 3 mL of a solution of Sulpyrine Hydrate (1 in 15): a deep blue color develops at first, but the color immediately turns red, then gradually changes to yellow.

(2) Boil 5 mL of a solution of Sulpyrine Hydrate (1 in 25) with 3 mL of dilute hydrochloric acid: the odor of sulfur dioxide is perceptible at first, and on further boiling, the odor of formaldehyde is perceptible.

(3) A solution of Sulpyrine Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Clarity of solution, and acidity or alkalinity—Dissolve 1.0 g of Sulpyrine Hydrate in 10 mL of water: the solution is clear and neutral.

(2) Sulfate <1.14>—Dissolve 0.20 g of Sulpyrine Hydrate in 0.05 mol/L hydrochloric acid VS to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS and 0.05 mol/L hydrochloric acid VS to make 50 mL (not more than 0.120%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Sulpyrine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Merbuline—Transfer 0.10 g of Sulpyrine Hydrate with 2 mL of water and 1 mL of dilute sulfuric acid into a flask, cover with a funnel, and boil gently for 15 minutes. Cool, add 2 mL of a solution of sodium acetate trihydrate (1 in 2) and water to make 5 mL, shake this solution with 5 mL of benzaldehyde-saturated solution, and allow to stand for 5 minutes: the solution is clear.

(5) Chloroform-soluble substances—Mix, by frequent shaking, 1.0 g of Sulpyrine Hydrate and 10 mL of chloroform for 30 minutes. Collect the precipitate, wash with two 5-mL portions of chloroform, combine the washings with the filtrate, and evaporate on a water bath to dryness. Dry the residue at 105°C for 4 hours: the mass of the residue is not more than 5.0 mg.

Loss on drying <2.4> Not more than 6.0% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.25 g of Sulpyrine Hydrate, dissolve in 100 mL of diluted hydrochloric acid (1 in 20), previously cooled below 10°C. Titrate <2.50> immediately with 0.05 mol/L iodine VS while keeping the temperature between 5°C and 10°C, until the color of the solution remains blue upon shaking vigorously for 1 minute after the addition of 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS = 16.67 mg of C_{13}H_{16}N_3NaO_4S

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Sulpyrine Injection

スルピリン注射液

Sulpyrine Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sulpyrine hydrate (C_{13}H_{16}N_3NaO_4S.H_2O: 351.35).

Method of preparation Prepare as directed under Injections, with Sulpyrine Hydrate.

Description Sulpyrine Injection is a clear, colorless or pale yellow liquid.

pH: 5.0 – 8.5

Identification (1) To a volume of Sulpyrine Injection, equivalent to 0.2 g of Sulpyrine Hydrate according to the labeled amount, add water to make 3 mL, then add 2 drops of dilute sulfuric acid and 1 mL of chlorinated lime TS: a deep blue color develops at first, and the color immediately turns red and gradually changes to yellow.

(2) To a volume of Sulpyrine Injection, equivalent to 0.2 g of Sulpyrine Hydrate according to the labeled amount, add water to make 5 mL, and boil this solution with 3 mL of dilute hydrochloric acid: the odor of sulfur dioxide is perceptible at first, and on further boiling the odor of formaldehyde is perceptible.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet 2 mL of Sulpyrine Injection, dilute with water to exactly 100 mL. Measure exactly a volume (V mL) of this solution, equivalent to about 50 mg of sulpyrine hydrate (C_{13}H_{16}N_3NaO_4S.H_2O), and add water to make exactly 100
mL. Pipet 5 mL of this solution, add water to exactly 100 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of sulpyrine for assay (previously determine the loss on drying $<2.4\%$ in the same conditions as Sulpyrine Hydrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and standard solution into separate 25-mL volumetric flasks, add 5 mL of ethanol (95), 2 mL each of the sample solution and standard solution into 100 mL, and use this solution as the standard solution. Pipet solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 2 mL of water in the same manner as the blank. Determine the absorbance, $A_1$ and $A_2$, of the subsequent solutions of the sample solution and the standard solution at 510 nm.

Amount (mg) of sulpyrine hydrate ($C_{13}H_{16}N_3NaO_4S.H_2O$) in 1 mL of Sulpyrine Injection:

$$M_s = A_1/A_2 \times V/V \times 1.054$$

$M_s$: Amount (mg) of sulpyrine for assay, calculated on the dried basis

Containers and storage—Containers—Hermetic containers, and colored containers may be used. Storage—Light-resistant, and under nitrogen atmosphere.

### Sultamicillin Tosilate Hydrate

スルタミシリントシル酸塩水和物

C$_{25}$H$_{30}$N$_4$O$_9$S$_2$.C$_7$H$_8$O$_3$S.2H$_2$O: 802.89

$\text{[2S,5R]-3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2-ylcarbonyloxy)methyl}$

$\text{(2S,5R,6R)-6-[1(2R)-2-amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate mono-4-toluenesulfonate dihydrate [83105-70-8, anhydride]}$

Sultamicillin Tosilate Hydrate contains not less than 698 μg (potency) and not more than 800 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of residual solvent. The potency of Sultamicillin Tosilate Hydrate is expressed as mass (potency) of sultamicillin ($C_{25}H_{30}N_4O_9S_2$: 594.66).

**Description** Sultamicillin Tosilate Hydrate occurs as a white to yellowish white crystalline powder. It is freely soluble in acetonitrile, in methanol and in ethanol (99.5), and very slightly soluble in water.

**Identification** Determine the infrared absorption spectrum of Sultamicillin Tosilate Hydrate as directed in the past method under Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum or the spectrum of Sultamicillin Tosilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** $<2.49>$ $\left[\alpha\right]_D^2 = +173 - +187^\circ$ (0.5 g calculated on the anhydrous bases, a mixture of water and acetonitrile (3:2), 25 mL, 100 mm).

**Purity (1)** Heavy metals $<1.07>$—Proceed with 1.0 g of Sultamicillin Tosilate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL Standard Lead Solution (not more than 20 ppm).

**Purity (2)** Arsenic $<1.11>$—Prepare the test solution with 1.0 g of Sultamicillin Tosilate Hydrate, according to Method 3, and perform the test (not more than 2 ppm).

**Purity (3)** Ampicillin—Perform the procedure rapidly. Weigh accurately about 20 mg of Sultamicillin Tosilate Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 20 mg (potency), dissolve in the mobile phase to make exactly 100 mL. Pipet 6 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and determine the area of the peak of ampicillin by the automatic integration method: the peak area from the sample solution is not larger than that from the standard solution.

**Operating conditions**—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in about 750 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL. To 80 mL of acetonitrile for liquid chromatography add this solution to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 14 minutes.

**System suitability**—

System performance: Dissolve 12 mg of Ampicillin RS, 4 mg of Sulbactam RS and 4 mg of p-toluenesulfonic acid monohydrate in 1000 mL of the mobile phase. When the procedure is run with 25 μL of this solution under the above operating conditions, sulbactam, p-toluenesulfonic acid and ampicillin are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ampicillin is not more than 2.0%.

**(4)** Sulbactam—Perform the procedure rapidly. Weigh accurately about 20 mg of Sultamicillin Tosilate Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Sulbactam RS, equivalent to about 20 mg (potency), dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following con-
ditions, and determine the area of the peak of sulbactam by the automatic integration method: the peak area from the sample solution is not larger than that from the standard solution.

**Operating conditions—**
- Proceed as directed in the operating conditions in the Purity (3).

**System suitability—**
- Proceed as directed in the system suitability in the Purity (3).

(5) Penicilloic acids—Weigh accurately about 25 mg of Sultamicillin Tosilate Hydrate, dissolve in 1 mL of acetonitrile, and add 25 mL of 0.02 mol/L phosphate buffer solution, pH 3.0, in a 100-mL flask with stopper. Add exactly 5 mL of 0.005 mol/L iodine VS, and allow to stand the stopped flask for 5 minutes. Titrate <2.50> with 0.005 mol/L sodium thiosulfate VS (indicator: 1.0 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction. Calculate the amount of penicilloic acid (C₂₅H₃₄N₄O₁₁S₂: 630.70) by using the following equation: it is not more than 3.0%.

\[
\text{Each mL of 0.005 mol/L sodium thiosulfate VS} = 0.2585 \text{ mg of C₂₅H₃₄N₄O₁₁S₂}
\]

(6) Residual solvent <2.40>—Weigh accurately about 0.1 g of Sultamicillin Tosilate Hydrate, dissolve in 2 mL of methanol, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of ethyl acetate, and mix with water to make exactly 200 mL. Pipet 2 mL of this solution, add 10 mL of methanol and water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas, A₁ and Aₛ, of ethyl acetate of these solutions. Calculate the amount of ethyl acetate by the following equation: not more than 2.0%.

\[
\text{Amount (mg) of ethyl acetate} = \frac{Mₛ}{M₁} \times \frac{A₁}{Aₛ} \times 1/5
\]

**Containers and storage**
- Containers—Tight containers.

**Operating conditions—**
- Detector: A hydrogen flame-ionization detector.
- Column: A column 3 mm in inside diameter and 1 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0085 μm, 300 – 400 m²/g) (150 to 180 μm in particle diameter).
- Column temperature: A constant temperature of about 155°C.
- Carrier gas: Nitrogen.
- Flow rate: Adjust the flow rate so that the retention time of ethyl acetate is about 6 minutes.

**System suitability—**
- System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ethyl acetate are not less than 500 steps and not more than 1.5, respectively.
- System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ethyl acetate is not more than 5%.

**Water <2.48>**
- 4.0 – 6.0% (0.5 g, volumetric titration, direct titration).

**Residue on ignition <2.44>**
- Not more than 0.2% (1 g).

**Assay**
- Perform the procedure rapidly. Weigh accurately an amount of Sultamicillin Tosilate Hydrate and Sultamicillin Tosilate RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 50 mL. Pipet 5 mL of each of these solutions, add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Qₛ, of the peak area of sultamicillin to that of the internal standard of each solution.

\[
\text{Amount [μg (potency)] of sultamicillin (C₂₅H₃₀N₄O₉S₂)} = \frac{Mₛ}{M₁} \times Q₁/Qₛ \times 1000
\]

**Official Monographs**
Sultiame

スルチアム

\[ \text{C}_{10}\text{H}_{14}\text{N}_{2}\text{O}_{4}\text{S}_{2} : 290.36 \]
\[ 4-(3,4,5,6-\text{Tetrahydro-2H-1,2-thiazin-2-yl})\text{benzenesulfonamide} \text{ S, S-dioxide} \]

Sultiame, when dried, contains not less than 98.5\% of \text{C}_{10}\text{H}_{14}\text{N}_{2}\text{O}_{4}\text{S}_{2}.

**Description**

Sultiame occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in \( N \),\( N \)-dimethylformamide, freely soluble in \( n \)-butylamine, slightly soluble in methanol and in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

**Identification** (1) Dissolve 0.02 g of Sultiame in 5 mL of water and 1 mL of \( n \)-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. To this solution add 5 mL of chloroform, shake, and allow to stand: a green color develops in the chloroform layer.

(2) Mix 0.1 g of Sultiame with 0.5 g of sodium carbonate decahydrate, and melt carefully: the gas evolved changes moistened red litmus paper to blue. After cooling, crush the fused substance with a glass rod, stir with 10 mL of water, and filter. To 4 mL of the filtrate add 2 drops of hydrogen peroxide (30), 5 mL of diluted hydrochloric acid (1 in 5) and 2 to 3 drops of barium chloride TS: a white precipitate is formed.

(3) Determine the absorption spectrum of a solution of Sultiame in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point** <2.60> 185 – 188°C

**Purity** (1) Chloride <1.03>—Dissolve 1.0 g of Sultiame in 20 mL of sodium hydroxide TS by warming, cool, and add 2 mL of acetic acid (100) and water to make 100 mL. After shaking, filter, and discard the first 10 mL of the filtrate. To 40 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 8 mL of sodium hydroxide TS, 4.2 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Sultiame in 20 mL of sodium hydroxide TS by warming, cool, and add 8 mL of dilute hydrochloric acid and water to make 100 mL. After shaking, filter, and discard the first 10 mL of the filtrate. To 40 mL of the subsequent filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Sultiame according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sultiame according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Sultiame in methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of sulfanilamide in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 \( \mu \)L of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (30:8:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.43> Not more than 0.5\% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1\% (1 g).

**Assay** Weigh accurately about 0.8 g of Sultiame, previously dried, dissolve in 70 mL of \( N \),\( N \)-dimethylformamide, and titrate <2.50> with 0.2 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.2 mol/L tetramethylammonium hydroxide VS
\[ = 58.07 \text{ mg of C}_{10}\text{H}_{14}\text{N}_{2}\text{O}_{4}\text{S}_{2} \]

**Containers and storage** Containers—Well-closed containers.

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**Suxamethonium Chloride Hydrate**

スキサメトニウム塩化物水和物

\[ \text{C}_{14}\text{H}_{30}\text{Cl}_{2}\text{N}_{2}\text{O}_{4} : 361.31 \]

Suxamethonium Chloride Hydrate contains not less than 98.0\% of suxamethonium chloride (\text{C}_{14}\text{H}_{16}\text{Cl}_{2}\text{N}_{2}\text{O}_{4}: 361.31), calculated on the anhydrous basis.

**Description**

Suxamethonium Chloride Hydrate occurs as a
white, crystalline powder.

It is freely soluble in water, in methanol and in acetic acid (100), slightly soluble in ethanol (95), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

**Identification (1)** Determine the infrared absorption spectrum of Suxamethonium Chloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Suxamethonium Chloride Hydrate (1 in 20) responds to the Qualitative Tests $<1.09>$ for chloride.

**pH** $<2.54>$ The pH of a solution of Suxamethonium Chloride Hydrate (1 in 100) is between 4.0 and 5.0.

**Melting point** $<2.60>$ 159 – 164°C (hydrate form).

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Suxamethonium Chloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 0.25 g of Suxamethonium Chloride Hydrate in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.05>$. Spot 1 $\mu$L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium acetate (1 in 100), acetone, n-butanol and formic acid (20:20:20:1) to a distance of about 10 cm, and dry the plate at 105°C for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)–potassium iodide TS on the plate: the spots obtained from the sample solution and standard solution are not more intense than the spot from the standard solution.

**Water** $<2.48>$ 8.0 – 10.0% (0.4 g, volumetric titration, direct titration).

**Residue on ignition** $<2.44>$ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Suxamethonium Chloride Hydrate, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate $<2.50>$ with 0.1 mol/L perchloric acid VS (potentiometric titration).

Each mL of 0.1 mol/L perchloric acid VS $= 18.07$ mg of $C_{14}H_{30}Cl_2N_2O_4$

**Containers and storage** Containers—Tight containers.

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**Suxamethonium Chloride for Injection**

注射用スキサメトニウム塩化物

Suxamethonium Chloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$; 361.31).

The concentration of Suxamethonium Chloride for Injection should be stated as the amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$).

**Method of preparation** Prepare as directed under Injections, with Suxamethonium Chloride Hydrate.

**Description** Suxamethonium Chloride for Injection occurs as a white, crystalline powder or mass.

**Identification** Take an amount of Suxamethonium Chloride for Injection, equivalent to 0.05 g of Suxamethonium Chloride Hydrate according to the labeled amount, dissolve in water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of suxamethonium chloride for thin-layer chromatography in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.05>$. Spot 1 $\mu$L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium acetate (1 in 100), acetone, 1-butanol and formic acid (20:20:20:1) to a distance of about 10 cm, and dry the plate at 105°C for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)–potassium iodide TS on the plate: the spots obtained from the sample solution and standard solution are blue-purple in color and have similar $Rf$ value.

**pH** $<2.54>$ The pH of a solution of Suxamethonium Chloride for Injection (1 in 100) is between 4.0 and 5.0.

**Purity** Related substances—Take an amount of Suxamethonium Chloride for Injection, equivalent to 0.25 g of Suxamethonium Chloride Hydrate according to the labeled amount, and proceed as directed in the Purity (2) under Suxamethonium Chloride Hydrate.

**Bacterial endotoxins** $<4.01>$ Less than 1.5 EU/mg.

**Uniformity of dosage units** $<6.02>$ It meets the requirement of the Mass variation test.

**Foreign insoluble matter** $<6.06>$ Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** $<6.07>$ It meets the requirement.

**Sterility** $<4.06>$ Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Weigh accurately about 0.5 g of the contents, and proceed as directed in the Assay under Suxamethonium Chloride Hydrate.

Each mL of 0.1 mol/L perchloric acid VS $= 18.07$ mg of $C_{14}H_{30}Cl_2N_2O_4$

**Containers and storage** Containers—Hermetic containers.
**Suxamethonium Chloride Injection**

**スキサメトニウム塩化物注射液**

Suxamethonium Chloride Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of suxamethonium chloride (C_{14}H_{30}Cl_{2}N_{2}O_{4}: 361.31).

The concentration of Suxamethonium Chloride Injection should be stated as the amount of suxamethonium chloride (C_{14}H_{30}Cl_{2}N_{2}O_{4}).

**Method of preparation** Prepare as directed under Injections, with Suxamethonium Chloride Hydrate.

**Description** Suxamethonium Chloride Injection is a clear, colorless liquid.

**Identification** Take a volume of Suxamethonium Chloride Injection, equivalent to 0.05 g of Suxamethonium Chloride Hydrate according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of suxamethonium chloride for thin-layer chromatography in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 1 µL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium acetate (1 in 100), acetone, 1-butanol and formic acid (20:20:20:1) to a distance of about 10 cm, and dry the plate at 105°C for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots obtained from the sample solution and the standard solution are blue-purple in color and have similar Rf value.

**pH** <2.54> 3.0 – 5.0

**Purity** Hydrolysis products—Perform the preliminary neutralization with 0.1 mol/L sodium hydroxide VS in the Assay: not more than 0.7 mL of 0.1 mol/L sodium hydroxide VS is required for each 200 mg of Suxamethonium Chloride (C_{14}H_{30}Cl_{2}N_{2}O_{4}) taken.

**Bacterial endotoxins** <4.01> Less than 2.0 EU/mg.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Transfer to a separator an accurately measured volume of Suxamethonium Chloride Injection, equivalent to about 0.2 g of suxamethonium chloride (C_{14}H_{30}Cl_{2}N_{2}O_{4}), add 30 mL of freshly boiled and cooled water, and wash the solution with five 20-mL portions of diethyl ether. Combine the diethyl ether washings, and extract the combined diethyl ether layer with two 10-mL portions of freshly boiled and cooled water. Wash the combined water extracts with two 10-mL portions of diethyl ether. Combine the solution and the water extracts, add 2 drops of bromothymol blue TS, and neutralize with 0.1 mol/L sodium hydroxide VS. Add accurately measured 25 mL of 0.1 mol/L sodium hydroxide VS, and boil for 40 minutes under a reflux condenser, and cool. Titrate <2.25> the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS. Transfer 50 mL of the freshly boiled and cooled water to a flask, add 2 drops of bromothymol blue TS, neutralize the solution with 0.1 mol/L sodium hydroxide VS, and perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS = 18.07 mg of C_{14}H_{30}Cl_{2}N_{2}O_{4}

**Containers and storage** Containers—Hermetic containers. Storage—Not exceeding 5°C, and avoid freezing.

**Expiration date** 12 months after preparation.

**Tacrolimus Hydrate**

**タクロリムス水和物**

Tacrolimus Hydrate contains not less than 98.0% and not more than 102.0% of tacrolimus (C_{44}H_{69}NO_{12}: 804.02), calculated on the anhydrous basis.

**Description** Tacrolimus Hydrate occurs as a white crystal or crystalline powder.

It is very soluble in methanol and in ethanol (99.5), freely soluble in N,N-dimethylformamide and in ethanol (95), and practically insoluble in water.

**Identification** (1) Dissolve 5 mg of Tacrolimus Hydrate in 1 mL of ethanol (95), add 1 mL of 1,3-dinitrobenzene TS and 1 mL of sodium hydroxide TS, and shake: a red-purple color develops.

(2) Determine the infrared absorption spectrum of Tacrolimus Hydrate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Tacrolimus RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

<table>
<thead>
<tr>
<th>Chemical Formula</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{44}H_{69}NO_{12}·H_{2}O</td>
<td>822.03</td>
</tr>
</tbody>
</table>

The concentration of Suxamethonium Chloride Injection is stated as the amount of suxamethonium chloride (C_{14}H_{30}Cl_{2}N_{2}O_{4}).
Talampicillin Hydrochloride

Ampicillinphthalidyl Hydrochloride

Talampicillin Hydrochloride is the hydrochloride of ampicillin phthalidyl ester. It contains not less than 600 μg (potency) and not more than 700 μg (potency) per mg, calculated on the anhydrous basis. The potency of Talampicillin Hydrochloride is expressed as mass (potency) of ampicillin (C_{16}H_{18}N_{3}O_{4}S: 349.40).

Description
Talampicillin Hydrochloride occurs as a white to light yellowish white powder. It is very soluble in methanol, and freely soluble in water and in ethanol (99.5).

Identification
(1) To 1 mL of a solution of Talampicillin Hydrochloride (1 in 30) add 1 mL of sodium hydroxide TS, mix, allow to stand for 5 minutes, and add 2 mL of dilute sulfuric acid and 2 to 3 drops of 2,4-dinitrophenylhydrazine TS: an orange-yellow precipitate is formed.

(2) Determine the infrared absorption spectrum of Talampicillin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum or the spectrum of Talampicillin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 10 mL of a solution of Talampicillin Hydrochloride (1 in 30) add 1 mL of dilute nitric acid, and add silver nitrate TS: a white precipitate is formed.

Optical rotation \(\{2.49\} [\alpha]_{D}^{25}: +151 – +171^\circ \) (0.2 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Purity

(1) Heavy metals \(\{1.07\}\)—Proceed with 2.0 g of Talampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Talampicillin Hydrochloride in ethanol (99.5) to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL, 2 mL.
and 3 mL of the sample solution, add ethanol (99.5) to each to make exactly 100 mL, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 µL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of tetrhydrofuran, ethyl acetate, water and ethanol (95): (4:4:2:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (99.5) (1 in 500) on the plate, and heat at 110°C for 5 minutes: the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution (3), and the total of the amount of each spot other than the principal spot from the sample solution, which is calculated by the comparison with the spots obtained from the standard solutions (1), (2) and (3), is not more than 5%.

(4) 2-Formylbenzoic acid—Dissolve 50 mg of Talcamin Hydrochloride in ethanol (99.5) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 2-formylbenzoic acid in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of chloroform and acetic acid (100): (4:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly a solution of 2,4-dinitrophenylhydrazine in diluted sulfuric acid (6 in 25) (1 in 500): the spot of 2-formylbenzoic acid obtained from the sample solution is not more intense than that obtained from the standard solution.

Water <2.48> Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Talcamin Hydrochloride and Talcamin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in water to make exactly 20 mL each, and use these solutions as the sample solution and the standard solution. The standard solution should be prepared before use. Pipet 2 mL each of the sample solution and the standard solution in separate 100-mL glass-stoppered flasks, add 2.0 mL of sodium hydroxide TS, and allow them to stand for exactly 15 minutes. Add 2.0 mL of diluted hydrochloric acid (1 in 10) and exactly 10 mL of 0.005 mol/L iodine VS, allow them to stand for exactly 15 minutes, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the color of the solution is disappeared. If necessary, add 0.2 to 0.5 mL of starch TS. Separately, pipet 2 mL each of the sample solution and the standard solution in separate 100-mL glass-stoppered flasks, add exactly 10 mL of 0.005 mol/L iodine VS, titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the color of the solution is disappeared, and make any necessary correction. For this titration, add 0.2 to 0.5 mL of starch TS, if necessary. Calculate the amount (mL) of 0.005 mol/L iodine VS, V₁ and V₅, consumed by the sample solution and the standard solution, respectively.

\[
\text{Amount [µg (potency)] of ampicillin (C₁₆H₁₉N₃O₄S)} = \frac{M_S \times V_I}{V_5} \times 1000
\]

\(M_S\): Amount [mg (potency)] of Talcamin Hydrochloride RS

Containers and storage Containers—Tight containers.

Talc

タルク

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (•). Talc is a powdered, selected, natural, hydrated magnesium silicate. Pure talc is Mg₃Si₄O₁₀(OH)₂: 379.27. It may contain related mineral substances consisting chiefly of chlorite (hydrus magnesium aluminum silicate), magnesite (magnesium carbonate), calcite (calcium carbonate) and dolomite (calcium magnesium carbonate).

It contains no asbestos. It contains not less than 17.0% and not more than 19.5% of magnesium (Mg: 24.31).

*Description Talc occurs as a white to grayish white, fine, crystalline powder.

It is unctuous, and adheres readily to the skin.

It is practically insoluble in water and in ethanol (99.5).

Identification Determine the infrared absorption spectrum of Talc as directed in the potassium bromide disk method under Infrared Spectrophotometry (<2.25>); it exhibits absorption at the wave numbers of about 3680 cm⁻¹, 1018 cm⁻¹ and 669 cm⁻¹.

Purity (1) Acidity or alkalinity—To 2.5 g of Talc add 50 mL of freshly boiled and cooled water, and heat under a reflux condenser. Filter the liquid by suction, add 0.1 mL of bromothymol blue-sodium hydroxide-ethanol TS to 10 mL of the filtrate, and add 0.01 mol/L hydrochloric acid VS until the color of the solution changes: the necessary volume of the VS is not more than 0.4 mL. Separately, to 10 mL of the filtrate add 0.1 mL of phenolphthalein TS, and add 0.01 mol/L sodium hydroxide VS until the color of the solution changes to light red: the necessary volume of the VS is not more than 0.3 mL.

* (2) Acid-soluble substances—Weigh accurately about 1 g of Talc, heat with 20 mL of dilute hydrochloric acid at 50°C for 15 minutes with stirring. Cool, add water to make exactly 50 mL, and filter. Centrifuge, if necessary, until the filtrate becomes clear. To 25 mL of the filtrate add 1 mL of dilute sulfuric acid, evaporate to dryness, and ignite to constant mass at 800 ± 5°C: the amount of the residue is not more than 2.0%. *

(3) Water-soluble substances—To 10.0 g of Talc add 50 mL of water, weigh the mass, and boil for 30 minutes, supplying water lost by evaporation. Cool, add water to restore the original mass, and filter. Centrifuge, if necessary, until the filtrate becomes clear. Evaporate 20 mL of the filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 4.0 mg.

(4) Iron—Weigh accurately about 10 g of Talc, add 50 mL of 0.5 mol/L hydrochloric acid TS gently while stirring, and heat under a reflux condenser on a water bath for 30
minutes. After cooling, transfer the content to a beaker, and allow to settle the insoluble matter. Filter the supernatant liquid through a filter paper for quantitative analysis (No. 5B), leaving the precipitate in the beaker as much as possible, wash the remaining precipitate in the beaker with three 10-mL portions of hot water, and also wash the filter paper with 15 mL of hot water, and combine the washings and the filtrate. After cooling, add water to make exactly 100 mL, and use this solution as the sample stock solution. Pipet 2.5 mL of the stock solution, add 50 mL of 0.5 mol/L hydrochloric acid TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 50 mL each of 0.5 mol/L hydrochloric acid TS add exactly 2 mL, 2.5 mL, 3 mL and 4 mL of Standard Iron Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS add exactly 1 mL, 2 mL, 3 mL and 5 mL of Standard Calcium Solution, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry according to the following conditions, and calculate the amount of calcium from the calibration curve prepared from the absorbances of the standard solutions: not more than 0.5 mg/mL.

Gas: Combustible gas—Acetylene.
Lamp: Iron hollow-cathode lamp.
Wavelength: 248.3 nm.

(5) Aluminum—Pipet 5 mL of the sample stock solution obtained in the Assay, add 10 mL of cesium chloride TS and 10 mL of hydrochloric acid, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of cesium chloride TS add exactly 5 mL, 10 mL, 15 mL and 20 mL of Standard Aluminum Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry according to the following conditions, and calculate the amount of aluminum from the calibration curve prepared from the absorbances of the standard solutions: not more than 0.25 mg/mL.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Aluminum hollow-cathode lamp.
Wavelength: 309.3 nm.

(6) Lead—Use the sample stock solution obtained in (4) as the sample solution. Separately, to 50 mL of 0.5 mol/L hydrochloric acid TS add exactly 5 mL, 7.5 mL, 10 mL and 12.5 mL of Standard Lead Solution, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry according to the following conditions, and calculate the amount of lead from the calibration curve prepared from the absorbances of the standard solutions: not more than 10 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Lead hollow-cathode lamp.
Wavelength: 217.0 nm.

(7) Calcium—Pipet 5 mL of the sample stock solution obtained in the Assay, add 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS add exactly 1 mL, 2 mL, 3 mL and 5 mL of Standard Calcium Solution, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry according to the following conditions, and calculate the amount of calcium from the calibration curve prepared from the absorbances of the standard solutions: not more than 0.9 mg/mL.

Gas: Combustible gas—Acetylene.
Supporting gas—Nitrous oxide.
Lamp: Calcium hollow-cathode lamp.
Wavelength: 422.7 nm.

* Containers and storage  Containers—Well-closed containers.
Tamoxifen Citrate

C₂₆H₃₂N₂O₇·C₆H₈O₇: 563.64
2-[4-[(1Z)-1,2-Diphenylbut-1-en-1-yl]phenoxy]-N,N-dimethylamylamine monocitrate

[54965-24-1]

Tamoxifen Citrate, when dried, contains not less than 99.0% and not more than 101.0% of C₂₆H₃₂N₂O₇.

Description Tamoxifen Citrate occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Tamoxifen Citrate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tamoxifen Citrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tamoxifen Citrate (1 in 100) responds to the Qualitative Tests <1.09> (1) for citrate.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Tamoxifen Citrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure rapidly, using light-resistant vessels. Dissolve 15 mg of Tamoxifen Citrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than tamoxifen obtained from the sample solution is not larger than 3/10 times the peak area of tamoxifen from the standard solution, and the total area of the peaks other than the peak of tamoxifen from the sample solution is not larger than 4/5 times the peak area of tamoxifen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.8 g of N,N-dimethyl-n-octylamine in 1000 mL of water. Separately, dissolve 0.9 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water. Mix these solutions, and adjust to pH 3.0 with phosphoric acid. To 600 mL of this solution add 400 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tamoxifen is about 21 minutes.

Time span of measurement: About 2.5 times as long as the retention time of tamoxifen, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of tamoxifen obtained with 10 μL of this solution is equivalent to 8 to 12% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of tamoxifen are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tamoxifen is not more than 1.5%.

Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Tamoxifen Citrate, previously dried, dissolve in 150 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 56.36 mg of C₂₆H₃₂N₂O₇·C₆H₈O₇

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.
**Tamsulosin Hydrochloride**

**Description** Tamsulosin Hydrochloride occurs as white crystals.

It is freely soluble in formic acid, sparingly soluble in water, slightly soluble in acetic acid (100), and very slightly soluble in ethanol (95.9). Melting point: about 230°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Tamsulosin Hydrochloride (3 in 160,000) as directed under Ultraviolet-visible Spectrophotometry \( \lambda_\text{max} = 244 \text{ nm} \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tamsulosin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry \( \lambda_\text{max} = 245 \text{ cm}^{-1} \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of an ice cooled solution of Tamsulosin Hydrochloride (3 in 400) add 3 mL of dilute nitric acid, shake well, allow to stand at room temperature for 30 minutes, and filter: the filtrate responds to the Qualitative Tests \( \left< 1.09 \right> \) for chloride.

**Optical rotation** \( \left< 2.49 \right> \ [\alpha]_D^\circ = -17.5 \div -20.5^\circ \) (after drying, 0.15 g, water, warming, after cooling, 20 mL, 100 mm).

**Purity** (1) Heavy metals \( \left< 1.07 \right> \)—Proceed with 1.0 g of Tamsulosin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—

(i) Dissolve 50 mg of Tamsulosin Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( \left< 2.01 \right> \) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tamsulosin obtained from the sample solution is not larger than 1/2 times the peak area of tamsulosin from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.4 mL of perchloric acid and 1.5 g of sodium hydroxide in 950 mL of water, adjust the pH to 2.0 with sodium hydroxide TS, and add water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin is about 6 minutes.

Time span of measurement: Until tamsulosin is eluted, beginning after the solvent peak.

**System suitability—**

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of tamsulosin obtained from 10 \( \mu \)L of this solution is equivalent to 1.4 to 2.6% of that from 10 \( \mu \)L of the standard solution.

System performance: Dissolve 5 mg of Tamsulosin Hydrochloride and 10 mg of propyl parahydroxybenzoate in 20 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 10 \( \mu \)L of this solution under the above operating conditions, tamsulosin and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tamsulosin is not more than 4.0%.

(ii) Perform the test with 10 \( \mu \)L each of the sample solution and standard solution which are obtained in above (i) as directed under Liquid Chromatography \( \left< 2.01 \right> \) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tamsulosin obtained from the sample solution is not larger than 1/2 times the peak area of tamsulosin from the standard solution.

**Operating conditions—**

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity (2) (i).

Mobile phase: Dissolve 4.4 mL of perchloric acid and 1.5 g of sodium hydroxide in 950 mL of water, adjust the pH to 2.0 with sodium hydroxide TS, and add water to make 1000 mL. To this solution add 1000 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin is about 2.5 minutes.

Time span of measurement: About 5 times as long as the retention time of tamsulosin, beginning after the peak of tamsulosin.

**System suitability—**

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase used in the Purity (2) (i) to make exactly 50 mL. Confirm that the peak area of tamsulosin obtained from 10 \( \mu \)L of this solution is equivalent to 1.4 to 2.6% of that from 10 \( \mu \)L of the standard solution.
System performance: Proceed as directed in the system suitability in the Purity (2) (i).
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tamsulosin is not more than 4.0%.

**Loss on drying** <2.4> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.4> Not more than 0.1% (1 g).

**Assay**
Weigh accurately about 0.7 g of Tamsulosin Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 75 mL of a mixture of acetic acid (100) and acetic anhydride (3:2), and immediately titrate <2.5> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 44.50 mg of C_{20}H_{28}N_{2}O_{5}S.HCl

**Containers and storage**
Containers—Well-closed containers.

### Tamsulosin Hydrochloride Extended-release Tablets

タムスロシン塩酸塩徐放錠

Tamsulosin Hydrochloride Extended-release Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of tamsulosin hydrochloride (C_{20}H_{28}N_{2}O_{5}S.HCl: 444.97).

**Method of preparation**
Prepare as directed under Tablets, with Tamsulosin Hydrochloride.

**Identification**
To an amount of powdered Tamsulosin Hydrochloride Extended-release Tablets, equivalent to 1 mg of Tamsulosin Hydrochloride according to the labeled amount, add about 5 g of porcelain balls with about 5 mm in diameter, add 20 mL of 0.2 mol/L sodium hydroxide TS, warm at 50°C for 10 minutes, and shake vigorously for 15 minutes. Then, add 7 mL of acetonitrile, shake slightly, and centrifuge. Take the supernatant liquid, add 2.5 g of sodium chloride and 5 mL of ethyl acetate, shake vigorously for 5 minutes, and centrifuge. Take the supernatant liquid, evaporate to dryness at 50°C in a water bath, dissolve the residue with 20 mL of water, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits maxima between 222 nm and 226 nm, and between 278 nm and 282 nm.

**Uniformity of dosage units** <6.02>
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tamsulosin Hydrochloride Extended-release Tablets add about 5 g of porcelain balls with about 5 mm in diameter and 5 mL of water, and shake to disintegrate the tablet. Add 20 mL of a solution of sodium hydroxide (1 in 500), warm at 50°C for 10 minutes, shake vigorously for 30 minutes, and add 10 mL of acetonitrile and 5 mL of 0.2 mol/L hydrochloric acid TS. To this solution add exactly 5 mL of the internal standard solution for every 0.1 mg of tamsulosin hydrochloride, add the mobile phase to make 50 mL, shake slightly, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. To V mL of the filtrate add the mobile phase to make V mL so that each mL contains about 2 μg of tamsulosin hydrochloride (C_{20}H_{28}N_{2}O_{5}S.HCl), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of tamsulosin hydrochloride (C_{20}H_{28}N_{2}O_{5}S.HCl) = M_S × Q_s ÷ (V/V) × 1/100

M_S: Amount (mg) of tamsulosin hydrochloride for assay

**Internal standard solution**—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 25,000).

**Dissolution**
Being specified separately.

**Assay**
Weigh accurately about 10 mg of tamsulosin hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_s, of the peak area of tamsulosin to that of the internal standard.

Amount (mg) of tamsulosin hydrochloride (C_{20}H_{28}N_{2}O_{5}S.HCl) = M_S × Q_T ÷ Q_s × 1/100

M_S: Amount (mg) of tamsulosin hydrochloride for assay

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 225 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 4.4 mL of perchloric acid and 1.5 g of sodium hydroxide in 950 mL of water, adjust to pH 2.0 with sodium hydroxide TS, and add water to make 1000
mL. To 700 mL of this solution add 300 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin is about 6 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and tamsulosin are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tamsulosin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Tannic Acid
タンニン酸

Tannic Acid is the tannin usually obtained from nutgalls or rhusgalls.

Description Tannic Acid occurs as a yellowish white to light brown, amorphous powder, glistening leaflets, or spongy masses. It is odorless or has a faint, characteristic odor, and has a strongly astringent taste.

It is very soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) To 5 mL of a solution of Tannic Acid (1 in 400) add 2 drops of iron (III) chloride TS: a blue-black color develops. Allow the solution to stand: a blue-black precipitate is produced.

(2) To 5 mL of a solution of Tannic Acid (1 in 20) add 1 drop each of albumin TS, gelatin TS, or 1 mL of starch TS: a precipitate is produced in each solution.

Purity (1) Gum, dextrin and sucrose—Dissolve 3.0 g of Tannic Acid in 15 mL of boiling water: the solution is clear or slightly turbid. Cool, and filter the solution. To 5 mL of the filtrate add 5 mL of ethanol (95): no turbidity is produced. Add further 3 mL of diethyl ether to this solution: no turbidity is produced.

(2) Resinous substances—To 5 mL of the filtrate obtained in (1) add 10 mL of water: no turbidity is produced.

Loss on drying <2.41> Not more than 12.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 1.0% (0.5 g).

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Tartaric Acid
酒石酸

C₄H₆O₆: 150.09
(2R,3R)-2,3-Dihydroxybutanedioic acid [87-69-4]

Tartaric Acid, when dried, contains not less than 99.7% of C₄H₆O₆.

Description Tartaric Acid occurs as colorless crystals or a white, crystalline powder. It is odorless, and has a strong acid taste.

It is very soluble in water, freely soluble in ethanol (95), and slightly soluble in diethyl ether.

A solution of Tartaric Acid (1 in 10) is dextrorotatory.

Identification (1) Ignite Tartaric Acid gradually: it decomposes and an odor of burning sugar is perceptible.

(2) A solution of Tartaric Acid (1 in 10) changes blue litmus paper to red, and responds to the Qualitative Tests <1.09> for tartrate.

Purity (1) Sulfate <1.14>—Perform the test with 0.5 g of Tartaric Acid. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Oxalate—Dissolve 1.0 g of Tartaric Acid in 10 mL of water, and add 2 mL of calcium chloride TS: no turbidity is produced.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Tartaric Acid according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Calcium—Neutralize a solution of 1.0 g of Tartaric Acid in 10 mL of water with ammonia TS, and add 1 mL of ammonium oxalate TS: no turbidity is produced.

(5) Arsenic <1.11>—Prepare the test solution with 2.0 g of Tartaric Acid according to Method 1, and perform the test (not more than 1 ppm).

Loss on drying <2.41> Not more than 0.5% (3 g, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.05% (1 g).

Assay Weigh accurately about 1.5 g of Tartaric Acid, previously dried, dissolve in 40 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS = 75.05 mg of C₄H₆O₆

Containers and storage Containers—Well-closed containers.
Taurine

タウリン

\[
\text{C}_2\text{H}_7\text{NO}_3\text{S}: 125.15
\]
2-Aminoethanesulfonic acid

[107-35-7]

Taurine, when dried, contains not less than 99.0% and not more than 101.0% of \(\text{C}_2\text{H}_7\text{NO}_3\text{S}\).

Description Taurine occurs as colorless or white crystals, or a white crystalline powder.

It is soluble in water, and practically insoluble in ethanol (99.5).

The \(pH\) of a solution prepared by dissolving 1.0 g of Taurine in 20 mL of freshly boiled and cooled water is between 4.1 and 5.6.

Identification Determine the infrared absorption spectrum of Taurine as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Taurine in 20 mL of water is clear and colorless.

(2) Chloride \(<1.03\rangle—\)Perform the test with 1.0 g of Taurine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate \(<1.14\rangle—\)Perform the test with 2.0 g of Taurine. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(4) Ammonium \(<1.02\rangle—\)Perform the test with 0.25 g of Taurine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals \(<1.07\rangle—\)Proceed with 2.0 g of Taurine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron \(<1.10\rangle—\)Prepare the test solution with 2.0 g of Taurine according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 1.0 g of Taurine in 50 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.05\rangle\). Spot 5 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ethanol (99.5), 1-butanol and acetic acid (100) (150:150:100:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-butanol TS on the plate, and heat at 105°C for 5 minutes: the spot other than the principle spot with the sample solution is not one spot, and it is not more intense than the spot with the standard solution.

Loss on drying \(<2.41\rangle—\)Not more than 0.20% (1 g, 105°C, 2 hours).

Residue on ignition \(<2.44\rangle—\)Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Taurine, previously dried, dissolve in 50 mL of water, add 5 mL of formaldehyde solution, and titrate \(<2.50\rangle with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 12.52 mg of \(\text{C}_2\text{H}_7\text{NO}_3\text{S}\)

Containers and storage Containers—Well-closed containers.

Tazobactam

タゾバクタム

C\(_{10}\)H\(_{12}\)N\(_4\)O\(_5\)S: 300.29
(25,35,5R)-3-Methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide

[g]\(_{19}^0\) = +162° + 167° (1 g calculated on the anhydrous basis, N,N-dimethylformamide, 100 mL, 100 mm).
Purity (1) Clarity and color of solution—Dissolve 1.0 g of Tazobactam in 10 mL of sodium hydrogen carbonate (3 in 100): the solution is clear. Perform the test with the solution as directed under Ultraviolet-visible Spectrophotometry $<2.20>$: the absorbance at 420 nm is not more than 0.14.

(2) Heavy metals $<1.07>$—Proceed with 1.0 g of Tazobactam according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—This operation must be performed quickly. Dissolve 50 mg of Tazobactam in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 50 $\mu$L each of the sample solution, the standard solutions (1) and (2) as directed under the Liquid Chromatography $<2.01>$ according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.17 with respect to tazobactam, obtained from the sample solution is not larger than 4/5 times the peak area of tazobactam from the standard solution (1), and the peak having the relative retention time of about 0.17 with respect to tazobactam from the standard solution and the peak having the relative retention time of about 0.17 with respect to tazobactam from the sample solution is not larger than the peak area of tazobactam from the standard solution (2), and the total area of the peaks other than the peak of tazobactam and the peak having the relative retention time of about 0.17 with respect to tazobactam from the sample solution is not larger than 2 times the peak area of tazobactam from the standard solution (2).

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of tazobactam.

System suitability—
Test for required detectability: Pipet 1 mL of the standard solution (1), and add the mobile phase to make exactly 20 mL. Confirm that the peak area of tazobactam obtained from 50 $\mu$L of this solution is equivalent to 3 to 7% of that of tazobactam from 50 $\mu$L of the standard (1).

System performance: When the procedure is run with 50 $\mu$L of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of tazobactam are not less than 2000 and 0.8 – 1.2, respectively.

System repeatability: When the test is repeated 6 times with 50 $\mu$L of the standard solution (1) under the above operating conditions, the relative standard deviations of the peak area of tazobactam is not more than 1.0%.

(4) Residual solvent—Being specified separately.

Water $<2.48>$ Not more than 0.5% (1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (3:1) instead of methanol for water determination).

Residue on ignition $<2.44>$ Not more than 0.1% (1 g).

Bacterial endotoxins $<4.01>$ Less than 0.04 EU/mg (potency).

Assay Weigh accurately an amount of Tazobactam and Tazobactam RS, equivalent to about 50 mg (potency), dissolve each in exactly 10 mL of the internal standard solution, add water to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 $\mu$L each of the sample solution and standard solution as directed under the Liquid Chromatography $<2.01>$ according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak areas of tazobactam to that of the internal standard.

\[
\text{Amount} \ [\mu \text{g (potency)}] \ \text{of tazobactam} \ (\text{C}_{10}\text{H}_{12}\text{N}_{4}\text{O}_{5}\text{S}) = M_S \times \left( \frac{Q_T}{Q_S} \right) \times 1000
\]

$M_S$: Amount [mg (potency)] of Tazobactam RS

Internal standard solution—A solution of phenylalanine (1 in 400).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.32 g of diammonium hydrogen phosphate in 750 mL of water, adjust the pH to 2.5 with phosphoric acid, add water to make 1000 mL, and add 25 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tazobactam is about 10 minutes.

System suitability—
System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, the internal standard and tazobactam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of tazobactam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Expiration date 24 months after preparation.
Teceleukin (Genetical Recombination)

デセロイキン(遺伝子組換え)

Identification (1) Measure accurately an appropriate amount of Teceleukin (Genetical Recombination), add accurately to a concentration of 200 units per mL of culture medium for assay of teceleukin, and use this solution as the sample stock solution. Dilute reference anti-interleukin-2 antibody for teceleukin with culture medium for assay of teceleukin to a concentration of approximately 200 neutral units per mL and use this solution as the interleukin-2 neutral antibody solution. Accurately add an equivalent volume of the interleukin-2 neutral antibody solution to the sample stock solution, shake, and then leave for 1 hour in a 37°C incubator in air containing 5% carbon dioxide. This solution is the sample solution. Prepare a standard solution by accurately adding an equivalent volume of culture medium for assay of teceleukin to the sample stock solution, mixing, and then processing in the same way. Process the sample and standard solutions according to the assay method, determine their respective dilution coefficients, \( D_A \) and \( D_T \), and then calculate the neutralization rate, which should be at least 90%, using the following formula.

Neutralization rate (\( \% \)) = \( \frac{(D_T - D_A)}{D_T} \times 100 \)

However, please note if the mean values of the absorbance of the maximum uptake control solution and absorbance of the minimum uptake control solution do not fit the standard curve, the neutralization coefficient is to be determined within the following range.

Neutralization coefficient (\( \% \)) > \( \frac{(D_T - 2)}{D_T} \times 100 \)

(2) Place a volume of Teceleukin (Genetical Recombination) corresponding to approximately 50 \( \mu \)g of protein into 2 test tubes for hydrolysis, evaporate to dryness under vacuum, and use one as the sample (1). To the other, add 50 \( \mu \)L of a mixture of formic acid and hydrogen peroxide (30:9:1) that has been left at room temperature for one hour, cool for 4 hours in ice, add 0.5 mL of water, and then evaporate to dryness under vacuum to give the sample (2). To 1.3 mL of methanesulfonic acid add 3.7 mL of water, mix well, add and dissolve 10 mg of 3-(2-aminoethyl)indole, to make a 4 mol/L methanesulfonic acid solution. Dissolve 39.2 g of trisodium citrate dihydrate, 33 mL of hydrochloric acid, 40 mL of thioglycol, and 4 mL of lauroamcoagul solution (1 in 4) in 700 mL of water, adjust the pH to 2.2, add water to 1000 mL, add 100 mL of capric acid, and mix to make a sodium citrate solution for dilution. Add 50 \( \mu \)L of freshly prepared 4 mol/L methanesulfonic acid to the sample (1) and sample (2), add 0.05 mL of 0.4 mol/L sodium hydroxide TS followed by 0.4 mL of sodium citrate solution for dilution to make the sample solution (1) and sample solution (2). Separately, accurately measure 0.25 mmol amounts of L-asparagic acid, L-threonine, L-serine, L-glutamine acid, L-proline, glycine, L-alanine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine hydrochloride monohydrate, and L-arginine hydrochloride as well as 0.125 mmol of L-cysteine and then dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. This is the amino acid standard stock solution. Accurately measure 1 mL of this solution, and add sodium citrate solution for dilution to make exactly 25 mL. This is solution A. Accurately weigh approximately 20 mg of L-tryptophan and dissolve in water to make exactly 1000 mL. This is solution B. Accurately measure 10 mL of both solution A and solution B, combine together, and add sodium citrate solution for dilution to make exactly 50 mL. This is the amino acid standard solution. Separately, accurately weigh approximately 17 mg of L-cysteic acid and dissolve in sodium citrate solution for dilution to make exactly 50 mL. This is the cysteic acid standard solution. Accurately measure 0.25 mL of the sample solution (1), the sample solution (2), amino acid standard solution, and the cysteic acid standard solution. When the test is conducted by Liquid Chromatography \( <2.01> \) under the following conditions, peaks for the 18 amino acids are observed in the chromatogram obtained from the sample solution (1). Also, measure the peak area of each amino acid in the sample solution (1) and the amino acid standard solution, and taking the molar number of alanine in the sample solution (1) as 5.0, determine the concentrations of aspartic acid, glutamic acid, proline, glycine, methionine, leucine, tyrosine, phenylalanine, lysine, histidine, tryptophan, and arginine and then calculate the molar ratio for each amino acid. Also, measure the cysteic acid peak areas of the sample solution (2) and the cysteic acid standard solution, determine the concentration of cysteine, and, taking the molar number of alanine in the sample solution (2) as 5.0, calculate the molar ratio of cysteine. When determining the molar ratios of the respective amino acids, aspartic acid is 11.4 to 12.6, glutamic acid 17.1 to 18.9, proline 4.5 to 5.5, glycine 1.8 to 2.2, cystine 2.7 to 3.3, methionine 4.5 to 5.5, leucine 20.9 to 23.1, tyrosine 2.7 to 3.3, phenylalanine 5.4 to 6.6, lysine 10.5 to 11.6, histidine 2.7 to 3.3, tryptophan 0.7 to 1.2, and arginine 3.6 to 4.4.

Operating conditions—

Detector: Visible absorption photometer [wavelengths: 440 nm (proline) and 570 nm (amino acids other than proline)]
Column: A stainless steel column with an inside diameter of 4 mm and length of 25 cm packed with a strongly acidic ion exchange resin for liquid chromatography consisting of polystyrene to which sulphonate group binds.

Column temperature: A constant temperature of about 50°C when the sample is injected. After a certain time, increase the temperature to a constant temperature of about 62°C.

Reaction temperature: A constant temperature of about 98°C.

Time for color formation: Approximately 2 minutes.

Mobile phase: After preparing mobile phases A, B, and C according to the following table, add 0.1 mL of capric acid to each.

<table>
<thead>
<tr>
<th></th>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
<th>Mobile phase C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>18.70 g</td>
<td>10.50 g</td>
<td>7.10 g</td>
</tr>
<tr>
<td>monohydrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trisodium citrate</td>
<td>7.74 g</td>
<td>14.71 g</td>
<td>26.67 g</td>
</tr>
<tr>
<td>dihydrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>7.07 g</td>
<td>2.92 g</td>
<td>54.35 g</td>
</tr>
<tr>
<td>Ethanol (99.5%)</td>
<td>60 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiodiglycol</td>
<td>5 mL</td>
<td>5 mL</td>
<td>—</td>
</tr>
<tr>
<td>Lauromacrogol</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
</tr>
<tr>
<td>solution (1 in 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>3.2</td>
<td>4.3</td>
<td>4.7</td>
</tr>
<tr>
<td>Total volume</td>
<td>1000 mL</td>
<td>1000 mL</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Changing mobile phases and column temperature: When operating under the above conditions using 0.25 mL of amino acid standard solution, the amino acids will elute in the following order; aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine, tryptophan, and arginine. Switchover to mobile phase A, mobile phase B, and mobile phase C, in sequence so that the resolution between the peaks of cystine and valine is 2.0 or more and that between ammonia and histidine is 1.5 or more. Also, increase the temperature after a constant length of time so that the resolution between the peaks of glutamic acid and proline is at least 2.0.

Reaction reagents: Dissolve 408 g of lithium acetate dihydrate in water, and add 100 mL of acetic acid (100) and water to make 1000 mL. To this solution add 1200 mL of dimethylsulfoxide and 800 mL of 2-methoxyethanol. This is solution (I). Separately, mix together 600 mL of dimethylsulfoxide and 400 mL of 2-methoxyethanol and then add 80 g of ninhydrin and 0.15 g of sodium borohydride. This is solution (II). After gassing 3000 mL of the solution (I) for 20 minutes with nitrogen, rapidly add 1000 mL of the solution (II) and then mix by gassing for 10 minutes with nitrogen.

Mobile phase flow rate: About 0.275 mL every minute.

Reaction reagent flow rate: About 0.3 mL every minute.

System suitability—

System performance: When 0.25 mL of the amino acid standard solution is run under the above conditions, the resolution between the peaks of threonine and serine is at least 1.5.

(3) Dissolve 0.242 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 5.0 g of sodium lauryl sulfate, and 74 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 60 mL of water. After adjusting the pH to 8.0 using 1 mol/L hydrochloric acid TS, add water to make 100 mL. This is the molecular weight determination buffer solution. Accurately measure 20 μL of Teceleukin (Genetical Recombination), add exactly 20 μL of the molecular weight determination buffer solution and 2 μL of 2-mercaptoethanol, and then heat for 5 minutes on a 90 to 100°C water bath without allowing any water evaporation from the mixture. After cooling, add exactly 1 μL of bromophenol blue solution (1 in 2000) and then shake. This is the sample solution. Separately, accurately measure 5 μL of molecular weight marker for teceleukin, and add exactly 50 μL of water, 55 μL of the molecular weight determination buffer solution, and 5 μL of 2-mercaptoethanol, and then heat for 5 minutes on a 90 to 100°C water bath without allowing any water evaporation from the mixture. After cooling, add exactly 1 μL of bromophenol blue solution (1 in 2000), and shake well. This is the molecular weight standard solution. When conducting a test using SDS-polyacrylamide gel electrophoresis with 1 μL each of the sample solution and the molecular weight standard solution, the molecular weight of the main band is in the range of 14,000 and 16,000.

Operating conditions—

Equipment: Horizontal electrophoresis vessel equipped with a cooling unit, a device that accumulates load voltage over time, and a direct current power source device that controls the amperage, voltage, wattage.

Spotting of solutions: Solutions are spotted on concentrating gel of polyacrylamide gel sheets.

Electrophoresis conditions

Polyacrylamide gel sheet: Polyester sheet to which a polyacrylamide gel (width, about 43 mm, length, about 50 mm, and thickness, about 0.5 mm) is closely adhered. The polyacrylamide gel consists of a concentrating gel with a gel support concentration of 7.5% and a 3% degree of crosslinking and a separating gel with corresponding values of 20% and 2%. The gel contains pH 6.5 Tris- acetate buffer.

Buffer solution for electrode: Prepared by dissolving 35.83 g of tricine, 24.23 g of 2-amino-2-hydroxymethyl-1,3-propanediol, and 5.5 g of sodium lauryl sulfate in water to make 1000 mL.

Cooling temperature of gel support plate: 15°C.

Running conditions

Pre-electrophoresis and electrophoresis: The voltage, amperage, and wattage should not exceed 250 V, 10 mA, and 3 W, respectively. The amperage and wattage should be proportional to the number of polyacrylamide sheets.

Immediately after adding sample: The voltage, amperage, and wattage should not exceed 250 V, 1 mA, and 3 W, respectively. The amperage and wattage should be proportional to the number of polyacrylamide sheets.

Electrophoresis time

Before adding sample: Until value of load voltage integrated with respect to time reaches 60 V·h.

Immediately after adding sample: Until value of load voltage integrated with respect to time reaches 1 V·h.

Main electrophoresis: Until value of load voltage integrated with respect to time reaches 140 V·h.

Fixation and staining

Dissolve 25 g of anhydrous sodium carbonate anhydride and 0.8 mL of formaldehyde solution in water to make 1000 mL. This is the developing solution. After immersing
the polyacrylamide gel sheet in a mixture of ethanol (99.5), water and acetic acid (100) (5:4:1) for 2 minutes, immerse for 2 minutes in a mixture of water, ethanol (99.5) and acetic acid (100) (17:2:1). Change the mixture, immerse for another 4 minutes, immerse in water for 2 minutes to rinse the polyacrylamide gel sheet, and change the water to immerse for 2 minutes. This procedure is carried out with heating to 50°C. Next, while heating at 40°C, immerse for 10 to 15 minutes in diluted silver nitrate solution (1 in 7), warm to 30°C, and gently rinse the polyacrylamide gel sheet with water. While warming at 30°C, immerse the polyacrylamide gel sheet in freshly prepared developing solution. After obtaining adequate color formation, immerse the polyacrylamide gel sheet in diluted acetic acid (100) (1 in 20) to terminate the color formation.

Estimation of molecular weight

Plot graphs for each band obtained from the molecular weight standard solution, distance from the border of the concentrating gel and separating gel, and the logarithm of the molecular weight of proteins in each band. Calculate the molecular mass by reading the corresponding position of the major band obtained from the sample solution on the graph.

(4) The isoelectric point determined from the electrophoresis position is 7.4 to 7.9 when 3 μL of Teceleukin (Genetical Recombination) and 8 μL of isoelectric marker for teceleukin are tested by the polyacrylamide gel isoelectric method.

Operating conditions—

Equipment: Horizontal electrophoretic vessel with cooling unit and direct current power source that can perform constant wattage control.

Preparation of polyacrylamide gel: Dissolve 1.62 g of polyacrylamide and 50 mg of N,N’-methylenebisacrylamide in water to make 25 mL. Accurately measure 7.5 mL of this solution, 2 mL of a 10 mL solution prepared by adding water to 5 g of glycine, and 0.64 mL of a pH 3 to pH 10 amphoteric electrolyte solution, and degas under reduced pressure while stirring thoroughly. Next, accurately measure 74 μL of freshly prepared ammonium persulfate solution (1 in 50), 3 μL of N,N,N’,N’-tetramethylethylenediamine, and 50 μL of freshly prepared riboflavin sodium phosphate solution (1 in 1000), stir well, immediately pour on a gel preparation plate (10 cm wide, 11 cm long, and 0.8 mm thick), and then expose to a fluorescent light source for 60 minutes to gelate.

Spotting

Add Teceleukin (Genetical Recombination) or isoelectric marker for teceleukin 30 minutes after starting electrophoresis to wells in gel plates to which plastic tape (3.5 mm wide, 3.5 mm long, 0.4 mm thick) has been applied in advance and that have undergone gelation.

Electrophoresis conditions

Anode solution: α-D-aspartic acid solution (133 in 25,000).

Running conditions: After starting the electrophoresis, a constant wattage of 10 W for 20 minutes and 20 W thereafter. However, the voltage should be 3000 V or less.

Running time: 120 to 140 minutes while blowing Nitrogen into the electrophoresis vessel.

Fixation and washing

Dissolve 28.75 g of trichloroacetic acid and 8.65 g of 5-sulfosalicylic acid dihydrate in 75 mL of methanol and 175 mL of water. Immerse the gel in this solution for 60 minutes to fix the protein to the gel. After fixation, immerse for 10 minutes in a mixture of water, ethanol (99.5) and acetic acid (100) (67:25:8).

Staining and decolorization

Dissolve 0.11 g of Coomassie brilliant blue G-250 in 25 mL of ethanol (99.5), and add 8 mL of acetic acid (100) and water to make 100 mL. This is the staining solution. Immerse the gel for 10 minutes while heating at 60°C in freshly filtered staining solution. After staining, decolorize by immersing in a mixture of water, ethanol (99.5) and acetic acid (100) (67:25:8).

Determination of isoelectric point

Plot the protein isoelectric points and the distance from the cathode of each band obtained from the isoelectric markers for teceleukin. Calculate the isoelectric point from the corresponding position of the major bands obtained from the sample solution.

pH < 2.54: 2.7 – 3.5

Purity (I) Host cell-derived protein—Take an appropriate amount of Teceleukin (Genetical Recombination) and add an exact amount of diluted acetic acid (100) (1 in 350) to make a solution containing between 0.68 and 0.72 mg of protein in one mL. This is the sample stock solution. Dissolve 1.52 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 10.94 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 200 mL. Dissolve 0.5 g of bovine serum albumin in 25 mL of this solution. This is 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution. Accurately measure 0.5 mL of the sample stock solution, add exactly 30 μL of sodium carbonate TS, stir, and immediately add exactly 0.47 mL of the 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution to make the sample solution. Accurately measure 10 mL of dilute acetic acid (100) (1 in 350), add 0.6 mL of sodium carbonate TS, and then add 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution to make exactly 20 mL. This is the dilution solution. Add the E. coli protein stock solution to this dilution solution to make a solution containing 0.015 μg of E. coli protein in one mL. This is standard solution (1). Accurately dilute this solution serially two-fold with the dilution solution to make standard solutions (2) to (8) having different concentrations of E. coli protein. Dissolve 0.5 g of bovine serum albumin in 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS, pH 7.4. This is the wash solution. Accurately measure 0.1 mL of the sample solution, standard solutions (1) to (8), and dilution solution as a blank standard solution and place each in 3 wells in solid phase plates (place dilution solution in 6 wells), cover with plastic wrap, mix by shaking in a horizontal direction, and then leave standing at a constant temperature of about 25°C for 5 to 16 hours. Next, remove the solution from each well by aspiration, add 0.25 mL of the the wash solution, mix again by shaking in a horizontal direction, and then remove by aspiration. Repeat this procedure 2 more times by adding 0.25 mL of the wash solution to each well. Freshly dilute peroxidase marker antibody stock solution with 1 w/v% bovine serum albumin-phosphate buffer-sodium chloride TS, add exactly 0.1 mL to each well, cover with plastic wrap, mix by shaking in a horizontal direction, and then leave standing
at a constant temperature of about 25°C for 16 to 24 hours. Next, remove the solution in the wells by aspiration, add 0.25 mL of the wash solution, mix by shaking in a horizontal direction, and then remove the solution by aspiration. Using 0.25 mL of the wash solution, repeat this procedure 2 more times for each well. To each well, accurately add 0.1 mL of teceleukin chromophore solution, stir gently, and then shield from light and leave standing for 30 minutes at a constant temperature of about 25°C. Add exactly 0.1 mL of diluted sulfuric acid (3 in 50) to each well and then mix by gently shaking horizontally. Measure the absorbances of these solutions at 450 nm and 340 nm to determine the concentration of each standard solution in logarithmic scale on the horizontal axis and the absorbance values on the vertical axis. Match the values obtained from the sample solution to the standard curve, determine the concentration of E. coli protein (ng/mL) in logarithmic scale.

Prepare a standard curve by plotting a graph having the concentration of E. coli protein (ng/mL) in logarithmic scale on the horizontal axis and the absorbance values on the vertical axis. Match the values obtained from the sample solution to the standard curve, determine the concentration A of E. coli protein in the sample solution, and take the mean. The amount of E. coli protein is not more than 5 ng when the amount of E. coli protein per mg of protein is determined using the following formula.

\[ \text{Amount (ng) of E. coli-derived protein per mg protein} = \frac{A}{C} \]

C: Protein concentration (mg/mL) in sample solution

The test is valid if the E. coli protein concentration is 0.3 ng/mL or less when the concentration is obtained by fitting the absorbance value at detection limit, calculated from the following formula using absorbance value of the dilution solution, to the standard curve.

\[ \text{Absorbance at detection limit} = \bar{X} + 3.3 \times \left( \sum_{i=1}^{n} (X_i - \bar{X})^2 \right)^{1/2} / (6 - 1) \]

\( \bar{X} \): Individual absorbance values obtained from the dilution solution
\( \bar{X} \): The mean of absorbance values obtained from the dilution solution
6: The number of wells in the microplate containing dilution solution

(2) Tetracycline hydrochloride—Serially subculture through 2 passages at 35 to 37°C the test bacteria Micrococcus luteus ATCC9341 in a slant culture of test bacteria inoculation media for teceleukin and then dilute this 100-fold by adding sterilized purified water. This is the test bacteria solution. Store the test bacteria solution at 5°C or less and use the solution within 5 days. Dilute the test bacteria solution serially by adding sterilized purified water, add an appropriate amount to 100 mL of normal agar medium for teceleukin, conduct a preliminary test, and determine the amount of tetracycline hydrochloride that shows an inhibition zone corresponding to standard solution containing 0.5 μg (potency) in 1 mL. Add this amount to 100 mL of normal agar medium for teceleukin dissolved and then cooled to 45 to 50°C and mix. Pipet 25 mL of this solution into square Petri dishes (135 × 95 mm) and spread horizontally to solidify. Prepare plates for testing by making an appropriate number of wells in this agar medium. The volume of the test bacteria solution to which 100 mL of normal agar medium for teceleukin has been added is 0.25 to 1.0 mL. Accurately measure an appropriate amount of Tetracycline Hydrochloride RS and dilute accurately with water to make a clear solution with a concentration of 1 mg (potency)/mL. Accurately measure an appropriate amount of this solution and dilute precisely with water to make standard solutions with concentrations of 0.8, 0.4, 0.2, and 0.1 mg/mL. Prepare a standard curve by plotting a graph having the concentration of tetracycline hydrochloride per mg of protein determined using the following formula, the amount is not more than 0.7 μg. However, if an inhibition zone is not seen, or is seen but the diameter is smaller than 0.5 μg/mL on the standard curve, A is taken as being 0.5 μg/mL or less.

\[ \text{Amount [μg (potency)] of tetracycline hydrochloride (C11H8N2O8.HCl) per mg of protein} = \frac{A}{P} \]

P: The protein concentration (mg/mL) of the sample solution.

(3) Desmethionyl form—Add water to an appropriate amount of teceleukin to make a sample solution with a protein concentration of about 0.17 mg/mL. Perform the test with 1.2 mL of this solution as directed under Liquid Chromatography 2.01 under the following conditions. Determine using automatic integration the peak area, A2, of teceleukin and the peak area of the desmethionyl form with a relative retention time of about 0.8 relative to teceleukin, A1. The content of the desmethionyl form is not more than 1.0% when determined using the following formula.

\[ \text{Amount (%) of desmethionyl form} = \frac{A_1}{A_1 + A_2} \times 100 \]

Operating conditions—

Detector: Ultravioletabsorption photometer (wavelength: 280 nm)

Columns: Two stainless steel columns with inside diameters of 7.5 mm and lengths of 7.5 cm connected in sequence and packed with 10 μm synthetic polymer bound to diethylaminoethyl base for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Mix 0.658 g of diethanolamine in 400 mL of water, adjust the pH to 9.0 by adding 1 mol/L hydrochloric acid TS, and then add water to make 500 mL.

Mobile phase B: Add 300 mL of water to 2.6 mL of a pH 6 to 9 amphoteric electrolyte solution and 0.5 mL of a pH 8 to 10.5 amphoteric electrolyte solution, adjust to pH 7 with diluted hydrochloric acid (9 in 100), and then add water to
make 400 mL.

Switching mobile phases and sample injection: Inject the sample solution while running the mobile phase A. Repeatedly inject 10 times a sample solution volume of 0.11 mL followed by a single injection of 100 μL. After injecting the entire volume and running mobile phase A for 60 minutes, switch to mobile phase B. After measuring the sample solution and after running 1 mol/L sodium chloride TS for 10 minutes for posttreatment and cleaning of the columns, inject 100 μL of sodium hydroxide TS while running the mobile phase A and then 55 minutes later start injection of the next sample solution.

Flow: Adjust the flow of the mobile phase B so that the retention time for teceleukin is 45 to 65 minutes. Measure the retention time from the point at which the mobile phase is switched to the mobile phase B.

System suitability—

System performance: Dissolve in water a mixture of two kinds of equine heart-derived myoglobin whose isoelectric points are 6.76 and 7.16 to make a concentration of approximately 0.5 mg/mL. Mix together 50 μL of this solution, 50 μL of Teceleukin (Genetical Recombination), and 1.47 mL of water. When 1.2 mL of this solution is run under the above conditions, myoglobin and teceleukin are eluted in this order, and their respective peaks are completely separated.

(4) Dimer—Prepare a sample solution by adding 20 μL of 0.2% sodium laurylsulfate TS to 20 μL of Teceleukin (Genetical Recombination). Perform the test as directed under Liquid Chromatography <2.01> using 20 μL of this solution under the following conditions. Determine using automated integration the teceleukin peak area, A1, and the peak area, A2, of the dimer with a relative retention time of 0.8 to 0.9 in relation to teceleukin. The amount of the dimer is not more than 1.0% by the following formula.

\[
\text{Amount (\% of dimer) } = \frac{A_1}{(A_1 + A_2)} \times 100
\]

Operating conditions—

Detector: Ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column with an inside diameter of 7.5 mm and 60 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 0.1 mol/L sodium phosphate buffer, pH 7.0, to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of teceleukin is between 30 and 40 minutes.

System suitability—

System performance: Add 20 μL of 0.2% sodium lauryl sulfate TS to 20 μL of a solution consisting of 5 mg of carbonic anhydrase and 5 mg of α-lactoalbuminin dissolved in 100 mL of water. When 20 μL of this solution is tested under the above conditions, the relative standard deviation of the teceleukin peak area is not more than 7%.

(5) Other related proteins—Perform the test on 5 μL of Teceleukin (Genetical Recombination) as directed under Liquid Chromatography <2.01> under the following conditions, and measure the area of each peak using automatic integration. When the amounts are determined by the area percent method, the total amount of peaks other than the teceleukin and solvent peaks is not more than 1.0%.

Operating conditions—

Detector: Ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column with an inside diameter of 4.6 mm and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: A solution of trifluoroacetic acid in a mixture of water and acetonitrile (19:1) (1 in 1000).

Mobile phase B: A solution of trifluoroacetic acid in acetonitrile (7 in 10,000).

Mobile phase flow: Control the concentration gradient by changing the mobile phase A and mobile phase B as shown in the table below.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ~ 12</td>
<td>60 → 50</td>
<td>40 → 50</td>
</tr>
<tr>
<td>12 ~ 25</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>25 ~ 45</td>
<td>50 → 0</td>
<td>50 → 100</td>
</tr>
<tr>
<td>45 ~ 50</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL/min.

Time span of measurement: a range that is approximately 1.2-fold the retention time of teceleukin.

System suitability—

System performance: Add 3.8 μL of water and 16.6 μL of polysorbet 80 solution (1 in 100) to 83.6 μL of Teceleukin (Genetical Recombination) and let stand for at least one hour. When 5 μL of this solution is tested by running under the above conditions, there is complete separation between the teceleukin peak and the peak with a relative retention time of about 0.98 in relation to the teceleukin peak.

(6) Acetic acid—Measure exactly 0.25 mL of Teceleukin (Genetical Recombination) and add exactly 0.25 mL of the internal standard solution to make the sample solution. Separately, measure exactly 3 mL of acetic acid (100) and add water to make exactly 100 mL. Take exactly 10 μL of this solution and add water to make exactly 100 mL. Measure exactly 2 mL of this solution and add exactly 2 mL of the internal standard solution to make the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution by Gas Chromatography <2.02> under the following conditions. Calculate the ratios of the peak area of acetic acid to that of the internal standard, Qx and Qh, and the amount of acetic acid (C2H4O2) in 1 mL of Teceleukin (Genetical Recombination) determined by the following formula is between 2.85 and 3.15 mg.
Amount (mg) of acetic acid (C₂H₄O₂) in 1 mL of Teceleukin (Genetical Recombination)

\[
Q_2 = \frac{Q_1}{Q_3} \times 1.5 \times 1.049 \times 2
\]

1.5: Concentration (µL/mL) of acetic acid (100) in the standard solution

1.049: Density (mg/µL) of acetic acid (100) at 25°C

2: Dilution coefficient

**Internal standard solution—Diluted propionic acid (1 in 500).**

**Operating conditions—**

Detector: Hydrogen flame ionization detector.

Column: A glass column with an inside diameter of 1.2 mm and 40 m in length, whose inside is covered with chemically-bound polyethylene glycol for gas chromatography 1.0 μm in thickness.

Column temperature: A constant temperature of about 110°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 8 minutes.

**System suitability—**

System performance: When 1 µL of the standard solution is run under the above conditions, acetic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeatedly run 6 times under the above conditions using 1 µL of standard solution, the relative standard deviation of the ratio of the acetic acid peak area to the internal standard peak area is not more than 5%.

**Bacterial endotoxins** Less than 5EU per mg of protein.

**Specific activity** Accurately measure an appropriate amount of Teceleukin (Genetical Recombination), and add water accurately so that 1 mL contains about 0.1 mg. This is the sample solution. Separately, measure precisely about 25 mg of human serum albumin for assay, dissolve in water, and add water to make 50 mL. Measure exactly an appropriate amount of this solution, and accurately dilute with water to make standard solutions with concentrations of 0.05, 0.10, and 0.15 mg/mL. Measure accurately 1 mL each of the sample solution, the standard solutions, and water, add 2.5 mL of alkaline copper solution, mix, leave for at least 10 minutes to dissolve, add exactly 2.5 mL of water and 0.5 mL of diluted Folin reagent (1 in 2), immediately shake vigorously, and then leave for 30 minutes at 37°C. Perform the test on these solutions, with water as a control, as directed under Ultraviolet-visible Spectrophotometry, and measure the absorbance at 750 nm. With the concentration of the standard solution as the x-axis and the absorbance as the y-axis, perform linear regression using their respective reciprocals, and calculate the protein content.

Calculate the ratio of the potency determined by Assay and the protein content.

**Assay** Accurately measure an appropriate amount of Teceleukin (Genetical Recombination) and, depending on the cell sensitivity, dilute precisely by adding culture medium for assay of teceleukin to a constant concentration of 10 to 50 units/mL (estimated value). This is the sample solution. Separately, dissolve Interleukin-2 Reference Substance in 1 mL of sterilized purified water, and, depending on the cell sensitivity, dilute precisely by adding culture medium for assay of teceleukin to a constant concentration of 10 to 50 units/mL. This is the standard solution. Add exactly 50 µL of culture medium for assay of teceleukin to all but 8 wells in a microtest plate. Add 50 µL of the sample solution and the standard solution to 2 wells each containing culture medium for assay of teceleukin. From these 4 wells, remove exactly 50 µL and add to 4 other wells containing culture medium for assay of teceleukin. From these 4 wells, remove exactly 50 µL and add to 4 other wells containing culture medium for assay of teceleukin and repeat this procedure to prepare 2 wells that contain each of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, and 1/256 dilutions of the sample and standard solutions. Add 50 µL of the standard solution to each of the 8 empty wells to make maximum uptake controls. Eight wells containing only culture medium for assay of teceleukin serve as the minimum uptake controls. After adding exactly 50 µL of cell suspension solution for teceleukin to each well in a microtest plate, leave for 15 to 17 hours in an incubator at 37°C filled with air containing 5% carbon dioxide. After adding 25 µL of MTT TS to each of the wells in the plate, leave for 4 hours in an incubator at 37°C filled with air containing 5% carbon dioxide. Transfer the culture medium in all of the wells to empty wells in another microtest plate. To each of the empty wells from which the culture medium was removed, add 100 µL of hydrochloric acid-2-propanol TS, and then shake the plates horizontally for 5 minutes to elute the pigment. After returning the transferred culture medium to each original well, perform the test with the solution in each well, determine the difference in absorption at wavelength of 560 nm and 690 nm, and calculate the mean values of the identical respective solutions in the two wells (dilution solutions of the sample solution and standard solutions) as well as the 8 wells containing the maximum or minimum uptake controls. Prepare standard curves by plotting the values obtained from each dilution solution of the sample solution, with the dilution coefficient of the sample solution on the microtest plates in logarithmic scale on the horizontal axis and the absorbance on the vertical axis. Determine the mean absorbance values of the maximum and minimum uptake controls, find the values on the standard curve, and then calculate the dilution coefficient, Dns. Perform the same plot for the dilution solution of the standard solution, calculate the dilution coefficient, Dns, and then calculate the potency in 1 mL by the following formula.

Teceleukin potency (units) in 1 mL of Teceleukin (Genetical Recombination)

\[
S = \frac{D_T}{D_S} \times d
\]

S: Concentration of standard solution (units/mL)

D: Dilution coefficient when sample solution prepared
Teceleukin for Injection (Genetical Recombination)

Teceleukin for Injection (Genetical Recombination) is a preparation for injection which is dissolved before use.

It contains not less than 70.0% and not more than 150.0% of the labeled amount of teceleukin (genetical recombination) \( C_{699}H_{1127}N_{179}O_{204}S_{8} \times 15547.01 \).

**Method of preparation** Prepare as directed under Injection, with Teceleukin (Genetical Recombination).

**Description** Teceleukin for Injection (Genetical Recombination) occurs as a white, light mass or powder.

**Identification (1)** Dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of sterilized purified water, dilute exactly with culture medium for assay of teceleukin to make the sample stock solution containing about 200 units per mL (estimate). Proceed as directed in the Identification (1) under Teceleukin (Genetical Recombination).

**Method (2)** Dissolve 0.242 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 5.0 g of sodium lauryl sulfate and 74 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 60 mL of water. Adjust to pH 8.0 with 1 mol/L hydrochloric acid TS, add water to make 100 mL, and use this solution as the buffer solution for molecular mass determination. Separately, dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in exactly 1 mL of water. To exactly 100 µL of this solution add exactly 100 µL of the buffer solution for molecular mass determination and 10 µL of 2-mercaptoethanol, and heat on a water bath for 5 minutes without allowing any water evaporation from the mixture. After cooling, add exactly 1 µL of bromophenol blue solution (1 in 2000), mix, and use this solution as the sample solution. Proceed as directed in Identification (3) under Teceleukin (Genetical Recombination): a band appears in the range of molecular mass between 14,000 and 16,000.

**pH** \(< 2.54\) Dissolve the content of one vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of water: the pH of the solution is between 7.0 and 7.7.

**Purity** Clarity and color of solution—Dissolve the content of one vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of water: the solution is clear and colorless.

**Loss on drying** Transfer the content of the vial of Teceleukin for Injection (Genetical Recombination) to a weighing bottle under the atmosphere not exceeding 10% relative humidity, and perform the test as directed in the Water content determination described in the Minimum Requirements for Biological Products: not more than 5%.

**Bacterial endotoxins** \(< 4.0 \times 10^{-6}\) Less than 5 EU/350,000 units.

**Uniformity of dosage units** \( < 6.02\) It meets the requirement of the Mass variation test. Calculate as \( |M - A| = 0 \).

**Foreign insoluble matter** \( < 6.06\) Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** \( < 6.07\) It meets the requirement.

**Sterility** \( < 4.06\) Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in exactly 1 mL of sterilized purified water, dilute exactly with culture medium for assay of teceleukin to make the sample solution containing a definite concentration of 10 to 50 units/mL (estimate). Proceed as directed in the Assay under Teceleukin (Genetical Recombination), and calculate the amount (unit) of teceleukin in 1 vial by the following formula.

- Amount (unit) of teceleukin in 1 vial = \( S \times \frac{D_t}{D_s} \times d \times 1 \)
- \( S \): Concentration of the standard solution (unit/mL)
- \( d \): Dilution coefficient when sample solution prepared
- \( 1 \): Volume (mL) of the sample solution

**Containers and storage** Containers—Hermetic containers. Storage—Light-resistant, not exceeding 10°C, avoiding freezing.

***Tegafur***

Teceleukin for Injection (Genetical Recombination) occurs as a white, light mass or powder.

It contains not less than 70.0% and not more than 150.0% of the labeled amount of teceleukin (genetical recombination) (\( C_{699}H_{1127}N_{179}O_{204}S_{8} \times 15547.01 \)).

**Method of preparation** Prepare as directed under Injection, with Teceleukin (Genetical Recombination).

**Description** Tegafur occurs as a white, crystalline powder.

It is soluble in methanol and in acetone, and sparingly soluble in water and in ethanol (95).

It dissolves in dilute sodium hydroxide TS.

A solution of Tegafur in methanol (1 in 50) shows no optical rotation.

**Identification (1)** Prepare the test solution with 0.01 g of Tegafur as directed under Oxygen Flask Combustion Method \(< 1.06\), using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests \(< 1.09\) for fluoride.

**Identification (2)** Determine the absorption spectrum of a solution of Tegafur in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( < 2.34\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Identification (3)** Determine the infrared absorption spectrum of Tegafur, previously dried, as directed in the potassium bro-
mide disk method under Infrared Spectrophotometry \( <2.25 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample with a mixture of methanol and acetone (1:1), filter and dry the crystals, and perform the test with the crystals.

**pH \( <2.54 \)**—Dissolve 0.5 g of Tegafur in 50 mL of water: the pH of this solution is between 4.2 and 5.2.

**Melting point \( <2.60 \)**—166–171°C

**Purity (1)** Clarity and color of solution—Dissolve 0.2 g of Tegafur in 10 mL of dilute sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride \( <1.03 \)—Dissolve 0.8 g of Tegafur in 40 mL of water by warming, cool, filter if necessary, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Heavy metals \( <1.07 \)—Dissolve 1.0 g of Tegafur in 40 mL of water by warming, cool, filter if necessary, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic \( <1.11 \)—Prepare the test solution in a platinum crucible with 1.0 g of Tegafur according to Method 4, incinerating by ignition between 750°C and 850°C, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Tegafur in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.03 \). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95:5) (5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying \( <2.41 \)** Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition \( <2.44 \)** Not more than 0.1% (1 g, platinum crucible).

**Assay** Weigh accurately about 0.15 g of Tegafur, previously dried in an iodine bottle, dissolve in 75 mL of water, and add exactly 25 mL of 1/60 mol/L potassium bromate VS. Add rapidly 1.0 g of potassium iodide, shake gently, allow to stand for exactly 5 minutes, and titrate \( <2.50 \) the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination.

Each mL of 1/60 mol/L potassium bromate VS = 10.01 mg of C₈₈H₉₇Cl₂N₉O₃₃

### Teicoplanin

**Containers and storage** Containers—Tight containers.

#### Teicoplanin

![Teicoplanin Structure](image)

**Teicoplanin A₁**

C₈₈H₉₇Cl₂N₉O₃₃: 1877.64


**Teicoplanin A2**

C₈₈H₉₅Cl₂N₉O₃₃: 1879.66

Teicoplanin is a mixture of glycopeptide substances having antibacterial activity produced by the growth of *Actinoplanes teichomyceticus*.

It contains not less than 900 µg (potency) and not more than 1120 µg (potency) per 1 mg, calculated on the anhydrous, de-sodium chloride and de-residual solvents basis. The potency of Teicoplanin is expressed as mass (potency) of teicoplanin (C72H68Cl2N8O28: 1564.25).

**Description**

Teicoplanin occurs as a white to light yellowish white powder.

It is freely soluble in water, sparingly soluble in *N*,*N*-dimethylformamide, and practically insoluble in acetonitrile, in methanol, in ethanol (95), in acetone, in acetic acid (100) and in diethyl ether.

**Identification**

(1) To 1 mL of a solution of Teicoplanin (1 in 100) add 2 mL of ninhydrin TS, and warm for 5 minutes: a blue-purple color develops.

(2) To 1 mL of a solution of Teicoplanin (3 in 100) add slowly 2 mL of anthrone TS, and shake gently: a dark brown color develops.

(3) Determine the infrared absorption spectra of Teicoplanin and Teicoplanin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the spectrum of Teicoplanin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**pH**

$\text{pH} < 2.54$  

Dissolve 0.5 g of Teicoplanin in 10 mL of water: the pH of the solution is between 6.3 and 7.7.

**Content ratio of the active principle**

Dissolve about 20 mg of Teicoplanin in water to make 10 mL, and use this solution as the sample solution. Perform the test with 20 µL of the sample solution as directed under Liquid Chromatography 2.07D according to the following conditions, and calculate the sum of peak areas of teicoplanin A2 group, $S_a$, the sum of peak areas of teicoplanin A3 group, $S_p$, and the sum of peak areas of other contents, $S_b$, from the sample solution by the automatic integration method. Calculate the content ratio of them by the formula given below: teicoplanin A2 group, teicoplanin A3 group, and the other are not less than 80.0%, not more than 15.0% and not more than 5.0%, respectively.

The elution order of each content and the relative retention time of each content to the retention time of teicoplanin A2 are shown in the following table.

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<td>0.42 $&lt;, \neq 1.25$</td>
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<td>teicoplanin A3 group</td>
<td>4</td>
<td>0.91</td>
</tr>
<tr>
<td>teicoplanin A2 group</td>
<td>5</td>
<td>1.00</td>
</tr>
<tr>
<td>teicoplanin A3 group</td>
<td>6</td>
<td>1.04</td>
</tr>
<tr>
<td>others</td>
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(3) Determine the infrared absorption spectra of Teicoplanin and Teicoplanin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the spectrum of Teicoplanin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**pH**

$\text{pH} < 2.54$  

Dissolve 0.5 g of Teicoplanin in 10 mL of water: the pH of the solution is between 6.3 and 7.7.

**Content ratio of the active principle**

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**pH**

$\text{pH} < 2.54$  

Dissolve 0.5 g of Teicoplanin in 10 mL of water: the pH of the solution is between 6.3 and 7.7.

**Content ratio of the active principle**

Dissolve about 20 mg of Teicoplanin in water to make 10 mL, and use this solution as the sample solution. Perform the test with 20 µL of the sample solution as directed under Liquid Chromatography 2.07D according to the following conditions, and calculate the sum of peak areas of teicoplanin A2 group, $S_a$, the sum of peak areas of teicoplanin A3 group, $S_p$, and the sum of peak areas of other contents, $S_b$, from the sample solution by the automatic integration method. Calculate the content ratio of them by the formula given below: teicoplanin A2 group, teicoplanin A3 group, and the other are not less than 80.0%, not more than 15.0% and not more than 5.0%, respectively.

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Content ratio (% of teicoplanin A$_2$ group
  \[ S_2/(S_2 + 0.83S_b + S_z) \times 100 \]
Content ratio (% of teicoplanin A$_3$ group
  \[ 0.83S_z/(S_2 + 0.83S_b + S_z) \times 100 \]
Content ratio (% of others
  \[ S_z/(S_2 + 0.83S_b + S_z) \times 100 \]

Operating conditions—
  Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
  Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
  Column temperature: A constant temperature of about 25°C.
  Mobile phase A: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 1650 mL of water, add 300 mL of acetonitrile, adjust pH to 6.0 with sodium hydroxide TS, and add water to make 2000 mL.
  Mobile phase B: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 550 mL of water, add 1400 mL of acetonitrile, adjust the pH to 6.0 with sodium hydroxide TS, and add water to make 2000 mL.
  Flowing of the mobile phase: Flow mobile phase A for 10 minutes before injection. After injection, control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 32</td>
<td>100 → 70</td>
<td>0 → 30</td>
</tr>
<tr>
<td>32 – 40</td>
<td>70 → 50</td>
<td>30 → 50</td>
</tr>
<tr>
<td>40 – 42</td>
<td>50 → 100</td>
<td>50 → 0</td>
</tr>
</tbody>
</table>

Flow rate: 1.8 mL per minute.

Time span of measurement: About 1.7 times as long as the retention time of teicoplanin A$_2$, beginning after the solvent peak.

System suitability—
  Test for required detection: Confirm that peak height of teicoplanin A$_2$ obtained from the sample solution is equivalent to 90% of the full scale.
  System performance: When the procedure is run with 20 µL of the sample solution under the above operating conditions, the symmetry factor of the peak of teicoplanin A$_3$ is not more than 2.2.
  System repeatability: When the test is repeated 3 times with 20 µL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of teicoplanin A$_2$ is not more than 2.0%.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Sodium chloride—Weigh accurately about 0.5 g of Teicoplanin, dissolve in 50 mL of water, titrate 2.50 with 0.1 mol/L silver nitrate VS (indicator: 1 mL of potassium chromate TS), and calculate an amount of sodium chloride: not more than 5.0%.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

(3) Heavy metals ≤1.07—Being specified separately.
(4) Arsenic ≤1.17—Being specified separately.
(5) Residual solvents ≤2.47—Weigh accurately about 0.1 g of Teicoplanin, dissolve in N,N-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g each of methanol and acetone, and add N,N-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add N,N-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 4 µL each of the sample solution and standard solution as directed under Gas Chromatography 2.02 according to the following conditions. Determine the peak area of methanol, A$_1$, and the peak area of acetone, A$_2$, obtained from the sample solution, and the peak area of methanol, A$_{51}$, and the peak area of acetone, A$_{52}$, obtained from the standard solution by the automatic integration method, and calculate the amounts of methanol and acetone by the following formula: not more than 0.5% and not more than 1.0%, respectively.

\[
\text{Amount (µg) of methanol} = S_{51} \times A_1/A_{51} \times 0.001 \times 1/M_F \times 100
\]
\[
\text{Amount (µg) of acetone} = S_{52} \times A_2/A_{52} \times 0.001 \times 1/M_F \times 100
\]

M$_{51}$: Amount (g) of methanol
M$_{52}$: Amount (g) of acetone
M$_F$: Amount (g) of Teicoplanin

Operating conditions—
  Detector: Hydrogen flame-ionization detector.
  Column: A glass column 2 mm in inside diameter and 3 m in length, packed with graphite carbon for gas chromatography, 150 to 180 µm in particle diameter, coated with 0.1% of polyethylene glycol esterified.
  Column temperature: Inject the sample at a constant temperature of about 70°C, maintain the temperature for 4 minutes, then program to increase the temperature at the rate of 8°C per minute to 210°C.
  Detector temperature: A constant temperature of about 240°C.
  Carrier gas: Nitrogen.
  Flow rate: Adjust the flow rate so that the retention times of methanol and acetone are about 2 minutes and 5 minutes, respectively.
  System suitability—
  Test for required detection: Confirm that the peak height of acetone obtained from 4 µL of the standard solution is equivalent to about the full scale.
  System performance: When the procedure is run with 4 µL of the standard solution under the above operating conditions, methanol and acetone are eluted in this order with the resolution between these peaks being not less than 2.0.
  System repeatability: When the test is repeated 3 times with 4 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetone is not more than 3%.

Water ≤2.47 Not more than 15.0% (0.2 g, volumetric titration, direct titration).

Bacterial endotoxins ≤4.01 Less than 0.75 EU/mg (potency).
Blood pressure depressant  Being specified separately.

Assay  Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics \(
<4.02\)
 according to the following conditions.

(i)  Test organism—
*Bacillus subtilis* ATCC 6633

(ii)  Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii)  Standard solutions—Weigh accurately an amount of Teicoplanin RS equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of this solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 160 µg (potency) and 40 µg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv)  Sample solutions—Weigh accurately an amount of Teicoplanin equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 160 µg (potency) and 40 µg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage  Containers—Tight containers. Storage—Light-resistant, and not exceeding 5°C.

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**Temocapril Hydrochloride**

テモカプリル塩酸塩

\[
\text{C}_{23}\text{H}_{28}\text{N}_{2}\text{O}_{5}\text{S}_{2}\text{HCl}: 513.07
\]

\[2-\{(2S,6R)-6-\{1\text{S}-1\text{(Ethoxy carbonyl)-3-phenylpropyl} \}\text{amino}\}-5\text{-oxo-2-(thiophen-2-yl)-2,3,6,7-tetrahydro-1,4-thiazepin-4\text{(5H)-yl}\}\text{acetic acid monohydrochloride}\]

\[\text{[110221-44-8]}\]

Temocapril Hydrochloride contains not less than 99.0% and not more than 101.0% of \(\text{C}_{23}\text{H}_{28}\text{N}_{2}\text{O}_{5}\text{S}_{2}\) HCl, calculated on the anhydrous basis.

Description  Temocapril Hydrochloride occurs as a white crystalline powder.

It is freely soluble in ethanol (99.5), and very slightly soluble in water.

Identification  (1) Determine the absorption spectrum of a solution of Temocapril Hydrochloride in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\)
, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Temocapril Hydrochloride as directed in the paste method under Infrared Spectrophotometry \(<2.25\)
, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Temocapril Hydrochloride in ethanol (99.5) (1 in 100) responds to the Qualitative Tests \(<1.09\)
 (2) for chloride.

Optical rotation  \(<2.49\) \([\alpha]_{D}^{20}: +60 - +64^\circ (0.2 \text{ g}, \text{ calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm})\).

Purity  (1) Heavy metals  \(<1.07\)  Proceed with 1.0 g of Temocapril Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Temocapril Hydrochloride in 100 mL of diluted acetonitrile (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted acetonitrile (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\)
 according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than temocapril obtained from the sample solution is not larger than 1/5 times the peak area of temocapril from the standard solution, and the total area of the peaks other than temocapril from the sample solution is not larger than 1/2 times the peak area of temocapril from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile (63:37). Flow rate: Adjust the flow rate so that the retention time of temocapril is about 11 minutes.

Time span of measurement: About 4 times as long as the retention time of temocapril, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add diluted acetonitrile (1 in 2) to make exactly 10 mL. Confirm that the peak area of temocapril obtained with 10 µL of this solution is equivalent to 7 to 13% of that with 10 µL of the standard solution.

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of temocapril are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of temocapril is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water  \(<2.48\)  Not more than 1.0% (0.3 g, coulometric titration).
Residue on ignition <2.4% Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Temocapril Hydrochloride, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.5% with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 51.31 mg of C\textsubscript{23}H\textsubscript{28}N\textsubscript{2}O\textsubscript{5}S\textsubscript{2}.HCl

Containers and storage Containers—Well-closed containers.

Temocapril Hydrochloride Tablets

テモカプリル塩酸塩

Temocapril Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of temocapril hydrochloride (C\textsubscript{23}H\textsubscript{28}N\textsubscript{2}O\textsubscript{5}S\textsubscript{2}.HCl: 513.07).

Method of preparation Prepare as directed under Tablets, with Temocapril Hydrochloride.

Identification To an amount of powdered Temocapril Hydrochloride Tablets, equivalent to 2.5 mg of Temocapril Hydrochloride according to the labeled amount, add 25 mL of diluted acetonitrile (1 in 2), shake vigorously for 10 minutes, and centrifuge. To 5 mL of the supernatant liquid add diluted acetonitrile (1 in 2) to make 25 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.4%: it exhibits a maximum between 232 nm and 236 nm.

Uniformity of dosage units <6.02% Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Temocapril Hydrochloride Tablets add exactly 20 mL of diluted acetonitrile (1 in 2), and agitate for 10 minutes with the aid of ultrasonic waves. Furthermore, shake for 10 minutes, and centrifuge. Pipet V mL of the supernatant liquid equivalent to about 0.8 mg of temocapril hydrochloride (C\textsubscript{23}H\textsubscript{28}N\textsubscript{2}O\textsubscript{5}S\textsubscript{2}.HCl) according to the labeled amount, and add diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of temocapril hydrochloride for assay (separately determine the water <2.4% in the same manner as Temocapril Hydrochloride), and dissolve in diluted acetonitrile (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 mL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01% according to the following conditions, and determine the peak area, A\textsubscript{T} and A\textsubscript{S}, of temocapril of both solutions.

Dissolution <6.10% When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Temocapril Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Temocapril Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 1.1 μg of temocapril hydrochloride (C\textsubscript{23}H\textsubscript{28}N\textsubscript{2}O\textsubscript{5}S\textsubscript{2}.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of temocapril hydrochloride for assay (separately determine the water <2.4% in the same manner as Temocapril Hydrochloride), and dissolve in diluted acetonitrile (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01% according to the following conditions, and determine the peak area, A\textsubscript{T} and A\textsubscript{S}, of temocapril of both solutions.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, temocapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of temocapril to that of the internal standard is not more than 1.0%.

Amount (mg) of temocapril hydrochloride for assay, calculated on the anhydrous basis

M\textsubscript{S}: Amount (mg) of temocapril hydrochloride for assay,

\[ M_{S} = \frac{M_{S} \times Q_{T} / Q_{S} \times 1/V \times 2/5}{C} \]

C: Labeled amount (mg) of temocapril hydrochloride (C\textsubscript{23}H\textsubscript{28}N\textsubscript{2}O\textsubscript{5}S\textsubscript{2}.HCl) in 1 tablet

M\textsubscript{S}: Amount (mg) of temocapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parabenzoate in diluted acetonitrile (1 in 2) (1 in 3000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, temocapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of temocapril to that of the internal standard is not more than 1.0%.
ditions, the number of theoretical plates and the symmetry factor of the peak of temocapril are not less than 9000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of temocapril is not more than 2.0%.

**Assay**

Weigh accurately the mass of not less than 20 Temocapril Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of temocapril hydrochloride (C₂₃H₂₈N₂O₅S₂.HCl), add exactly 20 mL of the internal standard solution, and shake for 10 minutes with the aid of ultrasonic waves. Furthermore, shake for 10 minutes, and centrifuge. To 2 mL of the supernatant liquid add diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of temocapril hydrochloride (C₂₃H₂₈N₂O₅S₂.HCl), add exactly 10 mL of the internal standard solution, and agitate for 10 minutes with the aid of ultrasonic waves. Furthermore, shake for 10 minutes, and centrifuge. To 2 mL of the supernatant liquid add diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Qₛ and Qₛ, of the peak area of temocapril to that of the internal standard.

\[
M_s = \frac{S}{\frac{T}{Q_s}} \times \frac{1}{5}
\]

**Internal standard solution—**A solution of propyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 3000).

**Operating conditions—**

- Detector: An ultraviolet absorption spectrophotometer (wavelength: 234 nm).
- Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile (63:37).
- Flow rate: Adjust the flow rate so that the retention time of temocapril is about 10 minutes.

**System suitability—**

- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, temocapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of temocapril to that of the internal standard is not more than 1.0%.

**Containers and storage**

Containers—Well-closed containers.

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**Teprenone**

テプレノン

C₃₁H₅₂O: 330.55

(5E,9E,13E)-6,10,14,18-Tetramethylnonadeca-5,9,13,17-tetraen-2-one

【6809-52-5】

Teprenone contains not less than 97.0% and not more than 101.0% of C₃₁H₅₂O.

Teprenone is comprised of mono-cis and all-trans isomers, with their ratio being about 2:3.

**Description**

Teprenone occurs as a colorless to slightly yellowish clear oily liquid, with slight, characteristic odor. It is miscible with ethanol (99.5), with ethyl acetate and with hexane. It is practically insoluble in water. It is oxidized by air, and gradually turns yellow.

**Identification**

1. To 2 mL of a solution of Teprenone in ethanol (99.5) (1 in 100) add 1 mL of a solution of phosphomolybdic acid n-hydrate in acetic acid (100) (1 in 100), heat in a water bath for 5 minutes, and continue heating with addition of 5 to 6 drops of sulfuric acid: blue to bluish green color develops.

2. To 2 mL of a solution of Teprenone in ethanol (99.5) (1 in 100) add 2 mL of 2,4-dinitrophenylhydrazine TS, and shake: a yellow to orange-yellow precipitate is formed.

3. Determine the infrared absorption spectrum of Teprenone as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Teprenone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Refractive index**<2.45> \(n_\text{D}^20: 1.485 - 1.491\)

**Specific gravity**<2.56> \(d_\text{D}^20: 0.882 - 0.890\)

**Purity**

1. Clarity and color of solution—To 1.0 mL of Teprenone add 9 mL of ethanol (99.5) and shake: the solution is clear, and its absorbance at 400 nm determined as described under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.02.

2. Heavy metals <1.07>—Proceed with 1.0 g of Teprenone according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

3. Related substances—Dissolve 30 mg of Teprenone in 6 mL of hexane, and use this solution as the sample solution. Perform the test with 3 μL of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area from the sample
solution by the automatic integration method and calculate the amounts of them by the area percentage method: the peak area of the di-cis isomer of teprenone, having the relative retention time of about 0.8 with respect to the all-trans isomer of teprenone, is not more than 0.5%, and each area of the peaks for the mono-cis and all-trans isomers of the teprenone and for those other than mentioned above is not more than 0.2%. Furthermore, the total area of the peaks other than the mono-cis, all-trans and di-cis isomers of teprenone is not more than 1.0%.

Operating conditions—
Detector, column, column temperature, carrier gas and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 2 times as long as the retention time for the all-trans isomer of teprenone beginning after the solvent peak.

System suitability—
Test for required detectability: To 1 mL of the sample solution add hexane to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add hexane to make exactly 10 mL. Confirm that the sum of the peak areas of the mono-cis and all-trans isomers of teprenone obtained from 3 µL of this solution is 7 to 13% of the peak areas of the mono-cis and all-trans isomers of teprenone from 3 µL of the solution for system suitability test.

System performance: When the procedure is run with 3 µL of the solution for system suitability test under the above operating conditions, the mono-cis and all-trans isomers of teprenone are eluted in this order with the resolution between these peaks being not less than 1.1.

System repeatability: When the test is repeated 6 times with 3 µL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the sum of the peak areas of the mono-cis and all-trans isomers of teprenone is not more than 3.0%.

(4) Residual solvent—Being specified separately.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Isomer ratio Dissolve 30 mg of Teprenone in 6 mL of hexane, and use this solution as the sample solution. Perform the test with 3 µL of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the areas of two adjacent peaks, $A_s$ and $A_b$, having retention times of about 18 minutes, where $A_s$ is the peak of the mono-cis isomer, having the shorter retention time, and $A_b$ is the peak area of the all-trans isomer, having the longer retention time: $A_s/A_b$ is 0.60 to 0.70.

Operating conditions—
Proceed as directed in the operating conditions in the Assay.

System suitability—
Proceed as directed in the system suitability in the Purity (3).

Assay Weigh accurately about 50 mg each of Teprenone and Teprenone RS, dissolve each in exactly 5 mL of the internal standard solution, add ethyl acetate to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 3 µL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, $Q_s$ and $Q_b$, of the peak area of teprenone (sum of the peak areas of mono-cis and all-trans isomers) to that of the internal standard.

Amount (mg) of teprenone ($C_{23}H_{32}O$) = $M_s \times \frac{Q_A}{Q_s}$

$M_s$: Amount (mg) of Teprenone RS

Internal standard solution—A solution of di-n-butyl phthalate in ethyl acetate (1 in 200).

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 4 mm in inside diameter and 2 m in length, packed with 149 to 177 µm siliceous earth for gas chromatography coated in 5% with polyethylene glycol 2-nitroterephthalate.
Column temperature: A constant temperature of about 210°C.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust the flow rate so that the retention time of the peak of the all-trans isomer of teprenone, the larger of the two main peaks, having the retention time about 18 minutes, becomes 19 minutes.
System suitability—
System performance: When the procedure is run with 3 µL of the standard solution under the above operating conditions, the internal standard and the mono-cis and all-trans isomers of teprenone are eluted in this order with the resolution between the mono-cis and all-trans isomers being not less than 1.1.
System repeatability: When the test is repeated 6 times with 3 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the sum of the peak areas of the mono-cis and all-trans isomers of teprenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight Containers.
Storage—Under Nitrogen atmosphere at 2 to 8°C.

Terbinafine Hydrochloride

#### Terbinafine Hydrochloride

C13H25N.HCl: 327.89

(2E)-N,6,6-Trimethyl-N-(naphthalen-1-ylmethyl)hept-2-en-4-yl-amine monohydrochloride

[78628-80-5]

Terbinafine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C13H25N.HCl.

Description Terbinafine Hydrochloride occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), and slightly soluble in water.

The pH of a solution of 1.0 g of Terbinafine Hydrochloro-
ride in 1000 mL of water is 3.5 to 4.5.
Melting point: about 205°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Terbinafine Hydrochloride in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Terbinafine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Terbinafine Hydrochloride in ethanol (99.5) (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Terbinafine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Terbinafine Hydrochloride in 100 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of a dimer, having the relative retention time of about 1.7 with respect to terbinafine obtained from the sample solution is not greater than 1/2 times the peak area of terbinafine from the standard solution, the area of the peak other than terbinafine and the dimer from the sample solution is not greater than the peak area of terbinafine from the standard solution, and the total area of the peaks other than terbinafine is not greater than 3 times the peak area of terbinafine from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 3 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase A: To 700 mL of a mixture of methanol and acetonitrile (3:2) add 300 mL of a solution of triethylamine (1 in 500) adjusted to pH 7.5 with dilute acetic acid.
Mobile phase B: To 950 mL of a mixture of methanol and acetonitrile (3:2) add 50 mL of a solution of triethylamine (1 in 500) adjusted to pH 7.5 with dilute acetic acid.
Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 4</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4 – 25</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>25 – 30</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of terbinafine is about 15 minutes.
Time span of measurement: About 2 times as long as the retention time of terbinafine, beginning after the solvent peak.

**System suitability—**
Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and acetonitrile (1:1) to make exactly 20 mL. Confirm that the peak area of terbinafine obtained with 20 μL of this solution is equivalent to 18 to 32% of that with 20 μL of the standard solution.

System performance: Dissolve 20 mg of Terbinafine Hydrochloride in 20 mL of a mixture of water and acetonitrile (1:1), and irradiate under a short-wave lamp (main wavelength: 254 nm) for 1 hour. When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peak of cis-terbinafine, having the relative retention time of about 0.94 with respect to terbinafine, and the peak of terbinafine is not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terbinafine is not more than 2.0%.

(3) Residual solvent—Being specified separately.

**Loss on drying <2.41>** Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition <2.44>** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.26 g of Terbinafine Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.79 mg of C₂₁H₂₅N.HCl

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

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**Terbinafine Hydrochloride Cream**

**タルビナフィン塩酸塩クリーム**

Terbinafine Hydrochloride Cream contains not less than 95.0% and not more than 105.0% of the labeled amount of terbinafine hydrochloride (C₂₁H₂₅N.HCl: 327.89).

**Method of preparation** Prepare as directed under Creams, with Terbinafine Hydrochloride.

**Identification** To quantity of Terbinafine Hydrochloride Cream, equivalent to 10 mg of Terbinafine Hydrochloride,
Terbinafine Hydrochloride Solution

**Japanese Name:** テルビナフィン塩酸塩液

Terbinafine Hydrochloride Solution is a liquid for external use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of terbinafine hydrochloride (C$_{21}$H$_{25}$N$\cdot$HCl: 327.89).

**Method of preparation** Prepare as directed under Liquids and Solutions for Cutaneous Application, with Terbinafine Hydrochloride.

**Identification** To a volume of Terbinafine Hydrochloride Solution, equivalent to 10 mg of Terbinafine Hydrochloride according to the labeled amount, add methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of terbinafine hydrochloride for assay in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of a mixture of 80 volumes of hexane, 20 volumes of ethyl acetate and 1 volume of ammonia solution (28) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution shows the same Rf value with the spot from the standard solution.

**pH** Being specified separately.

**Assay** Weigh accurately an amount of Terbinafine Hydrochloride Cream, equivalent to about 10 mg of terbinafine hydrochloride (C$_{21}$H$_{25}$N$\cdot$HCl), dissolve in 2-propanol to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 40 mg of terbinafine hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in 2-propanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the terbinafine peak areas, $A_1$ and $A_5$, of both solutions.

Amount (mg) of terbinafine hydrochloride (C$_{21}$H$_{25}$N$\cdot$HCl) = $M_5 \times A_1/A_5 \times 1/4$

$M_5$: Amount (mg) of terbinafine hydrochloride for assay

**Operating conditions**—

**Detector:** An ultraviolet absorption photometer (wavelength: 282 nm).

**Column:** A stainless steel column 4.0 mm in inside diameter and 125 mm in length, packed with octadeclsiilated silica gel for liquid chromatography (5 μm in particle diameter).

**Column temperature:** A constant temperature of about 25°C.

**Mobile phase:** A mixture of a solution of tetramethylammonium hydroxide (9 in 2000) adjusted to pH 8.0 with diluted phosphoric acid (1 in 25), acetonitrile and tetrahydrofuran (2:2:1).

**Flow rate:** Adjust the flow rate so that the retention time of terbinafine is about 8.5 minutes.

**System suitability**—

**System performance:** Dissolve 40 mg of terbinafine hydrochloride for assay and 3.5 mg of terphenyl in 200 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, terphenyl and terbinafine are eluted in this order with the resolution between these peaks being not less than 6.

**System repeatability:** When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terbinafine is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

According to the labeled amount, dissolve in 20 mL of 2-propanol, and use this solution as the sample solution. Separately, dissolve 10 mg of terbinafine hydrochloride for assay in 20 mL of 2-propanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of a mixture of 80 volumes of hexane, 20 volumes of ethyl acetate and 1 volume of ammonia solution (28) to a distance of about 15 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution shows the same Rf value with the spot from the standard solution.

Amount (mg) of terbinafine hydrochloride (C$_{21}$H$_{25}$N$\cdot$HCl) = $M_5 \times A_1/A_5 \times 1/4$

$M_5$: Amount (mg) of terbinafine hydrochloride for assay

**Optimizing conditions**—

**Detector:** An ultraviolet absorption photometer (wavelength: 282 nm).

**Column:** A stainless steel column 4.0 mm in inside diameter and 125 mm in length, packed with octadeclsiilated silica gel for liquid chromatography (5 μm in particle diameter).

**Column temperature:** A constant temperature of about 25°C.

**Mobile phase:** A mixture of a solution of tetramethylammonium hydroxide (9 in 2000) adjusted to pH 8.0 with diluted phosphoric acid (1 in 25), acetonitrile and tetrahydrofuran (2:2:1).
Flow rate: Adjust the flow rate so that the retention time of terbinafine is about 8.5 minutes.

System suitability—
System performance: Dissolve 40 mg of terbinafine hydrochloride for assay and 3.5 mg of terphenyl in 200 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, terphenyl and terbinafine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terbinafine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Terbinafine Hydrochloride Spray

Terbinafine Hydrochloride Spray contains not less than 95.0% and not more than 105.0% of the labeled amount of terbinafine hydrochloride \( \text{C}_21\text{H}_{25}\text{N.HCl} \): 327.89).

Method of preparation Prepare as directed under Pump Sprays for Cutaneous Application, with Terbinafine Hydrochloride.

Identification To an amount of Terbinafine Hydrochloride Spray, equivalent to 10 mg of Terbinafine Hydrochloride, add methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of terbinafine hydrochloride for assay in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of a mixture of 80 volumes of hexane, 20 volumes of ethyl acetate and 1 volume of ammonia solution \(28\) to a distance of about 15 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution shows the same \(R_f\) value with the spot from the standard solution.

pH Being specified separately.

Assay Weigh accurately an amount of Terbinafine Hydrochloride Spray, equivalent to about 10 mg of terbinafine hydrochloride \( \text{C}_21\text{H}_{25}\text{N.HCl} \), dissolve in methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of terbinafine hydrochloride for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and determine the terbinafine peak areas, \(A_1\) and \(A_2\), of both solutions.

Amount (mg) of terbinafine hydrochloride \( \text{C}_21\text{H}_{25}\text{N.HCl} \)

\[
= M_2 \times A_1 / A_2 \times 1 / 4
\]

\(M_2\): Amount (mg) of terbinafine hydrochloride for assay

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 125 mm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of tetramethylammonium hydroxide \(9\) in \(2000\) adjusted to \(\text{pH} 8.0\) with diluted phosphoric acid \(1\) in \(25\), acetonitrile and tetrahydrofuran \(2\) in \(2\).

Flow rate: Adjust the flow rate so that the retention time of terbinafine is about 8.5 minutes.

System suitability—
System performance: Dissolve 40 mg of terbinafine hydrochloride for assay and 3.5 mg of terphenyl in 200 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, terphenyl and terbinafine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terbinafine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Terbutaline Sulfate

Terbutaline Sulfate contains not less than 98.5% of \( \text{C}_{12}\text{H}_{19}\text{NO}_3\text{.H}_2\text{SO}_4 \), calculated on the anhydrous basis.

Description Terbutaline Sulfate is white to slightly brownish white crystals or crystalline powder. It is odorless or has a faint odor of acetic acid.

It is freely soluble in water, and practically insoluble in acetonitrile, in ethanol \(95\), in acetic acid \(100\), in chloroform, and in diethyl ether.

It is gradually colored by light and by air.

Melting point: about 255°C (with decomposition).

Identification (1) Dissolve 1 mg of Terbutaline Sulfate in 1 mL of water, and add 5 mL of Tris buffer solution, \(\text{pH} 9.5\), 0.5 mL of 4-aminoantipyrine solution \(1\) in \(50\) and 2 drops of potassium hexacyanoferrate \((III)\) solution \(2\) in \(25\): a reddish purple color is produced.

(2) Determine the absorption spectrum of a solution of
Terbutaline Sulfate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2,24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths. This maximum can be biphasic.

(3) A solution of Terbutaline Sulfate (1 in 50) responds to the Qualitative Tests <1.09> for sulfate.

**pH <2.54>** Dissolve Terbutaline Sulfate in 10 mL of water: the pH of this solution is between 4.0 and 4.8.

**Purity (1)** Clarity and color of solution—Dissolve 0.10 g of Terbutaline Sulfate in 10 mL of water: the solution is clear and colorless or slightly yellow.

(2) Chloride <1.07>—Perform the test with 2.0 g of Terbutaline Sulfate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.004%).

(3) Acetic acid—Dissolve 0.50 g of Terbutaline Sulfate in a solution of phosphoric acid (59 in 1000) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 1.50 g of acetic acid (100) in a solution of phosphoric acid (59 in 1000) to make exactly 100 mL. Dilute 2 mL of this solution, accurately measured, with a solution of phosphoric acid (59 in 1000) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following operating conditions. Measure the peak areas, $A_F$ and $A_S$, of acetic acid for the two solutions: $A_F$ is not larger than $A_S$.

**Operating conditions—**

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with 10% of macrocol 6000 on 180- to 250-μm terephthalic acid for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 5 minutes.

**System suitability—**

System performance: Mix 0.05 g each of acetic acid (100) and propionic acid in 100 mL of diluted phosphoric acid (59 in 1000). When the procedure is run with 2 μL of this solution under the above conditions, acetic acid and propionic acid are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetic acid is not more than 3.0%.

(4) 3,5-Dihydroxy-ω-tert-butylaminoacetophenone sulfate—Dissolve 0.50 g of Terbutaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and perform the test as directed under Ultraviolet-visible Spectrophotometry <2,24>—the absorbance at a wavelength of 330 nm does not exceed 0.47.

(5) Heavy metals <1.07>—Proceed with 2.0 g of Terbutaline Sulfate as directed under Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of Terbutaline Sulfate according to method 3, and perform the test (not more than 2 ppm).

**Water <2.48>** Not more than 0.5% (1 g, volumetric titration, direct titration).

**Residue on ignition <2.44>** Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.5 g of Terbutaline Sulfate, dissolve in 50 mL of a mixture of acetonitrile and acetic acid (100) (1:1) by stirring and warming. Allow to cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration, substituting a saturated solution of potassium chloride in methanol for the internal fluid).

Each mL of 0.1 mol/L perchloric acid VS = 54.87 mg of (C₂H₉NO₃)₂H₂SO₄

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Testosterone Enanthate**

テストステロンエナント酸エステル

C₁₈H₃₆O₃: 400.59
3-Oxoadrost-4-en-17β-yl heptanoate [313-37-7]

Testosterone Enanthate, when dried, contains not less than 95.0% and not more than 105.0% of C₁₈H₃₆O₃.

**Description** Testosterone Enanthate occurs as white to pale yellow crystals, crystalline powder or a pale yellow-brown, viscous liquid. It is odorless or has a slight, characteristic odor.

It is very soluble in ethanol (95), in 1,4-dioxane and in diethyl ether, and practically insoluble in water.

Melting point: about 36°C.

**Identification** Heat 25 mg of Testosterone Enanthate with 2 mL of a solution of potassium hydroxide in methanol (1 in 100) under a reflux condenser on a water bath for 1 hour, cool, and add 10 mL of water. Collect the produced precipitate by suction, wash with water until the last washing is neutral, and dry the precipitate in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the precipitate melts <2.60> between 151°C and 157°C.

**Optical rotation <2.49>** $\Phi_0^D = 77 - +88$° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

**Purity** Acidity—Dissolve 0.5 g of Testosterone Enanthate in 10 mL of ethanol (95) which has previously been rendered neutral to bromothymol blue TS, and add 2 drops of bromothymol blue TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is light blue.

**Loss on drying <2.41>** Not more than 0.5% (0.5 g, in vacu-
Ultraviolet-visible Spectrophotometry
make exactly 100 mL. Perform the test as directed under exactly 10 mL of this solution, and dilute with ethanol (95) to make exactly 100 mL. Measure exactly 10 mL of this solution, and dissolve in ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 10 mL of isoniazid TS, add methanol to make exactly 20 mL, and allow to stand for 45 minutes. Determine the absorbances, \( A_T \) and \( A_S \), of these solutions at 380 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution obtained by proceeding with 5 mL of chloroform as the blank.

Amount (mg) of testosterone enanthate (C26H40O3) = \( M_S \times \frac{A_T}{A_S} \times 1.163 \)

\( M_S \): Amount (mg) of Testosterone Propionate RS

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Testosterone Propionate
テストステロンプロピオン酸エステル

Testosterone Enanthate Injection is an oily solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of testosterone enanthate (C26H40O3; 400.59).

Method of preparation Prepare as directed under Injections, with Testosterone Enanthate.

Description Testosterone Enanthate Injection is a clear, colorless or pale yellow oily liquid.

Identification Measure a volume of Testosterone Enanthate Injection, equivalent to 0.05 g of Testosterone Enanthate according to the labeled amount, add 8 mL of petroleum ether, and extract with three 10-mL portions of diluted acetic acid (7 in 10) to 0.1 mL of the extract, and heat on a water bath for 5 minutes. Cool, and add 0.5 mL of iron (III) chloride-acetic acid TS: the color of the solution is blue.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 2: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure accurately a volume of Testosterone Enanthate Injection, equivalent to about 25 mg of testosterone enanthate (C26H40O3), and dissolve in chloroform to make exactly 25 mL. Pipet 3 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Testosterone Propionate RS, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 10 mL of isoniazid TS, add methanol to make exactly 20 mL, and allow to stand for 45 minutes. Determine the absorbances, \( A_T \) and \( A_S \), of these solutions at 380 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution obtained by proceeding with 5 mL of chloroform as the blank.

Amount (mg) of testosterone enanthate (C26H40O3) = \( M_S \times \frac{A_T}{A_S} \times 1.163 \)

\( M_S \): Amount (mg) of Testosterone Propionate RS

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Testosterone Propionate
テストステロンプロピオン酸エステル
Perform the test with 5 these solutions as the sample solution and standard solution. Exactly 5 mL of these solutions add exactly 5 mL of the inter-dried, and dissolve in methanol to make exactly 100 mL. To Propionate and Testosterone Propionate RS, previously Weigh accurately each about 10 mg of Testosterone um, phosphorus (V) oxide, 4 hours). Containers and storage

Loss on drying <2.4% Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.4% Not more than 0.1% (0.5 g).

Assay Weigh accurately each about 10 mg of Testosterone Propionate and Testosterone Propionate RS, previously dried, and dissolve in methanol to make exactly 100 mL. To exactly 5 mL of these solutions add exactly 5 mL of the internal standard solution and methanol to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of testosterone propionate to that of the internal standard.

$$\text{Amount (mg) of } \text{C}_22\text{H}_32\text{O}_3 = M_S \times Q_T / Q_S$$

$M_S$: Amount (mg) of Testosterone Propionate RS

Internal standard solution—A solution of progesterone in methanol (9 in 100,000). Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of testosterone propionate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 µL of the standard solution under the above operating conditions, the internal standard and testosterone propionate are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of testosterone propionate to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Tight containers.

Storage—Light-resistant.

### Testosterone Propionate Injection

テストステロンプロピオン酸エステル注射液

Testosterone Propionate Injection is an oily solution for injection.

It contains not less than 92.5% and not more than 107.5% of the labeled amount of testosterone propionate (C$_{22}$H$_{32}$O$_3$: 344.49).

Method of preparation Prepare as directed under Injections, with Testosterone Propionate.

Description Testosterone Propionate Injection is a clear, colorless or pale yellow oily liquid.

Identification Dissolve the residue obtained as directed in the procedure in the Assay in exactly 20 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of Testosterone Propionate RS in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03> . Spot 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the Rf values of the principal spot with the sample solution and of the spot with the standard solution are not different each other.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 2: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (i) Chromatographic tube A glass tube about 1 cm in inside diameter and about 18 cm in length, with a glass filter (G3) at the lower end.

(ii) Chromatographic column To about 2 g of silica gel for liquid chromatography add 5 mL of dichloromethane, and mix gently. Transfer and wash into the chromatographic tube with the aid of dichloromethane, allow to elute the dichloromethane through the column, and put a filter paper on the upper end of the silica gel.

(iii) Standard solution Weigh accurately about 10 mg of Testosterone Propionate RS, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 20 mL.

(iv) Sample stock solution To exactly a volume of Testosterone Propionate Injection, equivalent to about 20 mg of testosterone propionate (C$_{22}$H$_{32}$O$_3$), add dichloromethane to make 20 mL.

(v) Procedure Transfer exactly 2 mL of the sample stock solution into the chromatographic column, and elute to the upper surface of the silica gel. Wash the inner surface of the chromatographic tube with 15 mL of dichlorometh-
Testosterone Propionate.

Containers and storage
Containers—Hermetic containers.

Internal standard solution—A solution of progesterone in methanol (9 in 100,000).

Amount (mg) of testosterone propionate (C₂₂H₃₂O₃)

\[ M = \frac{M \times Q \times 2}{S} \]

where:
- \( M \) is the amount (mg) of testosterone propionate RS
- \( S \) is the amount (mg) of Testosterone Propionate
- \( Q \) is the concentration of the internal standard solution

Freeze-dried Tetanus Antitoxin, Equine

Freeze-dried Tetanus Antitoxin, Equine, is a preparation for injection which is dissolved before use. It contains tetanus antitoxin in immunoglobulin of horse origin. It conforms to the requirements of Freeze-dried Tetanus Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Tetanus Antitoxin, Equine, becomes a clear, colorless to light yellow-brown liquid or slightly white-turbid liquid on addition of solvent.

Adsorbed Tetanus Toxoid

Adsorbed Tetanus Toxoid is a liquid for injection containing tetanus toxoid prepared by treating tetanus toxoid with formaldehyde by a method involving no appreciable loss of the immunogenicity and rendered insoluble by the addition of aluminum salt. It conforms to the requirements of Adsorbed Tetanus Toxoid in the Minimum Requirements for Biological Products.

Description Adsorbed Tetanus Toxoid becomes a uniform white-turbid liquid on shaking.

Tetracaine Hydrochloride

Dry Tetracaine Hydrochloride, when dried, contains not less than 98.5% of C₁₅H₂₅N₂O₂·HCl.

Description Tetracaine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste followed by a sense of numbness on the tongue. It is very soluble in formic acid, freely soluble in water, soluble in ethanol (95), sparingly soluble in ethanol (99.5), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

A solution of Tetracaine Hydrochloride (1 in 10) is neutral.

Melting point: about 148°C.

Identification (1) Dissolve 0.5 g of Tetracaine Hydrochloride in 50 mL of water, add 5 mL of ammonia TS, shake, and allow to stand in a cold place. Collect the precipitate, wash with water until the washings is neutral, and dry in a desiccator (silica gel) for 24 hours: it melts \(< 2.60\) between 42°C and 44°C.

(2) Dissolve 0.1 g of Tetracaine Hydrochloride in 8 mL of water, and add 3 mL of ammonium thiocyanate TS: a crystalline precipitate is produced. Collect the precipitate, recrystallize from water, and dry at 80°C for 2 hours: it melts \(< 2.60\) between 130°C and 132°C.

(3) Determine the absorption spectrum of a solution of Tetracaine Hydrochloride in ethanol (99.5) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry \(< 2.24\)%, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Tetracaine Hydrochloride (1 in 10) responds to the Qualitative Tests \(< 1.09\) for chloride.

Purity Heavy metals \(< 1.07\) Proceed with 1.0 g of Tetracaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying \(< 2.47\) Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition \(< 2.44\) Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tetracaine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 80 mL of acetic anhydride, allow to stand at 30°C on a water bath for 15 minutes, cool, and titrate \(< 2.50\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.
Each mL of 0.1 mol/L perchloric acid VS = 30.08 mg of C15H24N2O2.HCl

Containers and storage  Containers—Tight containers.

Tetracycline Hydrochloride

Tetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of *Streptomyces aureofaciens*.

It contains not less than 95.0% (potency) and not more than 101.0% (potency) per mg, calculated on the dried basis. The potency of Tetracycline Hydrochloride is expressed as mass (potency) of tetracycline hydrochloride (C22H24N2O8.HCl).

**Description**  Tetracycline Hydrochloride occurs as a yellow to pale brownish yellow crystalline powder.

It is freely soluble in water, and sparingly soluble in ethanol (95).

**Identification (1)**  Determine the absorption spectrum of a solution of Tetracycline Hydrochloride (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.2P>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tetracycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tetracycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.2S>, and compare the spectrum with the Reference Spectrum or the spectrum of Tetracycline Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tetracycline Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

**pH** <2.54>  Dissolve 1.0 g of Tetracycline Hydrochloride in 100 mL of water: the pH of the solution is between 1.8 and 2.8.

**Purity (1)**  Heavy metals <1.07>—Proceed with 1.0 g of Tetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tetracycline Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 25 mg of Tetracycline Hydrochloride in 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the areas of each peak by the automatic integration method: each peak area other than tetracycline from the sample solution is not larger than the peak area of tetracycline from the standard solution, and the total area of the peaks other than tetracycline from the sample solution is not larger than 3 times of the peak area of tetracycline from the standard solution.

**System suitability**—

Test for required detection: Pipet 3 mL of the standard solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and confirm that the peak area of tetracycline obtained from 20 µL of this solution is equivalent to 1 to 5% of that from 20 µL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tetracycline is not more than 1.0%.

**Loss on drying** <2.41>  Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** <2.42>  Not more than 0.3% (1.0 g).

**Assay**  Weigh accurately an amount of Tetracycline Hydrochloride and Tetracycline Hydrochloride RS, equivalent to about 25 mg (potency), and dissolve each in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 µL each of these solutions as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak area, A1 and A5, of tetracycline of each solution.

Amount [µg (potency)] of C22H24N2O8.HCl = M5 × A1/A5 × 1000

M5: Amount [mg (potency)] of Tetracycline Hydrochloride RS

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (0.01 µm in pore diameter).

Column temperature: A constant temperature of about 60°C.
Mobile phase: Dissolve 3.5 g of dipotassium hydrogenphosphate and 0.4 g of disodium dihydrogen ethylenediamine tetracetate dihydrate in 300 mL of water, adjust to pH 9.0 with sodium hydroxide TS, add 90.0 g of t-butanol, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of tetracycline is about 5 minutes.

System suitability—
System performance: Dissolve 0.05 g of Tetracycline Hydrochloride RS in water to make 25 mL. Heat 5 mL of this solution on a water bath for 60 minutes, then add water to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the retention time of 4-epitetracycline is about 3 minutes, and 4-epitetracycline and tetracycline are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When, the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Thallium (201Tl) Chloride Injection
塩化タリウム (201Tl) 注射液

Thallium (201Tl) Chloride Injection is an aqueous solution for injection.
It contains thallium-201 (201Tl) in the form of thallous chloride.
It conforms to the requirements of Thallium (201Tl) Chloride Injection in the Minimum Requirements for Radiopharmaceuticals.
Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Thallium (201Tl) Chloride Injection is a clear, colorless liquid.

Theophylline
デオフィリン

It is soluble in N,N-dimethylformamide, and slightly soluble in water and in ethanol (99.5).
It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the absorption spectrum of a solution of Theophylline in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry 2.2.4, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Theophylline, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point 2.6–271 – 275°C

Purity (1) Acidity—To 0.5 g of Theophylline add 75 mL of water, 2.0 mL of 0.01 mol/L sodium hydroxide VS and 1 drop of methyl red TS; a yellow color develops.
(2) Heavy metals 1.07—Proceed with 1.0 g of Theophylline according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(3) Arsenic 1.11—Prepare the test solution with 1.0 g of Theophylline according to Method 3, and perform the test (not more than 2 ppm).
(4) Related substances—Dissolve 0.10 g of Theophylline in 3 mL of N,N-dimethylformamide, add 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.6.3. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, chloroform, methanol, 1-butanol and ammonia solution (28) (3:3:2.2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying 2.4– Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition 2.2.4 Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Theophylline, previously dried, and dissolve in 100 mL of water, add exactly 20 mL of 0.1 mol/L silver nitrate VS, shake the mixture, and titrate 2.5 with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.
Each mL of 0.1 mol/L sodium hydroxide VS = 18.02 mg of C7H8N4O2

Containers and storage Containers—Well-closed containers.
Thiamazole

**Description** Thiamazole occurs as white to pale yellowish white crystals or crystalline powder. It has a faint, characteristic odor, and has a bitter taste.

It is freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

The pH of the solution (1 in 50) is between 5.0 and 7.0.

**Identification**

1. Dissolve 5 mg of Thiamazole in 1 mL of water, shake with 1 mL of sodium hydroxide TS, and add 3 drops of sodium pentacyanonitrosylferrate (III) TS: a yellow color develops, and it gradually changes to yellow-green to green. To this solution add 1 mL of acetic acid (31): it changes to blue.

2. To 2 mL of a solution of Thiamazole (1 in 200) add 1 mL of sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 5): a deep blue color develops.

**Meltting point**<br>144 – 147°C

**Purity**

1. Selenium—Proceed with 0.10 g of Thiamazole as directed under Oxygen Flask Combustion Method <1.06>, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and transfer the test solution to a beaker. Wash C, B and the inner side of A with 25 mL of water, and combine the washings with the test solution. Boil gently for 10 minutes, cool to room temperature, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 40 mg of selenium, dissolve in 100 mL of diluted nitric acid (1 in 2), heat to dissolve on a water bath if necessary, and add water to make exactly 1000 mL. Pipet 5 mL of this solution, and add water to make exactly 200 mL. To 2 mL of this solution, exactly measured, add diluted nitric acid (1 in 60) to make exactly 50 mL, and use this solution as the standard solution. Pipet 40 mL each of the sample solution and standard solution into separate beakers, and adjust each solution with ammonia solution (28) to a pH of 1.8 to 2.2. To each solution add 0.2 g of hydroxylymmonium chloride, shake gently to dissolve. To these solutions add 5 mL of a solution prepared by dissolving 0.10 g of 2,3-diaminonaphthalene and 0.05 g of hydroxylymmonium chloride in 0.1 mol/L hydrochloric acid TS to make 100 mL, shake, and allow to stand for 100 minutes. Transfer these solutions to corresponding separators, rinse the beakers with 10 mL of water, combine the rinsings in the respective separators, shake well with 5.0 mL of cyclohexane for 2 minutes, and extract. Centrifuge the cyclohexane extracts to remove any water remaining in these solutions. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 40 mL of diluted nitric acid (1 in 60) in the same manner as the blank. The absorbance of the sample solution at the wavelength of maximum absorbance at about 378 nm does not exceed the absorbance of the standard solution.

2. Heavy metals <1.07>—Proceed with 1.0 g of Thiamazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

3. Arsenic <1.11>—Prepare the test solution with 1.0 g of Thiamazole according to Method 1, and perform the test (not more than 2 ppm).

**Loss on drying**<br>Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition**<br>Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.25 g of Thiamazole, previously dried, dissolve in 75 mL of water, add 15 mL of 0.1 mol/L sodium hydroxide VS from a burette, and add 30 mL of 0.1 mol/L silver nitrate VS with stirring. Add 1 mL of bromothymol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS, until a persistent blue-green color is produced. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS = 11.42 mg of C₄H₆N₂S

**Containers and storage**

Containers—Well-closed containers.

Storage—Light-resistant.

**Thiamazole Tablets**

Thiamazole Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of thiamazole (C₄H₆N₂S: 114.17).

**Method of preparation** Prepare as directed under Tablets, with Thiamazole.

**Identification**

1. To a quantity of powdered Thiamazole Tablets, equivalent to 0.05 g of Thiamazole according to the labeled amount, add 20 mL of hot ethanol (95), shake for 15 minutes, filter, and evaporate the filtrate on a water bath to dryness. Dissolve the residue in 10 mL of water, filter if necessary, and use this solution as the sample solution. To 1 mL of the sample solution add 1 mL of sodium hydroxide TS, shake, and add 3 drops of sodium pentacyanonitrosylferrate (III) TS: a yellow color develops, and it gradually changes to yellow-green to green. To this solution add 1 mL of acetic acid (31): it changes to blue.

2. With 2 mL of the sample solution obtained in (1), proceed as directed in the Identification (2) under Thiamazole.

**Assay**

Weigh accurately and powder not less than 20 Thiamazole Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.15 g of thiamazole (C₄H₆N₂S), add 80 mL of water, shake for 15 minutes, add
water to make exactly 100 mL, and centrifuge. Filter, discard the first 20 mL of the filtrate, pipet 50 mL of the subsequent filtrate, add 1 mL of bromothymol blue TS, and if a blue color develops, neutralize with 0.1 mol/L hydrochloric acid VS until the color of the solution changes to green. To this solution add 4.5 mL of 0.1 mol/L sodium hydroxide VS from a burette, add 15 mL of 0.1 mol/L silver nitrate VS while stirring, and titrate 2.50 μL with 0.1 mol/L sodium hydroxide VS. Continue the titration until a persistent blue-green color is produced, and determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS = 11.42 mg of C₄H₆N₂S

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

### Thiamine Chloride Hydrochloride

#### Vitamin B₁, Hydrochloride

チアミン塩化物塩酸塩

\[
\text{C}_{12}\text{H}_{17}\text{ClN}_{4}\text{OS.HCl}: 337.27
\]

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium chloride monohydrochloride [67-03-9]

Thiamine Chloride Hydrochloride contains not less than 98.5% of \( \text{C}_{12}\text{H}_{17}\text{ClN}_{4}\text{OS.HCl} \), calculated on the anhydrous basis.

**Description** Thiamine Chloride Hydrochloride occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95%), and practically insoluble in diethyl ether.

Melting point: about 245°C (with decomposition).

**Identification (1)** To 5 mL of a solution of Thiamine Chloride Hydrochloride (1 in 500) add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline.

(2) Determine the absorption spectrum of a solution of Thiamine Chloride Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Thiamine Chloride Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Thiamine Chloride Hydrochloride, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum, or the spectrum of Thiamine Chloride Hydrochloride RS previously dried at 105°C for 2 hours: both spectra exhibit similar intensities of absorption at the same wave numbers. In case when some differences are found between the spectra, repeat the test with residues obtained by dissolving these substances in water, evaporating to dryness, and drying at 105°C for 2 hours.

(4) A solution of Thiamine Chloride Hydrochloride (1 in 500) responds to the Qualitative Tests 1.09 for chloride.

\[ \text{pH} < 2.54 \]

Dissolve 1.0 g of Thiamine Chloride Hydrochloride in 100 mL of water: the pH of this solution is between 2.7 and 3.4.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Thiamine Chloride Hydrochloride in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 1.5 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

(2) Sulfate 1.14—Weigh 1.5 g of Thiamine Chloride Hydrochloride, and perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Nitrate—Dissolve 0.5 g of Thiamine Chloride Hydrochloride in 25 mL of water. Add 2 mL of sulfuric acid to 2 mL of this solution, shake, cool, and superimpose iron (II) sulfate TS: no dark brown ring is produced at the junction of the two layers.

(4) Heavy metals 1.07—Proceed with 1.0 g of Thiamine Chloride Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Related substances—Dissolve 0.10 g of Thiamine Chloride Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution, as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.25, according to the following conditions, and determine the area of each peak by the automatic integration method: the total area of the peaks other than thiamine obtained from sample solution is not larger than the peak area of thiamine from the standard solution.

**Operating conditions—**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of thiamine.

**System suitability—**

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of thiamine obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of thiamine is not more than 1.0%.

**Water** 2.48 Not more than 5.0% (30 mg, coulometric titration).

**Residue on ignition** 2.48 Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.1 g each of Thiamine Chloride Hydrochloride and Thiamine Chloride Hydrochloride RS (separately determine the water 2.48 in the same manner as Thiamine Chloride Hydrochloride), and dissolve them in the mobile phase to make exactly 50 mL. To 10 mL each of the solutions, accurately measured, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography 2.0 to according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of thiamine to that of the internal standard.

\[
\text{Amount (mg) of } C_{12}H_{17}ClN_4OS.HCl = M_S \times Q_T/Q_S
\]

\( M_S \): Amount (mg) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis

**Internal standard solution**—A solution methyl benzoate in methanol (1 in 50).

**Operating conditions**—

- **Detector:** An ultraviolet absorption photometer (wavelength: 254 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecysilanized silica gel for liquid chromatography (5 µm in particle diameter).
- **Column temperature:** A constant temperature of about 25°C.
- **Mobile phase:** Dissolve 1.1 g of sodium 1-octanesulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 600 mL of this solution add 400 mL of a mixture of methanol and acetonitrile (3:2).
- **Flow rate:** Adjust the flow rate so that the retention time of thiamine is about 12 minutes.

**System suitability**—

- **System performance:** When the procedure is run with 10 µL of the standard solution under the above operating conditions, thiamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.
- **System repeatability:** When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of thiamine to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

**Storage**—Light-resistant.

---

**Thiamine Chloride Hydrochloride Injection**

**Vitamin B_1 Hydrochloride Injection**

チアミン塩化物塩酸塩注射液

Thiamine Chloride Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 115.0% of the labeled amount of thiamine Chloride hydrochloride (C_{12}H_{17}ClN_4OS.HCl: 337.27).

**Method of preparation** Prepare as directed under Injections, with Thiamine Chloride Hydrochloride.

**Description** Thiamine Chloride Hydrochloride Injection is a clear, colorless liquid.

**pH** 2.5 – 4.5

**Identification** To a volume of Thiamine Chloride Hydrochloride Injection, equivalent to 0.05 g of Thiamine Chloride Hydrochloride according to the labeled amount, add water to make 25 mL. Proceed with 5 mL of this solution as directed in the Identification (1) under Thiamine Chloride Hydrochloride.

**Bacterial endotoxins** 4.01 Less than 6.0 EU/mg.

**Extractable volume** 6.05 It meets the requirement.

**Foreign insoluble matter** 6.06 Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** 6.07 It meets the requirement.

**Sterility** 4.06 Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Dilute with 0.001 mol/L hydrochloric acid TS if necessary, then measure exactly a volume of Thiamine Chloride Hydrochloride Injection, equivalent to about 20 mg of thiamine chloride hydrochloride (C_{12}H_{17}ClN_4OS.HCl), and add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Thiamine Chloride Hydrochloride RS (separately determine the water 2.48 in the same manner as Thiamine Chloride Hydrochloride), and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 50 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, and 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay under Thiamine Chloride Hydrochloride.

\[
\text{Amount (mg) of thiamine chloride hydrochloride} (C_{12}H_{17}ClN_4OS.HCl) = M_T \times Q_T/Q_S \times 1/5
\]

\( M_T \): Amount (mg) of Thiamine Chloride Hydrochloride
RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of methyl benzoate in methanol (1 in 200).

**Containers and storage**  Containers—Tight containers.

**Thiamine Chloride Hydrochloride Powder**

**Vitamin B₁ Hydrochloride Powder**

チアミン塩化物塩酸塩

Thiamine Chloride Hydrochloride Powder contains not less than 95.0% and not more than 115.0% of the labeled amount of thiamine chloride hydrochloride (C₁₂H₁₇ClN₄OS.HCl: 337.27).

**Method of preparation**  Prepare as directed under Powders, with Thiamine Chloride Hydrochloride.

**Identification**  To a portion of Thiamine Chloride Hydrochloride Powder, equivalent to 0.02 g of Thiamine Chloride Hydrochloride according to the labeled amount, add 50 mL of water and 10 mL of dilute acetic acid, shake, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Thiamine Chloride Hydrochloride.

**Purity**  Rancidity—Thiamine Chloride Hydrochloride Powder has no unpleasant or rancid odor. It is tasteless.

**Assay**  Weigh accurately a quantity of Thiamine Chloride Hydrochloride Powder, equivalent to about 20 mg of thiamine chloride hydrochloride (C₁₂H₁₇ClN₄OS.HCl), add 60 mL of 0.01 mol/L hydrochloric acid TS, and heat on a water bath for 30 minutes. Shake vigorously for 10 minutes, cool, add methanol to make exactly 100 mL, and centrifuge. Pipet 25 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Thiamine Chloride Hydrochloride RS (separately determine the water of hydration). Proceed as directed in the Assay under Thiamine Chloride Hydrochloride.

Amount (mg) of thiamine chloride hydrochloride (C₁₂H₁₇ClN₄OS.HCl)

\[ M_{S} = \frac{M_{S}}{Q_{S}} Q_{S} \times \frac{1}{5} \]

**Internal standard solution**—A solution of methyl benzoate in methanol (1 in 200).

**Thiamine Nitrate**

**Vitamin B₁ Nitrate**

チアミン硝化物

C₁₂H₁₇N₅O₄S: 327.36
3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium nitrate [532-43-4]

Thiamine Nitrate, when dried, contains not less than 98.0% and not more than 102.0% of C₁₂H₁₇N₅O₄S.

**Description**  Thiamine Nitrate occurs as white crystals or crystalline powder. It is odorless or a slight, characteristic odor.

It is sparingly soluble in water, and very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 193°C (with decomposition).

**Identification**  (1) Take 2-mL portions of a solution of Thiamine Nitrate (1 in 500), and add 2 to 3 drops of iodine TS: a red-brown precipitate or turbidity is produced. Upon further addition of 1 mL of 2,4,6-trinitrophenol TS, a yellow precipitate or turbidity is produced.

(2) To 1 mL of a solution of Thiamine Nitrate (1 in 500) add 1 mL of lead (II) acetate TS and 1 mL of a solution of sodium hydroxide (1 in 10), and warm: the color of the solution changes through yellow to brown, and on standing, a black-brown precipitate is produced.

(3) To 5 mL of a solution of Thiamine Nitrate (1 in 500) add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline.

(4) A solution of Thiamine Nitrate (1 in 50) responds to the Qualitative Tests <1.09> (1) and (2) for nitrate.

**pH** <2.54>  Dissolve 1.0 g of Thiamine Nitrate in 100 mL of water: the pH of this solution is between 6.5 and 8.0.

**Purity**  (1) Chloride <1.03>—Perform the test with 0.20 g of Thiamine Nitrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(2) Sulfate <1.14>—Dissolve 1.5 g of Thiamine Nitrate in 30 mL of water and 2 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS and 2 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.011%).
Thiamylal Sodium

(3) Heavy metals \(<1.07\)—Dissolve 1.0 g of Thiamine Nitrate in 30 mL of water by warming, cool, and add 12 mL of 6 mol/L acetic acid TS and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** \(<2.41\) Not more than 1.0% (0.5 g, 105°C, 2 hours).

**Residue on ignition** \(<2.44\) Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.1 g each of Thiamine Nitrate, previously dried, and Thiamine Chloride Hydrochloride RS (separately determine the water content of the sample to that of the internal standard. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\>) according to the following conditions and calculate the ratios, Q1 and Q2, of the peak area of thiamine to that of the internal standard.

Amount (mg) of C₁₂H₁₇N₂NaO₂S = Mₛ × Q₁/Q₂ × 0.971

**Internal standard solution**—A solution of methyl benzoate in methanol (1 in 50).

**Operating conditions**—

Detector: An ultraviolet spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.1 g of sodium l-octanesulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 600 mL of this solution add 400 mL of a mixture of methanol and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of thiamine is about 12 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, thiamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of thiamine to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers. Storage—Light-resistant.

**Thiamylal Sodium**

C₁₂H₁₇N₂NaO₂S: 276.33
Monosodium 5-allyl-5-[(1RS)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydropyrimidine-2-thiolate [337-47-3]

Thiamylal Sodium contains not less than 97.5% and not more than 101.0% of C₁₂H₁₇N₂NaO₂S, calculated on the dried basis.

**Description** Thiamylal Sodium occurs as light yellow crystals or powder.

It is very soluble in water, and freely soluble in ethanol (95).

The pH of a solution of Thiamylal Sodium (1 in 10) is between 10.0 and 11.0.

It is hygroscopic.

It is gradually decomposed by light.

Its solution in ethanol (95) (1 in 10) shows no optical rotation.

**Identification** (1) Determine the absorption spectrum of a solution of Thiamylal Sodium in ethanol (95) (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.25\>) and compare with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Thiamylal Sodium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\>) and compare with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Thiamylal Sodium (1 in 10) responds to Qualitative Tests \(<1.09\> for sodium salt.

**Purity** (1) Clarity and color of solution—To 1.0 g of Thiamylal Sodium in a 11- to 13-mL glass-stoppered test tube add 10 mL of freshly boiled and cooled water, stopper tightly, allow to stand, and dissolve by occasional gentle shaking: the solution is clear and light yellow.

(2) Heavy metals \(<1.07\)—Proceed with 1.0 g of Thiamylal Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Thiamylal Sodium in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL and 3 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\>) and compare the results with those obtained with 10 μL of each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography, develop...
with a mixture of toluene, methanol and ethyl acetate (40:7:3) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for a night: the spot appeared around Rf value 0.1 obtained with the sample solution is not more intense than the spot with the standard solution (2), and the spot other than the principal spot, the spot at origin and the spot mentioned above obtained with the sample solution is not more intense than the spot with the standard solution (1).

**Loss on drying** Not more than 2.0% (1 g, 105°C, 1 hour).

**Assay** Weigh accurately about 0.25 g of Thiamylal Sodium, dissolve in 50 mL of methanol and 5 mL of dilute hydrochloric acid, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Thiamylal RS, previously dried at 105°C for 1 hour, dissolve in 50 mL of methanol and 5 mL of dilute hydrochloric acid, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make 200 mL, and use this solution as the sample solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q₁ and Q₃, of the peak area of thiamylal to that of the internal standard.

\[ M_2 = \text{Amount (mg) of } C_{12}H_{17}N_2NaO_2S \]
\[ M_3 = 10 \times 1.086 \]

\[ M_2 \times Q_1 / Q_3 \]

**Internal standard solution**—A solution of phenyl benzoate in methanol (3 in 500).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.6 (13:7).

Flow rate: Adjust the flow rate so that the retention time of thiamylal is about 6 minutes.

**System suitability**—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, thiamylal and the internal standard are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of thiamylal to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.
Storage—Light-resistant.

### Thiamylal Sodium for Injection

Thiamylal Sodium for Injection is a preparation for injection which is dissolved before use.
It contains not less than 93.0% and not more than 107.0% of the labeled amount of thiamylal sodium (C₁₂H₁₇N₂NaO₂S: 276.33).

**Method of preparation**—Prepare as directed under Injections, with 100 parts of Thiamylal Sodium and 7 parts of Dried Sodium Carbonate in mass.

**Description**—Thiamylal Sodium for Injection occurs as light yellow crystals, powder or masses.
It is hygroscopic.
It is gradually decomposed by light.

**Identification** (1) To 1.0 g of Thiamylal Sodium for Injection add 20 mL of ethanol (95), shake vigorously, and filter. Subtract the precipitate so obtained in 1 mL of water, and add 1 mL of barium chloride TS: a white precipitate is produced. Centrifuge this solution, take off the supernatant liquid, and to the precipitate add dilute hydrochloric acid dropwise: the precipitate dissolves with effervescence.

(2) To 50 mg of Thiamylal Sodium for Injection add 100 mL of ethanol (95), shake vigorously, and filter. To 3 mL of the filtrate add ethanol (95) to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 236 nm and 240 nm, and between 287 nm and 291 nm.

**pH** Not more than 2.54: The pH of a solution obtained by dissolving 1.0 g of Thiamylal Sodium for Injection in 40 mL of water is between 10.5 and 11.5.

**Purity**—Related substances—To 0.10 g of Thiamylal Sodium for Injection add 10 mL of ethanol (95), shake vigorously, and filter. To 3 mL of the filtrate add ethanol (95) to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 236 nm and 240 nm, and between 287 nm and 291 nm.

**Bacterial endotoxins** Less than 1.0 EU/mg.

**Uniformity of dosage units**—It meets the requirement of the Mass variation test.

**Foreign insoluble matter**—Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter**—It meets the requirement.

**Sterility**—Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay**—Open carefully 10 containers of Thiamylal Sodium for Injection, dissolve the contents with water, wash out the inside of each container with water, combine them, and add water to make exactly V mL so that each mL contains about 5 mg of thiamylal sodium (C₁₂H₁₇N₂NaO₂S). Pipet 5 mL of this solution, and add 0.5 mL of dilute hydrochloric acid and methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make 200 mL, and use this solution as the sample solution. Proceed the test with the sample solu-
Thianthol / Official Monographs

Thianthol Combustion Method

Weigh accurately about 10 mg of Thianthol, and add 2 to 3 drops of barium chloride TS: no opalescence is produced.

Containers—Tight containers.

Containers and storage—Containers—Tight containers.

Storage—Light-resistant.

Thianthol

チアントール

Thianthol consists of dimethylthianthrene and ditoluene disulfide.

It contains not less than 23.5% and not more than 26.5% of sulfur (S: 32.07).

Description Thianthol is a yellowish, viscous liquid. It has a faint, agreeable odor.

It is freely soluble in diethyl ether, slightly soluble in ethanol (95), and practically insoluble in water.

It, when cold, may separate crystals, which melt on warming.

Specific gravity $d_20^\circ$: 1.19 - 1.23

Identification To 0.1 g of Thianthol add cautiously 5 mL of sulfuric acid: a blue-purple color develops. Add 5 to 6 drops of nitric acid to the solution: the color of the solution changes to yellow-red with evolution of gas.

Purity (1) Acidity or alkalinity—Shake 10 g of Thianthol with 20 mL of water, allow to stand, and separate the water layer. The solution is neutral.

(2) Sulfate—To 10 mL of the water layer obtained in (1) add 2 to 3 drops of barium chloride TS: no opalescence is produced.

Residue on ignition $<2.44$ Not more than 0.1% (1 g).

Assay Weigh accurately about 10 mg of Thianthol, and proceed as directed in the sulfur determination of Oxygen Flask Combustion Method $<1.065$, using a mixture of 5 mL of diluted sodium hydroxide TS (1 in 10) and 1.0 mL of hydrogen peroxide TS as an absorbing liquid.

Containers and storage—Containers—Tight containers.

Compound Thianthol and Salicylic Acid Solution

複方チアントール・サリチル酸液

Compound Thianthol and Salicylic Acid Solution contains not less than 1.8 w/v% and not more than 2.2 w/v% of salicylic acid ($C_9H_8O_3$: 138.12), and not less than 1.8 w/v% and not more than 2.2 w/v% of phenol ($C_7H_6O_3$: 138.12).

Method of preparation

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thianthol</td>
<td>200 mL</td>
</tr>
<tr>
<td>Salicylic Acid</td>
<td>20 g</td>
</tr>
<tr>
<td>Phenol</td>
<td>20 g</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>50 mL</td>
</tr>
<tr>
<td>Ether</td>
<td>100 mL</td>
</tr>
<tr>
<td>Petroleum Benzin</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Dissolve Salicylic Acid and Phenol in Ether, add Thianthol, Olive Oil and Petroleum Benzin to this solution, mix and dissolve to make 1000 mL.

Description Compound Thianthol and Salicylic Acid Solution is a light yellow liquid, having a characteristic odor.

Identification (1) Place 1 mL of Compound Thianthol and Salicylic Acid Solution to a porcelain dish, and evaporate on a water bath to dryness. To the residue add cautiously 5 mL of sulfuric acid: the color of the solution changes to yellow-red with evolution of gas (thianthol).

(2) Shake 10 mL of Compound Thianthol and Salicylic Acid Solution with 10 mL of sodium hydrogen carbonate TS, and separate the water layer. To 0.5 mL of the water layer add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 50 mL, and to 5 mL of this solution add 5 mL of a solution of ion (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(3) Wash the upper phase obtained in (2) with 10 mL of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrate TS and 1 mL of dilute hydrochloric acid, and add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

(4) To 1 mL of Compound Thianthol and Salicylic Acid Solution add 10 mL of ethanol (95), mix, and use this solution as the sample solution. Dissolve 0.01 g each of salicylic acid, phenol and thianthol in 5 mL each of ethanol (95), and use each solution as standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 5 mL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100:45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): three spots obtained from the sample solution and the corresponding spots of standard solutions (1), (2) and (3) show the same $R_f$ value. Spraye eveny iron (III) chloride TS on the plate: the spot from standard solution (1) and the corresponding spot from the sample solution reveal a purple color.

Assay Measure exactly 2 mL of Compound Thianthol and Salicylic Acid Solution, add exactly 10 mL of the internal standard solution, then add 70 mL of diluted methanol (1 in 2), mix well, and add diluted methanol (1 in 2) to make 100 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and about 0.2 g of phenol for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and diluted methanol (1 in...
2) to make 100 mL, and use this solution as the standard solution. With 5 µL each of the sample solution and standard solution, perform the test as directed under Liquid Chromatography $<$2.01$>$ according to the following conditions, and calculate the ratios, $Q_{Ta}$ and $Q_{Tb}$, of the peak area of salicylic acid and phenol to that of the internal standard in the sample solution, and the ratios, $Q_{Sa}$ and $Q_{Sb}$, of the peak area of salicylic acid and phenol to that of the internal standard in the standard solution.

Amount (mg) of salicylic acid (C$_7$H$_6$O$_3$)

$$M_{Sa} = \frac{Q_{Ta}}{Q_{Tb}} \times \frac{Tb}{Ta} \times \frac{Sa}{Sb} \times \frac{1}{5}$$

Amount (mg) of phenol (C$_{12}$H$_{18}$O$_2$)

$$M_{Sb} = \frac{Q_{Sa}}{Q_{Sb}} \times \frac{Sa}{Sb} \times \frac{Tb}{Ta} \times \frac{1}{5}$$

$M_{Sa}$: Amount (mg) of salicylic acid for assay

$M_{Sb}$: Amount (mg) of phenol for assay

Internal standard solution—A solution of theophylline in methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, and methanol (3:1).

Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 6 minutes.

Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 0.05 g of theophylline in 100 mL of diluted methanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with 10 µL of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

Containers and storage—Containers—Tight containers.

Storage—Light-resistant, and not exceeding 25°C.

Thiopental Sodium

チオペンタルナトリウム

C$_{11}$H$_{17}$N$_2$NaO$_2$S: 264.32

Monosodium 5-ethyl-5-[(1RS)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydropyrimidine-2-thiolate [71-73-8]

Thiopental Sodium, when dried, contains not less than 97.0% of C$_{11}$H$_{17}$N$_2$NaO$_2$S.

Description Thiopental Sodium occurs as a light yellow powder. It has a faint, characteristic odor.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Thiopental Sodium (1 in 10) is alkaline. It is hygroscopic. Its solution gradually decomposes on standing.

Identification (1) Dissolve 0.2 g of Thiopental Sodium in 5 mL of sodium hydroxide TS, and add 2 mL of lead (II) acetate TS: a white precipitate, which dissolves upon heating, is produced. Boil the solution thus obtained: a black precipitate forms gradually, and the precipitate responds to the Qualitative Tests $<$1.09$>$ for sulfide.

(2) Dissolve 0.5 g of Thiopental Sodium in 15 mL of water, add 10 mL of dilute hydrochloric acid to produce white precipitate, and extract with four 25-mL portions of chloroform. Combine the chloroform extracts, evaporate on a water bath, and dry at 105°C for 2 hours: the residue melts $<$2.0$>$ between 157°C and 162°C.

(3) A solution of Thiopental Sodium (1 in 10) responds to the Qualitative Tests $<$1.09$>$ (1) and (2) for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Thiopental Sodium in 10 mL of freshly boiled and cooled water: the solution is clear and light yellow.

(2) Heavy metals $<$1.07$>$—Dissolve 2.0 g of Thiopental Sodium in 76 mL of water, add 4 mL of dilute hydrochloric acid, shake, and filter through a glass filter (G4). To 40 mL of the filtrate add 2 mL of ammonium acetate TS, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare a control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, 2 mL of ammonium acetate TS and water to make 50 mL (not more than 20 ppm).

(3) Neutral and basic substances—Weigh accurately about 1 g of Thiopental Sodium, dissolve in 10 mL of water and 5 mL of sodium hydroxide TS, and shake vigorously with 40 mL of chloroform. Separate the chloroform layer, wash with two 5-mL portions of water, filter, and evaporate the filtrate on a water bath to dryness. Dry the residue at 105°C for 1 hour: the amount of the residue is not more than 0.5%.

(4) Related substances—Dissolve 50 mg of Thiopental Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and the standard solution as directed under Liquid Chromatography $<$2.01$>$ according to the following conditions. Measure each peak area of each solution by the automatic integration method: the total area of peaks other than thiopental from the sample solution is not larger than the peak area of thiopental from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To 700 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time...
of thiopental is about 15 minutes.

Time span of measurement: About 1.5 times as long as the retention time of thiopental.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of thiopental obtained from 20 μL of this solution is equivalent to 15 to 25% of that from 20 μL of the standard solution.

System performance: Dissolve 5 mg each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 50 mL of acetonitrile, and add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, isopropyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of thiopental is not more than 2.0%.

Loss on drying <2.4% Not more than 2.0% (1 g, in vacuum, 80°C, 4 hours).

Assay Weigh accurately about 0.5 g of Thiopental Sodium, previously dried, transfer to a separator, dissolve in 20 mL of water, add 5 mL of ethanol (95) and 10 mL of dilute hydrochloric acid, and extract with 50 mL of chloroform, then with 25 mL portions of chloroform. Combine the chloroform extracts, wash with two 5-mL portions of water, and extract the washings with two 10-mL portions of chloroform. Filter the combined chloroform extracts into a conical flask, and wash the filter paper with three 5-mL portions of chloroform. Filter the washings with the former solution, and add water to make 100 mL. Measure exactly a 1-mL volume (V mL) of this solution, equivalent to about 15 mg of thiopental sodium (C₁₁H₁₇N₂NaO₂S), and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 26.43 mg of C₁₁H₁₇N₂NaO₂S

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

**Thiopental Sodium for Injection**

注射用チオペンタールナトリウム

Thiopental Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of thiopental sodium (C₁₁H₁₇N₂NaO₂S; 264.32).

**Method of preparation** Prepare as directed under Injections, with 100 parts of Thiopental Sodium and 6 parts of Dried Sodium Carbonate in mass.

**Description** Thiopental Sodium for Injection is a light yellow powder or mass, and has a slight, characteristic odor.

It is very soluble in water, and practically insoluble in dehydrated diethyl ether.

It is hygroscopic.

**Identification** (1) Dissolve 0.1 g of Thiopental Sodium for Injection in 10 mL of water, and add 0.5 mL of barium chloride TS: a white precipitate is formed. Collect the precipitate, and add dilute hydrochloric acid dropwise: the precipitate dissolves with effervescence.

(2) Proceed as directed in the Identification under Thiopental Sodium.

**pH** <2.54 Dissolve 1.0 g of Thiopental Sodium for Injection in 40 mL of water: the pH of this solution is between 10.2 and 11.2.

**Purity** Proceed as directed in the Purity under Thiopental Sodium.

**Loss on drying** <2.4% Not more than 2.0% (1 g, in vacuum, 80°C, 4 hours).

**Bacterial endotoxins** <4.01 Less than 0.30 EU/mg.

**Uniformity of dosage units** <6.02 It meets the requirement of the Mass variation test.

**Foreign insoluble matter** <6.07 Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** <6.07 It meets the requirement.

**Sterility** <4.06 Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Take 10 samples of Thiopental Sodium for Injection, and open each container carefully. Dissolve each content with water, wash each container with water, combine the washings with the former solution, and add water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Measure exactly a volume (V mL) of this solution, equivalent to about 15 mg of thiopental sodium (C₁₁H₁₇N₂NaO₂S), and add water to make exactly 1000 mL. Pipet 10 mL of this solution, add 15 mL of diluted sodium hydroxide TS (1 in 100), add water to make exactly 30 mL, and use this solution as the sample solution. Separately, weigh accurately about 46 mg of thiopental for assay, previously dried at 105°C for 3 hours, dissolve in 50 mL of dilute sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, Aₜ and Aₛ, at 304 nm.

Amount (mg) of thiopental sodium (C₁₁H₁₇N₂NaO₂S) in each sample of Thiopental Sodium for Injection

\[ Mₜ = \frac{Mₛ \times Aₜ}{Aₛ \times 300/V \times 1.091} \]

Mₛ: Amount (mg) of thiopental sodium for assay.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.
Thioridazine Hydrochloride

Thioridazine Hydrochloride, when dried, contains not less than 99.0% of C$_{21}$H$_{26}$N$_2$S$_2$.HCl.

**Description**  Thioridazine Hydrochloride occurs as a white to pale yellow, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Thioridazine Hydrochloride (1 in 100) is between 4.2 and 5.2.

It is gradually colored by light.

**Identification** (1)  Dissolve 0.01 g of Thioridazine Hydrochloride in 2 mL of sulfuric acid: a deep blue color develops.

(2)  Dissolve 0.01 g of Thioridazine Hydrochloride in 2 mL of water, and add 1 drop of cerium (IV) tetraammonium sulfate TS: a blue color develops, and the color disappears on the addition of excess of the reagent.

(3)  Determine the infrared absorption spectrum of Thioridazine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4)  To 5 mL of a solution of Thioridazine Hydrochloride (1 in 100) add 2 mL of ammonia TS, and heat on a water bath for 5 minutes. After cooling, filter, and acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

**Melting point** <2.60> 159 - 164°C

**Purity** (1)  Heavy metals <1.07>—Proceed with 1.0 g of Thioridazine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2)  Arsenic <1.11>—Prepare the test solution with 1.0 g of Thioridazine Hydrochloride, according to Method 3, and perform the test (not more than 2 ppm).

(3)  Related substances—Conduct this procedure under the protection from the sunlight. Dissolve 0.10 g of Thioridazine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, 2-propanol and ammonia solution (28) (74:25:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41>  Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44>  Not more than 0.1% (1 g).

**Assay**  Weigh accurately about 0.35 g of Thioridazine Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 40.70 mg of C$_{21}$H$_{26}$N$_2$S$_2$.HCl

**Containers and storage**  Containers—Tight containers. Storage—Light-resistant.

**Thiotepa**

Thiotepa, when dried, contains not less than 98.0% of C$_{6}$H$_{12}$N$_3$.PS.

**Description**  Thiotepa occurs as colorless or white crystals, or white, crystalline powder. It is odorless.

It is freely soluble in water, in ethanol (95) and in diethyl ether.

A solution of Thiotepa (1 in 10) is neutral.

**Identification** (1)  To 5 mL of a solution of Thiotepa (1 in 100) add 1 mL of hexammonium heptamolybdate TS, and allow to stand: a dark blue color develops slowly when the solution is cold, or quickly when warm.

(2)  To 5 mL of a solution of Thiotepa (1 in 100) add 1 mL of nitric acid: this solution responds to the Qualitative Tests <1.09> (2) for phosphate.

(3)  Dissolve 0.1 g of Thiotepa in a mixture of 1 mL of lead (II) acetate TS and 10 mL of sodium hydroxide TS, and boil: the gas evolved changes moistened red litmus paper to blue, and the solution shows a grayish red color.

**Melting point** <2.60> 52 – 57°C

**Purity** (1)  Clarity and color of solution—Dissolve 1.0 g of Thiotepa in 20 mL of water: the solution is clear and col-
orless.

(2) Heavy metals $<1.07>$—Proceed with 1.0 g of Thiotepa in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic $<1.11>$—Dissolve 0.20 g of Thiotepa in 5 mL of water, and add 1 mL of nitric acid and 1 mL of sulfuric acid. Take this solution, prepare the test solution according to Method 2, and perform the test (not more than 10 ppm).

**Loss on drying** $<2.41>$ Not more than 0.20% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** $<2.44>$ Not more than 0.1% (1 g, platinum crucible).

**Assay**

Weigh accurately about 0.1 g of Thiotepa, previously dried, dissolve in 50 mL of a solution of potassium thiocyanate (3 in 20), add 25 mL of 0.05 mol/L sulfuric acid VS, exactly measured, and allow to stand for 20 minutes with occasional shaking. Titrate $<2.50>$ the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from red to light yellow (indicator: 3 drops of methyl red TS). Perform a blank determination.

Each mL of 0.05 mol/L sulfuric acid VS $= 6.307$ mg of $\text{C}_6\text{H}_7\text{N}_3\text{PS}$

**Containers and storage**

Containers—Tight containers. Storage—Light-resistant, and in a cold place.

### L-Threonine

L-トレオニン

\[
\text{C}_6\text{H}_7\text{NO}_3: 119.12
\]

(2S,3R)-2-Amino-3-hydroxybutanoic acid [72-19-5]

L-Threonine, when dried, contains not less than 98.5% of $\text{C}_6\text{H}_7\text{NO}_3$.

**Description**

L-Threonine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slightly sweet taste.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (95).

**Identification**

Determine the infrared absorption spectrum of L-Threonine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** $<2.49>$ $[\alpha]_D ^{25}: -26.0 ^\circ$ to $-29.0 ^\circ$ (after drying, 1.5 g, water, 25 mL, 100 mm).

**pH** $<2.54>$ Dissolve 0.20 g of L-Threonine in 20 mL of water: the pH of this solution is between 5.2 and 6.2.

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of L-Threonine in 20 mL of water: the solution is clear and colorless.

(2) Chloride $<1.03>$—Perform the test with 0.5 g of L-Threonine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.02%).

(3) Sulfate $<1.44>$—Perform the test with 0.6 g of L-Threonine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.02%).

(4) Ammonium $<1.02>$—Perform the test with 0.25 g of L-Threonine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals $<1.07>$—Proceed with 1.0 g of L-Threonine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic $<1.11>$—Dissolve 1.0 g of L-Threonine in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.30 g of L-Threonine in 50 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 5 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50), and heat the plate at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** $<2.41>$ Not more than 0.20% (1 g, 105°C, 3 hours).

**Residue on ignition** $<2.44>$ Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.12 g of L-Threonine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate $<2.50>$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS $= 11.91$ mg of $\text{C}_6\text{H}_7\text{NO}_3$

**Containers and storage**

Containers—Tight containers.

### Thrombin

トロンピン

Thrombin is prepared from prothrombin obtained from blood of man or bull, through interaction with added thromboplastin in the presence of calcium ions, sterilized and lyophilized.

It contains not less than 80% and not more than 150% of the labeled Units of thrombin.

Each mg contains not less than 10 Units of thrombin.

**Description**

Thrombin is a white to light yellow, amor-
Thrombin (500 Units) dissolves in 1.0 mL of isotonic sodium chloride solution clearly or with slight turbidity within 1 minute.

**Loss on drying** (2.41) Not more than 3% (50 mg, in vacuum, phosphorus (V) oxide, 4 hours).

**Sterility** (4.06) It meets the requirement.

**Assay** (i) Fibrinogen solution—Weigh accurately about 30 mg of fibrinogen, and dissolve in 3 mL of isotonic sodium chloride solution. Allow the solution to clot sufficiently with frequent shaking after the addition of about 3 Units of thrombin. Wash the precipitated clot thoroughly until the washings yield no turbidity on addition of silver nitrate TS, weigh the clot after drying at 105°C for 3 hours, and calculate the percentage of the clot in the fibrinogen. Dissolve the fibrinogen in isotonic sodium chloride solution so that the clot should be 0.20%, adjust the pH of the solution between 7.0 and 7.4 by addition of 0.05 mol/L dibasic sodium phosphate TS (or if necessary, use 0.5 mol/L disodium hydrogen phosphate TS), and dilute with isotonic sodium chloride solution to make a 0.10% solution.

(ii) Procedure—Dissolve Thrombin RS in isotonic sodium chloride solution, and prepare four kinds of standard solutions which contain 4.0, 5.0, 6.2, and 7.5 Units in 1 mL. Transfer accurately 0.10 mL each of the standard solutions maintained at a given degree ± 1°C between 20°C and 30°C to a small test tube, 10 mm in inside diameter, 100 mm in length, blow out 0.90 mL of the fibrinogen solution at the same temperature into the test tube from a pipet, start a stop watch simultaneously, shake the tube constantly, and determine the time for the first appearance of clot. Calculate the average values of five determinations for the four kinds of standard solutions, respectively. If the deviation between the maximum and the minimum values of five determinations is more than 10% of the average value, reject the whole run, and try the experiment again. The concentration of the standard solution may be changed appropriately within the range between 14 and 60 seconds of the clotting time. The determination proceeds at the same temperature described above. Next, weigh accurately the whole contents of a single container of Thrombin, dissolve it in isotonic sodium chloride solution to provide a solution which is presumed to contain about 5 Units in each mL, treat 0.10 mL of the solution with the same reagents in the same manner five times, determine the clotting times, and calculate the average value. Plot the average values of the clotting times of the four kinds of the standard solutions on a logarithmic graph, using Units as the abscissa and clotting times as the ordinate, and draw a calibration line which best fits the four plotted points. Using this line, read the Units U from the average value of the clotting times of the sample solution.

Units of 1 container of Thrombin = \( U \times 10 \times V \)

\( V \): The number of mL of the volume in which the contents of 1 container of Thrombin has been dissolved

Calculate the units for 1 mg of the contents.

**Containers and storage** Containers—Hermetic containers.

**Storage**—Not exceeding 10°C.

**Expiration date** 36 months after preparation.

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**Thymol**

学名: 5-Methyl-2-(1-methylethyl)phenol

化学式: C_{10}H_{14}O

熔点: 49–51°C

**Purity (1)** Non-volatile residue—Volatilize 2.0 g of Thymol by heating on a water bath, and dry the residue at 105°C for 2 hours: the mass is not more than 1.0 mg.

**(2)** Other phenols—Shake vigorously 1.0 g of Thymol with 20 mL of warm water for 1 minute, and filter. To 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a green color may develop, but no blue to purple color develops.

**Assay** Weigh accurately about 0.5 g of Thymol, dissolve in 10 mL of sodium hydroxide TS, and add water to make exactly 100 mL. Measure exactly 10 mL of the solution into an iodine flask, add 50 mL of water and 20 mL of dilute sulfuric acid, and cool in ice water for 30 minutes. Add exactly 20 mL of 0.05 mol/L bromine VS, stopper tightly immediately, allow to stand for 30 minutes in ice water with occasional shaking in a dark place, add 14 mL of potassium iodide TS and 5 mL of chloroform, stopper tightly, shake vigorously, and titrate \( <2.50 \) the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Stopper tightly, shake vigorously near the end point, and continue the titration until the blue color in the chloroform layer disappears. Perform a blank determination.

Each mL of 0.05 mol/L bromine VS = 3.756 mg of C_{10}H_{14}O
Dried Thyroid

乾燥甲状腺

Dried Thyroid is the fresh thyroid gland, previously deprived of connective tissue and fat, minced, dried rapidly at a temperature not above 50°C, and powdered, or diluted with suitable diluents. It is obtained from domesticated animals that are used for food by man.

It contains not less than 0.30% and not more than 0.35% of iodine (I: 126.90) in the form of organic compounds peculiar to the thyroid gland.

Description Dried Thyroid occurs as a light yellow to grayish brown powder. It has a slight, characteristic, meat-like odor.

Identification Mount Dried Thyroid in diluted formaldehyde solution (1 in 10), stain in hematoxylin TS for 10 to 30 minutes, wash with water, soak in a mixture of 1 mL of hydrochloric acid and 99 mL of diluted ethanol (7 in 10) for 5 to 10 seconds, and again wash with water for about 1 hour. Stain in a solution of eosin Y (1 in 100) for 1 to 5 minutes, wash with water, dehydrate, and soak successively in diluted ethanol (7 in 10) for 5 to 10 seconds, in diluted ethanol (4 in 5) for 5 to 10 seconds, in diluted ethanol (9 in 10) for 1 to 2 minutes, in ethanol (95) for 1 minute for 5 seconds then in ethanol (99.5) for 1 to 5 minutes. Intercrinate in xylene, seal with balsam, and examine under a microscope: epithelial nuclei forming follicles peculiar to the thyroid gland are observed.

Purity (1) Inorganic iodides—Mix 1.0 g of Dried Thyroid with 10 mL of a saturated solution of zinc sulfate, shake for 5 minutes, and filter. To 5 mL of the filtrate add 0.5 mL of starch TS, 4 drops of sodium nitrite TS and 4 drops of dilute sulfuric acid with thorough shaking: no blue color is produced.

(2) Fat—Extract 1.0 g of Dried Thyroid with diethyl ether for 2 hours using a Soxhlet extractor. Evaporate the diethyl ether extract, and dry the residue at 105°C to constant mass: the mass of the residue is not more than 30 mg.

Loss on drying <2.41> Not more than 6.0% (1 g, 105°C, constant mass).

Total ash <0.01> Not more than 5.0% (0.5 g).

Assay Transfer about 1 g of Dried Thyroid, accurately weighed, to a crucible, add 7 g of potassium carbonate, mix carefully, and gently tap the crucible on the table to compact the mixture. Overlap with 10 g of potassium carbonate, and compact again thoroughly by tapping. Place the crucible in a muffle furnace preheated to a temperature between 600°C and 700°C, and ignite the mixture for 25 minutes. Cool, add 20 mL of water, heat gently to boiling, and filter into a flask. To the residue add 20 mL of water, boil, and filter into the same flask. Rinse the crucible and the char on the funnel with boiling water until the filtrate measures 200 mL. Add slowly 7 mL of freshly prepared bromine TS, 40 mL of diluted phosphoric acid (1 in 2), and boil until starch iodide paper is no longer colored blue by the evolved gas. Wash down inside of the flask with water, and continue boiling for 5 minutes. During the boiling add water from time to time to maintain a volume at not less than 200 mL. Cool, add 5 mL of a solution of phenol (1 in 20), again rinse inside of the flask with water, and allow to stand for 5 minutes. Add 2 mL of diluted phosphoric acid (1 in 2) and 5 mL of potassium iodide TS, and titrate <2.50> immediately the liberated iodine with 0.01 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L sodium thiosulfate VS = 0.2115 mg of I

Containers and storage Containers—Tight containers.

Tiapride Hydrochloride

チアプリド塩酸塩

Tiapride Hydrochloride occurs as a white to slightly yellowish white crystal or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), soluble in methanol, slightly soluble in ethanol (99.5) and very slightly soluble in acetic anhydride.

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the absorption spectrum of a solution of Tiapride Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 10,000) as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tiapride Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tiapride Hydrochloride (1 in 20) responds to Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Tiapride Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.20 g of Tiapride Hydrochloride in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this
solution, add methanol to make exactly 10 mL, and use this solution as directed under Thin-layer Chromatography. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot rapidly 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography under a stream of nitrogen. Develop the plate with a mixture of water, 1-butanol and acetic acid (100:2:2:1) to a distance of about 10 cm, and air-dry, and then dry the plate at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(3) Residual solvent—Being specified separately.

Loss on drying \( <2.41\% \) Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition \( <2.44\% \) Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Tiapride Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate \( <2.50\% \) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 36.49 mg of C₁₅H₂₄N₂O₄S.HCl

Containers and storage Containers—Well-closed containers.

Tiapride Hydrochloride Tablets

チアブリッド塩酸塩錠

Tiapride Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tiapride (C₁₅H₂₄N₂O₄S: 328.43).

Method of preparation Prepare as directed under Tablets, with Tiapride Hydrochloride.

Identification To a quantity of powdered Tiapride Hydrochloride Tablets, equivalent to 10 mg of tiapride (C₁₅H₂₄N₂O₄S) according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 286 nm and 290 nm.

Uniformity of dosage units \( <6.02\% \) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tiapride Hydrochloride Tablets add \( V/10 \) mL of 0.1 mol/L hydrochloric acid TS, treat with ultrasonic waves until the tablet is disintegrated, and add \( 4V/10 \) mL of methanol. To this solution add exactly \( V/10 \) mL of the internal standard solution, shake for 30 minutes, and add methanol to make \( V \) mL so that each mL contains about 1 mg of tiapride (C₁₅H₂₄N₂O₄S). Centrifuge this solution for 10 minutes, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of tiapride (C₁₅H₂₄N₂O₄S) = \( M_S \times Q_t/Q_s \times 100 \times 0.900 \)

\( M_S \): Amount (mg) of tiapride chloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 500).

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Tiapride Hydrochloride Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of tiapride (C₁₅H₂₄N₂O₄S), add about 10 mL of 0.1 mol/L hydrochloric acid TS and 40 mL of methanol, add exactly 10 mL of the internal standard solution, shake for 30 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.11 g of tiapride chloride for assay, previously dried at 105°C for 2 hours, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, add exactly 10 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, \( Q_t \) and \( Q_s \), of the peak area of Tiapride to that of the internal standard.

Amount (mg) of tiapride (C₁₅H₂₄N₂O₄S) = \( M_S \times Q_t/Q_s \times 0.900 \)

\( M_S \): Amount (mg) of tiapride chloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 11.2 g of sodium perchlorate in 800 mL of water, add 5 mL of diluted perchloric acid (17 in 2000). To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tiapride is about 8 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, tiapride and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tiapride to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.


Tiaramide Hydrochloride

チアラミド塩酸塩

C_{15}H_{18}ClN_{3}O_{3}S.HCl: 392.30
5-Chloro-3-[2-[4-(2-hydroxyethyl)piperazin-1-yl]-2-oxoethyl]-1,3-benzothiazol-2(3H)-one monohydrochloride
[35941-71-0]

Tiaramide Hydrochloride, when dried, contains not less than 98.5% of C_{15}H_{18}ClN_{3}O_{3}S.HCl.

Description
Tiaramide Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in water, slightly soluble in ethanol (95%) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

The pH of a solution of Tiaramide Hydrochloride (1 in 20) is between 3.0 and 4.5.

Melting point: about 265°C (with decomposition).

Identification
(1) Dissolve 5 mg of Tiaramide Hydrochloride in 5 mL of 0.1 mol/L hydrochloric acid TS, and add 3 drops of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the infrared absorption spectrum of Tiaramide Hydrochloride, previously dried, as directed in the potassium chloride disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tiaramide Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity
(1) Clarity and color of solution—Dissolve 0.5 g of Tiaramide Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Tiaramide Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tiaramide Hydrochloride according to Method 1, and perform the test. In the procedure, add 20 mL of diluted hydrochloric acid (1 in 2) (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Tiaramide Hydrochloride in 10 mL of diluted ethanol (7 in 10), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add diluted ethanol (7 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, add diluted ethanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>.

Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. After air-drying, immediately develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, air-dry the plate, and then dry at 100°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution. Allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay
Weigh accurately about 0.5 g of Tiaramide Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from red through purple to blue-purple (indicator: 3 drops of neutral red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 39.23 mg of C_{15}H_{18}ClN_{3}O_{3}S.HCl

Containers and storage
Containers—Well-closed containers.

Tiaramide Hydrochloride Tablets

チアラミド塩酸塩錠

Tiaramide Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tiaramide (C_{15}H_{18}ClN_{3}O_{3}S: 355.84).

Method of preparation
Prepare as directed under Tablets, with Tiaramide Hydrochloride.

Identification
(1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 285 nm and 289 nm, and between 292 nm and 296 nm.

(2) To a quantity of powdered Tiaramide Hydrochloride Tablets, equivalent to 0.1 g of tiaramide according to the labeled amount, add 10 mL of diluted ethanol (7 in 10), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 0.11 g of tiaramide hydrochloride for assay in 10 mL of diluted ethanol (7 in 10), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>.

Spot 20 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly Dragendorff's TS for spraying followed by diluted nitric acid (1 in 50) on the plate: the principal spot obtained with the sample solution and the spot with the standard solution are yellow-red in color and have the same Rf value.

Uniformity of dosage units <6.02> Perform the test accord-
To 1 tablet of Tiaramide Hydrochloride Tablets add a volume of 0.1 mol/L hydrochloric acid TS, equivalent to 3/5 volume of V mL which makes a solution so that each mL contains about 1 mg of tiaramide (C_{15}H_{18}ClN_{3}O_{3}S), shake for 30 minutes, then add 0.1 mol/L hydrochloric acid TS to make exactly V mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 55 mg of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_{T} and A_{S}, of the sample solution and standard solution at 294 nm as directed under Ultraviolet-visible Spectrophotometry. Consider the following method: it meets the requirement of the Content uniformity test.

\[ M_{S} = M_{0} \times A_{T}/A_{S} \times \frac{V}{50} \times 0.907 \]

**Dissolution (6.10)** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates of a 50-mg tablet in 15 minutes and of a 100-mg tablet in 30 minutes are not less than 80%.

Start the test with 1 tablet of Tiaramide Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 \( \mu \)m. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 56 \( \mu \)g of tiaramide (C_{15}H_{18}ClN_{3}O_{3}S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_{T} and A_{S}, at 294 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry.

\[ M_{S} = M_{0} \times A_{T}/A_{S} \times \frac{V}{25} \times 0.907 \]

**Identification (1)** Determine the infrared absorption spectrum of Tiaramide Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity (1)** Heavy metals <1.07>—Proceed with 2.0 g of Tiaramide Hydrochloride according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Purity (2)** Arsenic <1.11>—Prepare the test solution with 1.0 g of Tiaramide Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

**Related substances**—Dissolve 0.5 g of Tiaramide Hydrochloride in 20 mL of a solution of hydrochloric acid in methanol (1 in 20,000), and use this solution as the sample solution. To exactly 5 mL of the sample solution add a solution of hydrochloric acid in methanol (1 in 20,000) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, pipet 1 mL of the sample solution, add a solution of hydrochloric acid in methanol (1 in 20,000) to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Consider the following method: it meets the requirement of the Content uniformity test.

\[ M_{S} = M_{0} \times A_{T}/A_{S} \times 0.907 \]

**Concentration of Tiaramide Hydrochloride Tablets**

**Tiaramide Hydrochloride Tablets**

**Usage**

**Purity**

**Identification**

**Purity**

**Related substances**—Proceed with 1.0 g of Ticlopidine Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).
Weigh accurately about 0.4 g of Ticlopidine Hydrochloride in 19.0 mL of water, add 1.0 mL of 4 mol/L sodium hydroxide TS, shake well, centrifuge, and filter the supernatant liquid. To 5.0 mL of the filtrate add 5.0 mL of acetylacetone TS, mix, and warm at 40°C for 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1). Allow Plate 2 to stand in an iodine vapor for 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (2).

(4) Formaldehyde—Dissolve 0.8 g of Ticlopidine Hydrochloride in 19.0 mL of water, add 1.0 mL of 4 mol/L sodium hydroxide TS, shake well, centrifuge, and filter the supernatant liquid. To 5.0 mL of the filtrate add 5.0 mL of acetylacetone TS, mix, and warm at 40°C for 40 minutes: the solution has no more color than the following control solution.

Control solution: Weigh exactly 0.54 g of formaldehyde solution, and add water to make exactly 1000 mL. To exactly 10 mL of this solution add water to make exactly 1000 mL. Prepare before use. To 8.0 mL of this solution add water to make 20.0 mL, and filter. To 5.0 mL of the filtrate add 5.0 mL of acetylacetone TS, and proceed in the same manner.

**Water** Not more than 1.0% (0.3 g, volumetric titration, direct titration).

**Residue on ignition** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Ticlopidine Hydrochloride, dissolve in 20 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 30.03 mg of C_{14}H_{14}ClNS.HCl

**Containers and storage** Containers—Well-closed containers.

**Timepidium Bromide Hydrate**

![Chemical Structure](image)

C_{17}H_{22}BrNOS_{2}.H_{2}O: 418.41

(5SR)-3-(Dithien-2-ylmethylene)-5-methoxy-1,1-dimethylpiperidinium bromide monohydrate [35035-05-3, anhydride]

Timepidium Bromide Hydrate contains not less than 98.5% of timepidium bromide (C_{17}H_{22}BrNOS_{2}.H_{2}O: 400.40), calculated on the anhydrous basis.

**Description** Timepidium Bromide Hydrate occurs as white crystals or crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in ethanol (99.5), sparingly soluble in water and in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Timepidium Bromide Hydrate in freshly boiled and cooled water (1 in 100) is between 5.3 and 5.5.

A solution of Timepidium Bromide Hydrate in methanol (1 in 20) shows no optical rotation.

**Identification** (1) To 1 mL of a solution of Timepidium Bromide Hydrate (1 in 100) add 1 mL of ninhydrin-sulfuric acid TS: a red purple color develops.

(2) Determine the absorption spectrum of a solution of Timepidium Bromide Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry 

(3) Determine the infrared absorption spectrum of Timepidium Bromide Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry 

(4) A solution of Timepidium Bromide Hydrate (1 in 100) responds to the Qualitative Tests <1.09> (1) for Bromide.

**Purity** (1) Clarity and color of solution—Dissolve 0.10 g of Timepidium Bromide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals.<1.07>—Proceed with 1.0 g of Timepidium Bromide Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Timepidium Bromide Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, water, acetic acid (100) and ethyl acetate (5:4:1:1:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Water** 3.5 – 5.0% (0.4 g, volumetric titration, direct titration).

**Residue on ignition** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.6 g of Timepidium Bromide Hydrate, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (2:1), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.
Each mL of 0.1 mol/L perchloric acid VS = 40.04 mg of C$_{17}$H$_{22}$BrNOS$_2$.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Timolol Maleate**

チモロールマレイン酸塩

\[\text{C}_{13}\text{H}_{24}\text{N}_{4}\text{O}_{3}\text{S} \cdot \text{C}_4\text{H}_4\text{O}_4: 432.49}\]

(2S)-1-[(1,1-Dimethylethyl)amino]-3-(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl-oxy)propan-2-ol monomaleate [26921-17-5]

Timolol Maleate, when dried, contains not less than 98.0% and not more than 101.0% of C$_{13}$H$_{24}$N$_4$O$_3$S. C$_4$H$_4$O$_4$.

**Description** Timolol Maleate occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in acetic acid (100), and soluble in water and in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Melting point: about 197°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Timolol Maleate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2,24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Timolol Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelength numbers.

(3) To 5 mL of a solution of Timolol Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the TS disappears immediately.

**Optical rotation** <2,49> \[\alpha_d^{20} = -5.7 - (-6.2)^\circ\] (after drying, 1.25 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** <2,50> The pH of a solution prepared by dissolving 1.0 g of Timolol Maleate in 20 mL of water is between 3.8 and 4.3.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Timolol Maleate in 20 mL of water: the solution is clear, and its absorbance at 440 nm, determined as directed under Ultraviolet-visible Spectrophotometry <2,24>, is not more than 0.05.

(2) Heavy metals <1,07>—Proceed with 2.0 g of Timolol Maleate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 30 mg of Timolol Maleate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 µL of each of the sample solution and standard solution as directed under Liquid Chromatography <2,8B> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than timolol and maleic acid obtained from sample solution is not larger than 1/5 times the peak area of timolol from the standard solution, and the total area of the peaks other than the peak of timolol and maleic acid is not larger than 1/2 times the peak area of timolol from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.9 g of sodium 1-hexanesulfonate in 1800 mL of water, add 6.0 mL of triethylamine and 8.0 mL of formic acid, adjust to pH 3.0 with formic acid, and add water to make 2000 mL. To 1400 mL of this solution add 500 mL of methanol and 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of timolol is about 18 minutes.

Time span of measurement: About 2 times as long as the retention time of timolol beginning after the solvent peak.

**System suitability**—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make 10 mL. Confirm that the peak area of timolol obtained from 25 µL of this solution is equivalent to 7 to 13% of that from 25 µL of the standard solution.

System performance: When the procedure is run with 25 µL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of timolol are not less than 1500 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 25 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of timolol is not more than 2.0%.

**Loss on drying** <2,41> Not more than 0.5% (1 g, in vacuum, 100°C, 3 hours).

**Residue on ignition** <2,44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.8 g of Timolol Maleate, previously dried, dissolve in 90 mL of acetic acid (100), and titrate <2,50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 43.25 mg of C$_{13}$H$_{22}$N$_4$O$_3$S. C$_4$H$_4$O$_4$.

**Containers and storage** Containers—Tight containers.
**Tinidazole**

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\[
\begin{align*}
\text{C}_8\text{H}_{13}\text{N}_3\text{O}_4\text{S} : 247.27 \\
& \text{I-[2-(Ethylsulfonyl)ethyl]-2-methyl-5-nitro-1H-imidazole} \\
& [19387-91-8]
\end{align*}
\]

Tinidazole, when dried, contains not less than 98.5% and not more than 101.0% of \( \text{C}_8\text{H}_{13}\text{N}_3\text{O}_4\text{S} \).

**Description**

Tinidazole occurs as a light yellow, crystalline powder.

It is soluble in acetic anhydride and in acetone, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

**Identification (1)**

Determine the absorption spectrum of a solution of Tinidazole in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Identification (2)**

Determine the infrared absorption spectrum of Tinidazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point**

<2.60> 125 – 129°C

**Purity (1)**

Sulfate <1.14>—To 2.0 g of Tinidazole add 100 mL of water, boil for 5 minutes, cool, add water to make 100 mL, and filter. Take 25 mL of the filtrate, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.043%).

**Identification (2)**

Heavy metals <1.07>—Proceed with 1.0 g of Tinidazole according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Arsenic <1.12>**—Prepare the test solution with 2.0 g of Tinidazole according to Method 3, and perform the test (not more than 1 ppm).

**Related substances**—Dissolve 50 mg of Tinidazole in 2 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diethylamine (19:1) to a distance of about 10 cm, air-dry the plate, heat at 100°C for 5 minute, and cool. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Residue on ignition** <2.44> Not more than 1.0% (1 g, 105°C, 2 hours).

**Loss on drying** <2.41> Not more than 1.0% (1 g).

**Assay**

Weigh accurately about 0.35 g of Tinidazole, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.73 mg of \( \text{C}_8\text{H}_{13}\text{N}_3\text{O}_4\text{S} \).

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant.

**Tipepidine Hibenzate**

チペピジンヒベンズ酸塩

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\begin{align*}
\text{C}_{15}\text{H}_{21}\text{NS}_2\cdot\text{C}_4\text{H}_{10}\text{O}_4 : 517.66 \\
& \text{3-(Dithien-2-ylmethylene)-1-methylpiperidine mono(2-(4-hydroxybenzoyl)benzoate)} \\
& [31139-87-4]
\end{align*}
\]

Tipepidine Hibenzate, when dried, contains not less than 98.5% of \( \text{C}_{15}\text{H}_{21}\text{NS}_2\cdot\text{C}_4\text{H}_{10}\text{O}_4 \).

**Description**

Tipepidine Hibenzate occurs as a white to light yellow, crystalline powder. It is odorless and tasteless.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

**Identification (1)**

Dissolve 0.01 g of Tipepidine Hibenzate in 5 mL of sulfuric acid: an orange-red color develops.

**Identification (2)**

Dissolve 0.3 g of Tipepidine Hibenzate in 10 mL of sodium hydroxide TS and 5 mL of water, and extract with two 20-mL portions of chloroform. Wash the chloroform extracts with 10 mL of water, and filter the chloroform layer. Evaporate the filtrate on a water bath to dryness, and dissolve the residue in 0.5 mL of 1 mol/L hydrochloric acid AS and 5 mL of water. To 2 mL of this solution add 5 mL of Reinecke salt TS: a light red precipitate is formed.

**Identification (3)**

Determine the absorption spectrum of a solution of Tipepidine Hibenzate in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum; both spectra exhibit similar intensities of absorption at the same wave numbers.

**Identification (4)**

Determine the infrared absorption spectrum of Tipepidine Hibenzate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point**

<2.60> 189 – 193°C
Purity (1) Clarity of solution—Dissolve 1.0 g of Tipepidine Hibenzate in 10 mL of acetic acid (100): the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: its absorbance at 400 nm is not more than 0.16.

(2) Heavy metals 2.07—Proceed with 2.0 g of Tipepidine Hibenzate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic 1.12—Prepare the test solution with 1.0 g of Tipepidine Hibenzate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—(i) Dissolve 10 mg of Tipepidine Hibenzate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of peaks other than hibenzic acid and tipepidine from the sample solution is not larger than the peak area of the tipepidine from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 50°C.
Mobile phase: A mixture of a solution of ammonium acetate (1 in 100) and tetrahydrofuran (32:13).
Flow rate: Adjust the flow rate so that the retention time of tipepidine is about 12 minutes.
Time span of measurement: As long as the retention time of tipepidine beginning after the solvent peak.

System suitability—
Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of tipepidine obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.
System performance: Dissolve 10 mg of Tipepidine Hibenzate and 3 mg of propyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, hibenzic acid, tipepidine and propyl parahydroxybenzoate are eluted in this order with the resolution between the peaks of tipepidine and propyl parahydroxybenzoate being not less than 3.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tipepidine is not more than 3.0%.

Loss on drying 2.41 Not more than 0.5% (1 g, 60°C, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition 2.44 Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Tipepidine Hibenzate, previously dried, dissolve in 40 mL of acetic acid (100), and titrate 2.59 with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 51.77 mg of C₁₅H₁₇NS₂.C₁₄H₁₀O₄

Containers and storage Containers—Well-closed containers.
Storagelight-resistant.
**Tipepidine Hibenzate Tablets**

**Identification (1)**

To a quantity of powdered Tipepidine Hibenzate Tablets, equivalent to 44 mg of Tipepidine Hibenzate according to the labeled amount, add 5 mL of water, shake for 1 minute, add 10 mL of sodium hydroxide TS, and extract with two 20-mL portions of chloroform. Combine the extracts, wash with 10 mL of water, and filter the chloroform layer. Evaporate the filtrate on a water bath to dryness, dissolve the residue in 0.2 mL of 1 mol/L hydrochloric acid TS and 2 mL of water, and add 5 mL of Reinecke salt TS: a light red precipitate is formed.

(2) To a quantity of powdered Tipepidine Hibenzate Tablets, equivalent to 11 mg of Tipepidine Hibenzate according to the labeled amount, add 30 mL of ethanol (99.5), and warm for 10 minutes with occasional shaking. After cooling, add ethanol (99.5) to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 282 nm and 286 nm.

**Uniformity of dosage units**

Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tipepidine Hibenzate Tablets add 5 mL of diluted acetic acid (100) (1 in 2) and 15 mL of methanol per 11 mg of tipepidine hibenzate (C_{15}H_{17}NS{2}.C_{14}H_{10}O_{4}), and warm for 15 minutes with occasional shaking. After cooling, add diluted methanol (1 in 2) to make exactly 50 mL, and filter. To 1 mL of the filtrate, add ethanol (99.5) to make 20 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 282 nm and 286 nm.

**Dissolution**

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Tipepidine Hibenzate Tablets is not less than 80%.

Start the test with 1 tablet of Tipepidine Hibenzate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of tipepidine hibenzate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 3 hours, and dissolve in 80 mL of distilled ethanol (3 in 4) by warming occasionally. After cooling, add diluted ethanol (3 in 4) to make exactly 100 mL, then pipet 20 mL of this solution, add water to make exactly 900 mL, and use this solution as the standard solution. Determine the absorbances, A_{T1} and A_{T2}, at 286 nm, and A_{S1} and A_{S2}, at 360 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry:

Dissolution rate (%) with respect to the labeled amount of tipepidine hibenzate (C_{15}H_{17}NS{2}.C_{14}H_{10}O_{4})

\[
M_5 \times \frac{(A_{T1} - A_{T2} - A_{S1} - A_{S2})}{(A_{T1} - A_{T2})} \times \frac{1}{C} \times 20
\]

\[M_5: \text{Amount (mg) of tipepidine hibenzate for assay}
\]

**Assay**

Weigh accurately and powder not less than 20 Tipepidine Hibenzate Tablets. Weigh accurately a portion of the powder, equivalent to about 22 mg of tipepidine hibenzate (C_{15}H_{17}NS{2}.C_{14}H_{10}O_{4}), add 10 mL of diluted acetic acid (100) (1 in 2) and 30 mL of methanol, and warm for 10 minutes with occasional shaking. After cooling, add diluted methanol (1 in 2) to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of tipepidine hibenzate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 3 hours, dissolve in 10 mL of diluted acetic acid (100) (1 in 2) and 30 mL of methanol, and add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 20 mL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_{T1} and Q_{S1}, of the peak area of tipepidine to that of the internal standard, respectively.

Amount (mg) of tipepidine hibenzate (C_{15}H_{17}NS{2}.C_{14}H_{10}O_{4}) in 1 tablet

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M_5 = M_3 \times \frac{Q_{T1}}{Q_{S1}}
\]

\[M_3: \text{Amount (mg) of tipepidine hibenzate for assay}
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**Internal standard solution**—A solution of dibucaine hydrochloride in methanol (1 in 2000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of sodium lauryl sulfate in diluted phosphoric acid (1 in 1000) (1 in 500), acetoneitrile and 2-propanol (3:2:1).

Flow rate: Adjust the flow rate so that the retention time of tipepidine is about 7 minutes.

**System suitability**—

System performance: When the procedure is run with 20
butyl acetate (1 in 500), shake for 10 minutes, and use this solution as the standard solution. Determine the absorbances of the peak area of tipepidine to that of the internal standard under Atomic Absorption Spectrophotometry of the sample solution and standard solution as directed.

Containers and storage  Containers—Tight containers.
Storage—Light-resistant.

Titanium Oxide

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TiO₂: 79.87

Titanium Oxide, when dried, contains not less than 98.5% of TiO₂.

Description  Titanium Oxide occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (99.5) and in diethyl ether.

It dissolves in hot sulfuric acid and in hydrofluoric acid, and does not dissolve in hydrochloric acid, in nitric acid and in dilute sulfuric acid.

When fused by heating with potassium hydrogen sulfate, with potassium hydroxide, or with potassium carbonate, it changes to soluble salts.

Shake 1 g of Titanium Oxide with 10 mL of water: the mixture is neutral.

Identification  Heat 0.5 g of Titanium Oxide with 5 mL of sulfuric acid until white fumes are evolved, cool, add cautiously water to make 100 mL, and filter. To 5 mL of the filtrate add 2 to 3 drops of hydrogen peroxide TS: a yellow-red color develops.

Purity  (1) Lead—Place 1.0 g of Titanium Oxide in a platinum crucible, add 10.0 g of potassium hydrogen sulfate, heat gently with caution at the beginning, then raise the temperature gradually, and heat strongly with occasional shaking until the contents fuse to yield a clear liquid. Cool, add 30 mL of a solution of diammonium hydrogen citrate (9 in 20) and 50 mL of water, dissolve by heating on a water bath, cool, add water to make 100 mL, and use this solution as the sample stock solution. Take 25 mL of the sample stock solution to a separator, add 10 mL of a solution of ammonium sulfate (2 in 5) and 5 drops of thymol blue TS, neutralize with ammonia TS, and add 2.5 mL of ammonia TS. To this solution add exactly 20 mL of a solution of dithizone in n-butyl acetate (1 in 500), shake for 10 minutes, and use this n-butyl acetate solution as the sample solution. Separately, place 6.0 mL of Standard Lead Solution in a platinum crucible, proceed as directed in the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions: the absorbance of the sample solution is smaller than that of the standard solution (not more than 60 ppm).

Gas: Combustible gas—Acetylene gas or gas hydrogen gas.

Supporting gas—Air.

Lamp: Lead hollow-cathode lamp.

Wavelength: 283.3 nm.

Arsenic  <111>—Perform the test with 20 mL of the sample stock solution obtained in (1) as the test solution: the color is not deeper than the following color standard.

Color standard: Proceed in the same manner without Titanium Oxide, transfer 20 mL of the obtained solution to a generator bottle, add 2.0 mL of Standard Arsenic Solution, and proceed in the same manner as the test with the test solution (not more than 10 ppm).

(3) Water-soluble substances—Shake thoroughly 4.0 g of Titanium Oxide with 50 mL of water, and allow to stand overnight. Shake thoroughly with 2 mL of ammonium chloride TS, add further 2 mL of ammonium chloride TS if necessary, and allow titanium oxide to settle. Add water to make 200 mL, shake thoroughly, and filter through double filter paper. Discard the first 10 mL of the filtrate, evaporate 100 mL of the clear filtrate on a water bath, and heat strongly at 650°C to constant mass: the mass of the residue is not more than 5.0 mg.

Loss on drying  <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Assay  Weigh accurately about 0.2 g of Titanium Oxide, previously dried, transfer to a crucible, and add 3 g of potassium disulfate. Cover, and heat gently at first, gradually raise the temperature, and then heat the fused contents for 30 minutes. Continue heating for 30 minutes at a higher temperature to make the fused mixture a deep yellow-red, almost clear liquid. Cool, transfer the contents of the crucible to a 250-mL beaker, wash the crucible with a mixture of 75 mL of water and 2.5 mL of sulfuric acid into the beaker, and heat on a water bath until the solution becomes almost clear. Dissolve 2 g of L-tartaric acid in the solution, add 2 to 3 drops of bromothymol blue TS, neutralize with ammonia TS, and acidify with 1 to 2 mL of diluted sulfuric acid (1 in 2). Pass hydrogen sulfide sufficiently through the solution, add 30 mL of ammonia TS, again saturate the solution with hydrogen sulfide, allow to stand for 10 minutes, and filter. Wash the precipitate on the filter paper with ten 25-mL portions of a mixture of ammonium L-tartrate solution (1 in 100) and ammonium sulfide TS (9:1). When the precipitate is filtered and washed, prevent iron (II) sulfide from oxidation by filling the solution on the filter paper. Combine the filtrate and the washings, add 40 mL of diluted sulfuric acid (1 in 2), and boil to expel hydrogen sulfide. Cool, and dilute with water to make 400 mL. Add gradually 40 mL of cupferron TS to the solution with stirring, and allow to stand. After sedimentation of a yellow precipitate, add again cupferron TS until a white precipitate is produced. Filter by slight suction using quantitative filter paper, wash with twenty portions of diluted hydrochloric acid (1 in 10), and remove water by stronger suction at the last washing. Dry the precipitate together with the filter paper at 70°C, transfer to a tared crucible, and heat very gently at first, and raise the temperature gradually after smoke stops evolving. Heat strongly between 900°C and 950°C to constant mass, cool, and weigh as titanium oxide (TiO₂).

Containers and storage  Containers—Well-closed contain-
Tizanidine Hydrochloride

Tizanidine Hydrochloride occurs as a white to light yellowish white crystalline powder.

It is soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetic anhydride and in acetic acid (100).

Melting point: about 290°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Tizanidine Hydrochloride in diluted 1 mol/L ammonia TS (1 in 10) (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tizanidine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tizanidine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Tizanidine Hydrochloride according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 60 mg of Tizanidine Hydrochloride in 10 mL of a mixture of water and acetonitrile (17:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (17:3) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tizanidine with the sample solution is not larger than 1/5 times the peak area of tizanidine with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm for about 3 minutes after sample injection and 318 nm subsequently).

Column: A stainless steel column 4.6 mm in inside diameter and 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water and formic acid (200:1), adjusted to pH 8.5 with ammonia water (28).

Mobile phase B: A mixture of acetonitrile and the mobile phase A (4:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>81 → 68</td>
<td>19 → 32</td>
</tr>
<tr>
<td>10 – 13</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>13 – 26</td>
<td>68 → 10</td>
<td>32 → 90</td>
</tr>
<tr>
<td>26 – 28</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of tizanidine is about 7 minute.

Time span of measurement: About 4 times as long as the retention time of tizanidine beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mixture of water and acetonitrile (17:3) to make exactly 10 mL. Confirm that the peak area of tizanidine obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.

System performance: Dissolve 2 mg each of Tizanidine Hydrochloride and p-toluenesulfonic acid monohydrate in 100 mL of the mixture of water and acetonitrile (17:3). When the procedure is run with 10 μL of this solution under the above operating conditions, p-toluenesulfonic acid and tizanidine are eluted in this order with the retention between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tizanidine is not more than 2.0%.

Loss on drying <2.47> Not more than 0.2% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Tizanidine Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) with the aid of warming. After cooling, titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 29.02 mg of C_{9}H_{8}ClN_{5}S.HCl

Containers and storage Containers—Well-closed containers.
Tobramycin トブラマイシン

\[
\text{C}_{18}\text{H}_{37}\text{N}_{5}\text{O}_{9}: 467.51 \\
3\text{-Amino-3-deoxy-\alpha-D-glucopyranosyl-(1→6)-} \\
[2,6\text{-diamino-2,3,6-trideoxy-\alpha-D-ribo-hexopyranosyl-} \\
(1→4)]\text{-2-deoxy-\alpha-D-streptamine} \[32986-56-4\]
\]

Tobramycin is an aminoglycoside substance having antibacterial activity produced by the growth of Streptomyces tenebrarius.

It contains not less than 900 \( \mu \text{g} \) (potency) and not more than 1060 \( \mu \text{g} \) (potency) per mg, calculated on the anhydrous basis.

It is hygroscopic.

Identification (1) Determine the \( ^1 \text{H} \) spectrum of a solution of Tobramycin in heavy water for nuclear magnetic resonance spectroscopy (1 in 125) as directed under Nuclear Magnetic Resonance Spectroscopy \(<2.21>\), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal A at around \( \delta 5.1 \text{ ppm} \), a multiple signal B between \( \delta 2.6 \text{ ppm} \) and \( \delta 4.0 \text{ ppm} \), and a multiple signal C between \( \delta 1.0 \text{ ppm} \) and \( \delta 2.1 \text{ ppm} \). The ratio of the integrated intensity of these signals, A:B:C, is about 1:8:2.

(2) Dissolve 10 mg each of Tobramycin and Tobramycin RS in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.07>\). Spot 4 \( \mu \text{L} \) of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution, ethanol (95) and 2-butanol (1:1:1) to a distance of about 10 cm, air-dry the plate, then further dry at 110°C for 10 minutes. Immediately spray evenly a mixture of water and sodium hypochlorite TS (4:1) on the plate, air-dry the plate, then spray potassium iodide-starch TS on the plate: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Tobramycin in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals \(<1.07>\)—Proceed with 1.0 g of Tobramycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Related substances—Dissolve 80 mg of Tobramycin in 10 mL of diluted ammonia solution (28) (1 in 250), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ammonia solution (28) (1 in 250) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.07>\). Spot 5 \( \mu \text{L} \) of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28), ethanol (95) and 2-butanol (1:1:1) to a distance of about 10 cm, air-dry the plate, then further dry at 110°C for 10 minutes. Immediately spray evenly a mixture of water and sodium hypochlorite TS (4:1) on the plate, air-dry the plate, then spray potassium iodide-starch TS on the plate: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Water \(<2.48>\) Not more than 11.0% (0.1 g, volumetric titration, direct titration). Use a mixture of formamide for water determination and methanol for water determination (3:1) instead of methanol for water determination.

Residue on ignition \(<2.44>\) Not more than 1.0% (0.5 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics \(<4.02>\) according to the following conditions.

(i) Test organism—\textit{Bacillus subtilis} ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Tobramycin RS, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5°C and 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 \( \mu \text{g} \) (potency) and 2 \( \mu \text{g} \) (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Tobramycin, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 \( \mu \text{g} \) (potency) and 2 \( \mu \text{g} \) (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Optical rotation \(<2.49>\) \([\alpha]_{D}^{20}: +138° - +148° \) (1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).
Containers and storage  Containers—Tight containers.

**Tobramycin Injection**

トブラマイシン注射液

Tobramycin Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of tobramycin (C_{18}H_{27}N_{3}O_{9}: 467.51).

**Method of preparation** Prepare as directed under Injections, with Tobramycin.

**Description** Tobramycin Injection occurs as a colorless or very pale yellow clear liquid.

**Identification** To a volume of Tobramycin Injection, equivalent to 10 mg (potency) of Tobramycin according to the labeled amount, add water to make 1 mL, and use this solution as the sample solution. Separately, dissolve 10 mg (potency) of Tobramycin RS in 1 mL of water, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Tobramycin.

**Osmotic pressure ratio** Being specified separately.

**pH** <2.54> 5.0 – 7.0

**Bacterial endotoxins** <4.01> Less than 0.50 EU/mg (potency).

**Extractable volume** <6.06> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Tobramycin.

(ii) Sample solutions—To exactly 5 mL of Tobramycin Injection add 0.1 mol/L phosphate buffer solution, pH 8.0 so that each mL contains 1 mg (potency) of Tobramycin. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μg (potency) and 2 μg (potency), and use these solutions as the concentration sample solution high and the low concentration sample solution, respectively.

Containers and storage  Containers—Hermetic containers.

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**Tocopherol**

Vitamin E
dl-α-Tocopherol

トコフェロール

C_{29}H_{50}O_{2}: 430.71
2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol

[10191-41-0]

Tocopherol contains not less than 96.0% and not more than 102.0% of C_{29}H_{50}O_{2}.

**Description** Tocopherol is a clear, yellow to red-brown, viscous liquid. It is odorless.

It is miscible with ethanol (99.5), with acetone, with chloroform, with diethyl ether and with vegetable oils.

It is freely soluble in ethanol (95), and practically insoluble in water.

It is optically inactive.

It is oxidized by air and light, and acquires a dark red color.

**Identification** (1) Dissolve 0.01 g of Tocopherol in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at 75°C for 15 minutes: a red to orange color develops.

(2) Determine the infrared absorption spectrum of Tocopherol as directed in the liquid film method under Infrared Spectrophotometry <2.19>, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Absorbance** <2.42> E_{1\% 1cm}^{	ext{292 nm}}: 71.0 – 76.0 (10 mg, ethanol (99.5), 200 mL).

**Refractive index** <2.45> n_{D}^{20}: 1.503 – 1.507

**Specific gravity** <2.56> d_{20}^{20}: 0.947 – 0.955

**Purity** (1) Clarity and color of solution—Dissolve 0.1 g of Tocopherol in 10 mL of ethanol (99.5): the solution is clear and has no more color than Matching Fluid C.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Tocopherol according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Assay** Dissolve about 50 mg each of Tocopherol and Tocopherol RS, accurately weighed, in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography <2.02> according to the following conditions, and determine the peak heights, H_T and H_S, of tocoferol in the sample solution and standard solution.

Amount (mg) of C_{29}H_{50}O_{2} = M_S × H_T/H_S
M₅: Amount (mg) of Tocopherol RS

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 292 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μL in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: A mixture of methanol and water (49:1).
Flow rate: Adjust the flow rate so that the retention time of tocopherol is about 10 minutes.

System suitability—
System performance: Dissolve 0.05 g each of Tocopherol and tocopherol acetate in 50 mL of ethanol (99.5). When the procedure is run with 20 μL of this solution under the above operating conditions, tocopherol and tocopherol acetate are eluted in this order with the resolution between these peaks being not less than 2.6.
System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of tocopherol is not more than 0.8%.

Containers and storage Containers—Light-resistant, and well-filled, or under nitrogen atmosphere.

Tocopherol Acetate

Vitamin E Acetate
dl-α-Tocopherol Acetate

Tocopherol Acetate contains not less than 96.0% and not more than 102.0% of C₃₁H₅₂O₃.

Description Tocopherol Acetate is a clear, colorless or yellow, viscous and odorless liquid.
It is miscible with ethanol (99.5), with acetone, with chloroform, with diethyl ether, with hexane and with fixed oils.
It is freely soluble in ethanol (95), and practically insoluble in water.
It is optically inactive.
It is affected by air and light.

Identification (1) Dissolve 0.05 g of Tocopherol Acetate in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at 75°C for 15 minutes: a red to orange color is produced.
(2) Determine the infrared absorption spectrum of Tocopherol Acetate as directed in the liquid film method under Infrared Spectrophotometry §2.25, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.25> E₁cm (284 nm): 41.0 – 45.0 (10 mg, ethanol (99.5), 100 mL).

Refractive index <2.45> nD: 1.494 – 1.499

Specific gravity <2.56> d₂₀ Español 0.952 – 0.966

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Tocopherol Acetate in 10 mL of ethanol (99.5): the solution is clear, and has no more color than the following control solution.
Control solution: To 0.5 mL of Iron (III) Chloride CS add 0.5 mol/L hydrochloric acid TS to make 100 mL.
(2) Heavy metals <1.07>—Carbonize 1.0 g of Tocopherol Acetate by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (20 ppm).
(3) α-Tocopherol—Dissolve 0.10 g of Tocopherol Acetate in exactly 10 mL of hexane, and use this solution as the sample solution. Separately, dissolve 50 mg of Tocopherol RS in hexane to make exactly 100 mL. Pipet 1 mL of this solution, add hexane to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography §2.02. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and acetic acid (100) (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of iron (III) chloride hexahydrate in ethanol (99.5) (1 in 500) on the plate, then spray evenly a solution of α,α’-dipyridyl in ethanol (99.5) (1 in 200) on the same plate, and allow to stand for 2 to 3 minutes: the spot from the sample solution corresponding to that from the standard solution is not larger and not more intense than the spot from the standard solution.

Assay Dissolve 50 mg each of Tocopherol Acetate and Tocopherol Acetate RS, accurately weighed, in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography §2.01 according to the following conditions, and determine the peak heights, H₀ and H₅, of tocopherol acetate in the sample solution and the standard solution, respectively.

Amount (mg) of C₃₁H₅₂O₃ = M₅ × H₀/H₅

M₅: Amount (mg) of Tocopherol Acetate RS

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 284 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: A mixture of methanol and water (49:1).
Flow rate: Adjust the flow rate so that the retention time of tocopherol acetate is about 12 minutes.

System suitability—

System performance: Dissolve 0.05 g each of Tocopherol Acetate and tocopherol in 50 mL of ethanol (99.5). When the procedure is run with 20 μL of this solution under the above operating conditions, tocopherol and tocopherol acetate are eluted in this order with the resolution between these peaks being not less than 2.6.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of tocopherol acetate is not more than 0.8%.

Containers and storage—Containers—Tight containers. Storage—Light-resistant.

**Tocopherol Calcium Succinate**

Vitamin E Calcium Succinate

トコフェロールコハク酸エステルカルシウム

C_{66}H_{106}CaO_{10} • 1099.62
Monocalcium bis[3-[2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yloxycarbonyl]propanoate]

[14638-18-7]

Tocopherol Calcium Succinate, when dried, contains not less than 96.0% and not more than 102.0% of C_{66}H_{106}CaO_{10}.

**Description**—Tocopherol Calcium Succinate occurs as a white to yellowish white powder. It is odorless.

It is freely soluble in chloroform and in carbon tetrachloride, and practically insoluble in water, in ethanol (95) and in acetone.

Shake 1 g of Tocopherol Calcium Succinate with 7 mL of acetic acid (100): it dissolves, and produces a turbidity after being allowed to stand for a while.

It dissolves in acetic acid (100).

It is optically inactive.

**Identification**—

(1) Dissolve 0.05 g of Tocopherol Calcium Succinate in 1 mL of acetic acid (100), add 9 mL of ethanol (99.5), and mix. To this solution add 2 mL of fuming nitric acid, and heat at 75°C for 15 minutes: a red to orange color develops.

(2) Dissolve 0.08 g of Tocopherol Calcium Succinate, previously dried, in 0.2 mL of carbon tetrachloride. Determine the infrared absorption spectrum of the solution as directed in the liquid film method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 5 g of Tocopherol Calcium Succinate in 30 mL of chloroform, add 10 mL of hydrochloric acid, shake for 10 minutes, then draw off the water layer, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> for calcium salt.

**Absorbance** E_{1%}^{1 cm} (286 nm): 36.0 – 40.0 (10 mg, chloroform, 100 mL).

**Purity**—

(1) Clarity and color of solution—Dissolve 0.10 g of Tocopherol Calcium Succinate in 10 mL of chloroform: the solution is clear, and has no more color than the following control solution.

Control solution: To 0.5 mL of Iron (III) Chloride CS add 0.5 mol/L hydrochloric acid TS to make 100 mL.

(2) Alkalinity—To 0.20 g of Tocopherol Calcium Succinate add 10 mL of diethyl ether, 2 mL of water, 1 drop of phenolphthalein TS and 0.10 mL of 0.1 mol/L hydrochloric acid VS, and shake: no red color develops in the water layer.

(3) Chloride <1.07>—Dissolve 0.10 g of Tocopherol Calcium Succinate in 4 mL of acetic acid (100), add 20 mL of water and 50 mL of diethyl ether, shake thoroughly, and collect the water layer. To the diethyl ether layer add 10 mL of water, shake, and collect the water layer. Combine the water layers, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution in the same manner using 0.60 mL of 0.01 mol/L hydrochloric acid VS in place of Tocopherol Calcium Succinate (not more than 0.212%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Tocopherol Calcium Succinate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tocopherol Calcium Succinate according to Method 3, and perform the test (not more than 2 ppm).

(6) α-Tocopherol—Dissolve 0.10 g of Tocopherol Calcium Succinate in exactly 10 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 50 mg of Tocopherol RS in chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and acetic acid (100) (19:1) at a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of iron (III) chloride hexahydrate in ethanol (99.5) (1 in 500) on the plate, then spray evenly a solution of α-α’-dipyridyl in ethanol (99.5) (1 in 200) on the same plate, and allow to stand for 2 to 3 minutes: the spots from the sample solution corresponding to the spots from the standard solution is not larger than and not more intense than the spots from the standard solution.

**Loss on drying** <2.41>—Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

**Assay**—Weigh accurately about 50 mg each of Tocopherol Calcium Succinate and Tocopherol Succinate RS, previously dried, dissolve in a mixture of ethanol (99.5) and dilute acetic acid (100) (1 in 5) (9:1) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Pipet exactly 20 μL each of the sample solution and standard solution, and perform the test as directed under Liquid
Tocopherol Nicotinate

Vitamin E Nicotinate

dl-α-Tocopherol Nicotinate

Identification (1) Determine the absorption spectrum of a solution of Tocopherol Nicotinate in ethanol (99.5) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tocopherol Nicotinate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Tocopherol Nicotinate, if necessary melt by warming, as directed in the liquid film method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol Nicotinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Tocopherol Nicotinate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tocopherol Nicotinate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Tocopherol Nicotinate in 50 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 7 mL of the sample solution, add ethanol (99.5) to make exactly 200 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than tocopherol nicotinate from the sample solution is not larger than the peak area of tocopherol nicotinate from the standard solution, and the area of a peak which has a retention time 0.8 to 0.9 times that of tocopherol nicotinate from the standard solution is not larger than 4/7 times the peak area of tocopherol nicotinate from the standard solution.

Operating conditions—
Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.
Mobile phase: A mixture of methanol and water (19:1).
Flow rate: Adjust the flow rate so that the retention time of tocopherol nicotinate is about 20 minutes.
Time span of measurement: About 1.5 times as long as the retention time of tocopherol nicotinate beginning after the solvent peak.

System suitability—
Test for required detection: To exactly 1 mL of the sample solution add ethanol (99.5) to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add ethanol (99.5) to make exactly 10 mL. Confirm that the peak area of tocopherol nicotinate obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.
System performance: Dissolve 0.05 g of Tocopherol Nicotinate and 0.25 g of tocopherol in 100 mL of ethanol (99.5). When the procedure is run with 10 μL of this solution under the above operating conditions, tocopherol and tocopherol nicotinate are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tocopherol nicotinate is not more than 2.0%.

Assay Weigh accurately about 50 mg each of Tocopherol Nicotinate and Tocopherol Nicotinate RS, dissolve each in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 5 μL each of the sample solution and the standard solution as directed under Liquid Chromatography @2.01 according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of tocopherol nicotinate of these solutions.

Amount (mg) of $C_{35}H_{53}NO_3 = M_S \times A_T/A_S$

$M_S$: Amount (mg) of Tocopherol Nicotinate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 264 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: Methanol.
Flow rate: Adjust the flow rate so that the retention time of tocopherol nicotinate is about 10 minutes.

System suitability—

System performance: Dissolve 0.05 g of Tocopherol Nicotinate and 0.25 g of tocopherol in 100 mL of ethanol (99.5). When the procedure is run with 5 μL of this solution under the above operating conditions, tocopherol and tocopherol nicotinate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions: the relative standard deviation of the peak areas of tocopherol nicotinate is not more than 0.8%

Containers and storage—

Containers—Tight containers.
Storage—Light-resistant.

Todralazine Hydrochloride Hydrate

Ecarazine Hydrochloride

トドララジン塩酸塩水和物

$C_{11}H_{12}N_4O_2\cdot HCl \cdot H_2O$: 286.71
Ethyl 2-(phthalazin-1-yl)hydrazinecarboxylate monohydrochloride monohydrate
[3778-76-5, anhydride]

Todralazine Hydrochloride Hydrate contains not less than 98.5% of todralazine hydrochloride ($C_{11}H_{12}N_2O_2 \cdot HCl$: 268.70), calculated on the anhydrous basis.

Description Todralazine Hydrochloride Hydrate occurs as white crystals or crystalline powder. It has a slight, characteristic odor, and has a bitter taste.

It is very soluble in formic acid, freely soluble in methanol, soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Todralazine Hydrochloride Hydrate (1 in 200) is between 3.0 and 4.0.

Identification (1) To 2 mL of a solution of Todralazine Hydrochloride Hydrate (1 in 200) add 5 mL of silver nitrate-ammonia TS: the solution becomes turbid, and a black precipitate is formed.

(2) Determine the absorption spectrum of a solution of Todralazine Hydrochloride Hydrate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry @2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the infrared absorption spectrum of Todralazine Hydrochloride Hydrate as directed in the potassium chloride disk method under the Infrared Spectrophotometry @2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Todralazine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests @1.09 (1) for chloride.

Purity (1) Clarity and color of solution—Dissolve 0.30 g of Todralazine Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Sulfate @1.14—Proceed the test with 2.0 g of Todralazine Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.012%).

(3) Heavy metals @1.07—Proceed with 1.0 g of Todralazine Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic @1.11—Proceed the test solution with 1.0 g of Todralazine Hydrochloride Hydrate according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 50 mg of Todralazine Hydrochloride Hydrate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography @2.01 according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of todralazine from the sample solution is not larger than the peak area of todralazine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about...
25°C.

Mobile phase: Dissolve 1.10 g of sodium 1-heptane sulphonate in 1000 mL of diluted methanol (2 in 5). Adjust the pH of the solution to between 3.0 and 3.5 with acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of todralazine is about 8 minutes.

Time span of measurement: About twice as long as the retention time of todralazine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of todralazine obtained from 10 μL of this solution is equivalent to 15 to 25% of that from 10 μL of the standard solution.

System performance: Dissolve 5 mg each of Todralazine Hydrochloride Hydrate and potassium biphthalate in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, phthalic acid and todralazine are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of todralazine is not more than 2.0%.

Water <2.48> 6.0 – 7.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.4> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Todralazine Hydrochloride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 26.87 mg of C_{11}H_{12}N_{4}O_{2}.HCl

Containers and storage Containers—Tight containers.

**Tofisopam**

トフィソパム

C_{22}H_{37}N_{5}O_{4}; 382.45
(5R)-1-(3,4-Dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-4-methyl-5H-2,3-benzodiazepine

Tofisopam, when dried, contains not less than 98.0% of C_{22}H_{36}N_{2}O_{4}.

**Description** Tofisopam occurs as a pale yellowish white, crystalline powder.

It is freely soluble in acetic acid (100), soluble in acetone, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Tofisopam in ethanol (95) (1 in 100) shows no optical rotation.

**Identification** (1) Determine the absorption spectrum of a solution of Tofisopam in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tofisopam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.6> 155 – 159°C

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Tofisopam according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 1.0 g of Tofisopam according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Tofisopam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 25 mL, pipet 1 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, methanol and formic acid (24:12:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.4> Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

Residue on ignition <2.4> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Tofisopam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 38.25 mg of C_{22}H_{36}N_{2}O_{4}.

Containers and storage Containers—Light-resistant.

**Containers and storage** Containers—Tight containers.

*Storage—Light-resistant.*
Tolazamide

トラザミド

\[
\text{C}_{14}\text{H}_{21}\text{N}_{3}\text{O}_{3}\text{S}: 311.40 \\
\text{N-(Azepan-1-ylcarbamoyl)-} \\
4\text{-methylbenzenesulfonamide}
\]

Tolazamide, when dried, contains not less than 97.5% and not more than 102.0% of \( \text{C}_{14}\text{H}_{21}\text{N}_{3}\text{O}_{3}\text{S} \).

**Description** Tolazamide occurs as a white to pale yellow, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in acetone, slightly soluble in ethanol (95) and in \( n \)-butylamine, and practically insoluble in water and in diethyl ether.

Melting point: about 168°C (with decomposition).

**Identification** (1) Dissolve 0.02 g of Tolazamide in 5 mL of water and 1 mL of \( n \)-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. Shake well this solution with 5 mL of chloroform, and allow to stand: a green color develops in the chloroform layer.

(2) Determine the absorption spectrum of a solution of Tolazamide in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tolazamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tolazamide, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry \( <2.25> \), and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Tolazamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** (1) Heavy metals \( <1.07> \)—Produce with 1.0 g of Tolazamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \( <1.11> \)—Prepare the test solution with 1.0 g of Tolazamide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.20 g of Tolazamide in acetone to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 20 mg of \( p \)-toluenesulfonamide in acetone to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.20> \). Spot 10 \( \mu \)L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, cyclohexane and dilute ammonia solution (28) (10 in 11) (200:100:60:23) to a distance of about 12 cm, and air-dry the plate. Heat the plate at 110°C for 10 minutes, and immediately expose to chlorine for 2 minutes. Expose the plate to cold wind until a very pale blue color develops when 1 drop of potassium iodide-starch TS is placed on a site below the starting line on the plate. Spray evenly potassium iodide-starch TS on the plate: the spot from the sample solution corresponding to the spot from the standard solution (2) is not more intense than the spot from the standard solution (2), and the spots other than the principal and above spots from the sample solution are not more intense than the spot from the standard solution (1).

(4) N-Aminohexamethyleneimine—To 0.50 g of Tolazamide add 2.0 mL of acetone, stopper the flask tightly, shake vigorously for 15 minutes. Add 8.0 mL of disodium hydrogenphosphate-citric acid buffer solution, pH 5.4, shake, allow to stand for 15 minutes, and filter. To the filtrate add 1.0 mL of trisodium ferrous pentacyanoamine TS, and shake: the color developing within 30 minutes is not deeper than that of the following control solution.

Control solution: Dissolve 0.125 g of \( N \)-aminohexamethyleneimine in acetone to make exactly 100 mL. Pipet 1 mL of this solution, and add acetone to make exactly 100 mL. To 2.0 mL of this solution add 8.0 mL of disodium hydrogenphosphate-citric acid buffer solution, pH 5.4, shake, and proceed in the same manner.

**Loss on drying** \( <2.47> \) Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** \( <2.48> \) Not more than 0.2% (1 g).

**Assay** Weigh accurately about 30 mg each of Tolazamide and Tolazamide RS, previously dried, dissolve each in exactly 10 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01> \) according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of tolazamide to that of the internal standard, respectively.

\[
M_S: \text{Amount (mg) of C}_{14}\text{H}_{21}\text{N}_{3}\text{O}_{3}\text{S} = M_S \times Q_T/Q_S
\]

**Internal standard solution**—A solution of tolbutamide in ethanol-free chloroform (3 in 2000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of hexane, water-saturated hexane, tetrahydrofuran, ethanol (95) and acetic acid (100) (475:475:20:15:9).
Flow rate: Adjust the flow rate so that the retention time of tolazamide is about 12 minutes.

**System suitability**—
System performance: When the procedure is run with 10 \( \mu \)L of the standard solution under the above operating conditions, the internal standard and tolazamide are eluted in this order with the resolution between these peaks being not
Tolbutamide

トルブタミド

\[
\text{C}_{12}\text{H}_{18}\text{N}_{2}\text{O}_{3}\text{S}: 270.35
\]

\[\text{N-(Butylcarbamoyl)-4-methylbenzenesulfonamide} \quad [64-77-7]\]

Tolbutamide, when dried, contains not less than 99.0% of \(\text{C}_{12}\text{H}_{18}\text{N}_{2}\text{O}_{3}\text{S}\).

**Description** Tolbutamide occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor. It is tasteless.

It is soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

**Identification** (1) Boil 0.2 g of Tolbutamide with 8 mL of diluted sulfuric acid (1 in 3) under a reflux condenser for 30 minutes. Cool the solution in ice water, collect the precipitated crystals, recrystallize from water, and dry at 105°C for 3 hours: the crystals melt \(<2.60\) between 135°C and 139°C.

(2) Render the filtrate obtained in (1) alkaline with about 20 mL of a solution of sodium hydroxide (1 in 5), and heat: an ammonia-like odor is perceptible.

**Melting point** \(<2.60\) 126 – 132°C

**Purity** (1) Acidity—Warm 3.0 g of Tolbutamide with 150 mL of water at 70°C for 5 minutes, allow to stand for 1 hour in ice water, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(2) Chloride \(<1.03\)—To 40 mL of the filtrate obtained in (1) add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate \(<1.14\)—To 40 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.021%).

(4) Heavy metals \(<1.07\)—Proceed with 2.0 g of Tolbutamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Loss on drying** \(<2.41\) Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** \(<2.44\) Not more than 0.1% (1 g).

**Assay** Weigh accurately and powder about 0.5 g of Tolbutamide, previously dried, and dissolve in 30 mL of neutralized ethanol. Add 20 mL of water, and titrate \(<2.50\) with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

\[
\text{Each mL of 0.1 mol/L sodium hydroxide VS} = 27.04 \text{ mg of C}_{12}\text{H}_{18}\text{N}_{2}\text{O}_{3}\text{S}
\]

**Containers and storage** Containers—Well-closed containers.

Tolbutamide Tablets

トルブタミド錠

Tolbutamide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tolbutamide (\(\text{C}_{12}\text{H}_{18}\text{N}_{2}\text{O}_{3}\text{S}: 270.35\)).

**Method of preparation** Prepare as directed under Tablets, with Tolbutamide.

**Identification** Shake a quantity of powdered Tolbutamide Tablets, equivalent to 0.5 g of Tolbutamide according to the labeled amount, with 50 mL of chloroform, filter, and evaporate the filtrate to dryness. Proceed with the residue as directed in the Identification under Tolbutamide.

**Uniformity of dosage units** \(<0.02\) It meets the requirement of the Mass variation test.

**Dissolution** \(<6.10\) When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of phosphate buffer solution, pH 7.4 as the dissolution medium, the dissolution rate in 30 minutes of Tolbutamide Tablets is not less than 80%.

Start the test with 1 tablet of Tolbutamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add water to make exactly \(V\) mL so that each mL contains about 10 \(\mu\)g of tolbutamide (\(\text{C}_{12}\text{H}_{18}\text{N}_{2}\text{O}_{3}\text{S}\)) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Tolbutamide RS, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), using water as the control, and determine the absorbances, \(A_T\) and \(A_S\), at 226 nm.

Dissolution rate (% with respect to the labeled amount of tolbutamide (\(\text{C}_{12}\text{H}_{18}\text{N}_{2}\text{O}_{3}\text{S}\))

\[
M_S = M_X \times A_T / A_S \times V/V \times 1/C \times 18
\]

\(M_S\): Amount (mg) of Tolbutamide RS

\(C\): Labeled amount (mg) of tolbutamide (\(\text{C}_{12}\text{H}_{18}\text{N}_{2}\text{O}_{3}\text{S}\)) in 1 tablet

**Assay** Weigh accurately and powder not less than 20 Tol-
butamide Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of tolbutamide (C₈H₁₈N₂O₄S₃), dissolve in 50 mL of neutralized ethanol, add 25 mL of water, and titrate ≤2.50 with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS = 27.04 mg of C₈H₁₈N₂O₄S₃.

Containers and storage Containers—Well-closed containers.

Tolnaftate
トルナフタート

C₁₉H₁₇NOS: 307.41
O-Naphthalen-2-yl N-methyl-N-(3-methylphenyl)thiocarbamate [2398-96-1]

Tolnaftate, when dried, contains not less than 98.0% of C₁₉H₁₇NOS.

Description Tolnaftate occurs as a white powder. It is odorless.

It is freely soluble in chloroform, sparingly soluble in diethyl ether, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) To 0.2 g of Tolnaftate add 20 mL of potassium hydroxide-ethanol TS and 5 mL of water, and heat under a reflux condenser for 3 hours. After cooling, to 10 mL of this solution add 2 mL of acetic acid (100), and shake with 1 mL of lead (II) acetate TS: a black precipitate is formed.

(2) Determine the absorption spectrum of a solution of Tolnaftate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tolnaftate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tolnaftate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Tolnaftate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 111 – 114°C (after drying).

Purity (1) Heavy metals ≤1.07—Carbonize 1.0 g of Tolnaftate by gentle heating. After cooling, add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat until white fumes are evolved. After cooling, add 2 mL of nitric acid and 0.5 mL of perchloric acid, and heat gradually until white fumes are evolved. Repeat this procedure twice, and heat until white fumes are no longer evolved. Incinerate the residue by igniting between 500°C and 600°C for 1 hour. Proceed according to Method 2, and perform the test with 50 mL of the test solution so obtained. Prepare the control solution as follows: to 11 mL of nitric acid add 1 mL of sulfuric acid, 1 mL of perchloric acid and 2 mL of hydrochloric acid, proceed in the same manner as the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Related substances—Dissolve 0.50 g of Tolnaftate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07b>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 5 minutes, and examine under ultraviolet light (wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 65°C, 3 hours).

Residue on ignition <2.44> Weigh accurately about 2.0 g of Tolnaftate, and carbonize by gradual heating. Moisten the substance with 1 mL of sulfuric acid, heat gradually until white fumes are no longer evolved, and ignite between 450°C and 550°C for about 2 hours to constant mass: the residue is not more than 0.1%.

Assay Weigh accurately about 50 mg of Tolnaftate and Tolnaftate RS, previously dried, dissolve each in 200 mL of methanol by warming in a water bath, cool, and add methanol to make exactly 250 mL. Pipet 5 mL of each of the solutions, to each add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A₁ and A₅, of the sample solution and standard solution at 257 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of C₁₉H₁₇NOS = Mₛ × A₁/A₅

Mₛ: Amount (mg) of Tolnaftate RS

Containers and storage Containers—Tight containers.

Tolnaftate Solution
トルナフタート液

Tolnaftate Solution contains not less than 90.0% and not more than 110.0% of the labeled amount of tolnaftate (C₁₉H₁₇NOS: 307.41).

Method of preparation Prepare as directed under Liquids and Solutions for Cutaneous Application, with Tolnaftate.

Identification (1) Spot 1 drop of Tolnaftate Solution on filter paper. Spray hydrogen hexachloroplatinate (IV)-potas-
about 20 mg of tolnaftate (C₁₉H₁₇NOS), add exactly 4 mL of Tolnaftate Solution, equivalent to Assay value.

25 in chloroform (3 to 200).

rately, weigh accurately about 0.4 g of Tolnaftate RS, previ- ously dried in vacuum at a pressure not exceeding 0.67 kPa for 3 hours, and dissolve in chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add chloroform to make 50 mL, and use this solution as the sample solution. Perform the test with 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and standard solution on a plate of silanized silica gel for liquid chromatography (5 to 10 cm in diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel) is between these peaks being not less than 5.

Internal standard solution—A solution of diphenyl phthalate in chloroform (3 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of tolnaftate is about 14 minutes.

Selection of column: Proceed with 10 µL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and tolnaftate in this order with the resolution between these peaks being not less than 5.

Containers and storage—Containers—Tight containers.

### Tolperisone Hydrochloride

**トルペリゾン塩酸塩**

C₁₆H₂₃NO.HCl: 281.82

(2RS)-2-Methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one monohydrochloride [3644-61-9]

Tolperisone Hydrochloride, when dried, contains not less than 98.5% of C₁₆H₂₃NO.HCl.

**Description**

Tolperisone Hydrochloride occurs as a white, crystalline powder. It has a slight, characteristic odor. It is very soluble in acetic acid (100), freely soluble in water and in ethanol (95), soluble in acetic anhydride, slightly soluble in acetone, and practically insoluble in diethyl ether.

The pH of a solution of Tolperisone Hydrochloride (1 in 20) is between 4.5 and 5.5. It is hygroscopic.

Melting point: 167 – 174°C

**Identification**

1. Dissolve 0.2 g of Tolperisone Hydrochloride in 2 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of sodium hydroxide TS, and heat: a red color develops.

2. To 5 mL of a solution of Tolperisone Hydrochloride (1 in 20) add 2 to 3 drops of iodine TS: a red-brown precipitate is produced.

3. Dissolve 0.5 g of Tolperisone Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and filter. Acidify 5 mL of the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

**Absorbance** <2,24> E₁%<sub>1% cm</sub>: 555 – 585 (after drying, 5 mg, ethanol (95), 500 mL).

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Tolperisone Hydrochloride in 10 mL of water: the solution is clear and colorless.

2. Sulfate <1.14>—Perform the test using 4.0 g of Tolperisone Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.005%).

3. Heavy metals <1.07>—Proceed with 1.0 g of Tolperisone Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

4. Piperidine hydrochloride—Dissolve 0.20 g of Tolperisone Hydrochloride in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of piperidine hydrochloride in water to make exactly 1000 mL, and use this solution as the standard solution. Transfer 5.0 mL each of the sample solution and standard solution to different separators, add 0.1 mL each of a solution of copper (II) sulfate pentahydrate (1 in 20), then add 0.1 mL each of ammonia solution (28) and exactly 10 mL each of a mixture of isooctane and carbon disulfide (3:1), and enantiomer.

C₁₆H₂₃NO.HCl: 281.82

(2RS)-2-Methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one monohydrochloride [3644-61-9]
and shake vigorously for 30 minutes. Immediately after allowing to stand, separate the isoctane-carbon disulfide mixture layer, and dehydrate with anhydrous sodium sulfate. Perform the test with these solutions as directed under Ultra-violet-visible Spectrophotometry \( <2.24> \): the absorbance of the sample solution at 438 nm is not more than that of the standard solution.

**Loss on drying** \( <2.41> \) Not more than 0.5% (1 g, in vacuum, silica gel, 3 hours).

**Residue on ignition** \( <2.44> \) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Tolperisone Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (1:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 28.18 mg \( \text{C}_{14}\text{H}_{24}\text{NO} \cdot \text{HCl} \)

**Containers and storage** Containers—Well-closed containers.

### Tosufloxacin Tosilate Hydrate

\[ \text{C}_{19}\text{H}_{15}\text{F}_{3}\text{N}_{4}\text{O}_{3} \cdot \text{C}_{7}\text{H}_{8}\text{O}_{3}\text{S} : 576.54 \]

- **Description** Tosufloxacin Tosilate Hydrate occurs as a white to pale yellowish white, crystalline powder.
- **Melting point** About 254°C (with decomposition).
- **Identification** (1) Tosufloxacin Tosilate Hydrate shows a light bluish-white fluorescence under ultraviolet light (main wavelength 254 nm).
- (2) Proceed 10 mg of Tosufloxacin Tosilate Hydrate as directed under Oxygen Flask Combustion Method \( <1.09> \), using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution responds to the Qualitative Tests \( <1.09> \) (2) for fluoride.
- (3) Determine the absorption spectrum of a solution of Tosufloxacin Tosilate Hydrate in a mixture of methanol and sodium hydroxide TS (49:1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24> \), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tosufloxacin Tosilate Hydrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.
- (4) Determine the infrared absorption spectrum of Tosufloxacin Tosilate Hydrate as directed in the paste method under Infrared Spectrophotometry \( <2.25> \), and compare the spectrum with the Reference Spectrum or the spectrum of Tosufloxacin Tosilate Hydrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** (1) Chloride \( <1.03> \)—Dissolve 1.0 g of Tosufloxacin Tosilate Hydrate in 40 mL of \( \text{N,N-dimethylformamide} \), and add 6 mL of dilute nitric acid and \( \text{N,N-dimethylformamide} \) to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and \( \text{N,N-dimethylformamide} \) to make 50 mL (not more than 0.007%).
- (2) Heavy metals \( <1.07> \)—Proceed with 1.0 g of Tosufloxacin Tosilate Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
- (3) Arsenic \( <1.11> \)—Prepare the test solution with 1.0 g of Tosufloxacin Tosilate Hydrate according to Method 4, and perform the test under the condition of the ignition temperature being between 750 and 850°C, and add 10 mL of diluted hydrochloric acid to residue (not more than 2 ppm).
- (4) Related substances—Dissolve 10 mg of Tosufloxacin Tosilate Hydrate in 12 mL of mobile phase B, add water to make 25 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add mobile phase A to make exactly 100 mL. Pipet 2 mL of this solution, add mobile phase A to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.07> \) according to the following conditions. Determine each peak area of both solutions by automatic integration method: the area of each peak other than tosylic acid and tosufloxacin obtained from the sample solution is not larger than 3/4 times the peak area of tosufloxacin from the standard solution, and the total area of the peaks other than tosylic acid and tosufloxacin from the sample solution is not larger than 2.5 times the peak area of tosufloxacin from the standard solution.

**Operating conditions**—
- Detector: An ultraviolet absorption photometer (wavelength: 272 nm).
- Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \text{m} \) in particle diameter).
- Column temperature: A constant temperature of about 35°C.
- Mobile phase A: To 300 to 500 mL of water add slowly 100 mL of methanesulfonic acid under ice-cooling, add slowly 100 mL of triethylamine under ice-cooling too, and
add water to make 1000 mL. To 10 mL of this solution add 143 mL of water, 40 mL of acetonitrile and 7 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

Mobile phase B: To 300 to 500 mL of water add slowly 100 mL of methanesulfonic acid under ice-cooling, add slowly 100 mL of triethylamine under ice-cooling too, and add water to make 1000 mL. To 10 mL of this solution add 100 mL of acetonitrile, 83 mL of water and 7 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1 – 16</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>16 – 35</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 0.5 mL per minute.

Time span of measurement: About 5 times as long as the retention time of tosufloxacin.

**System suitability**—
Test for required detectability: Pipet 5 mL of the standard solution, and add mobile phase A to make exactly 20 mL. Confirm that the peak area of tosufloxacin obtained from 20 μL of this solution is equivalent to 18 to 32% of the peak area of tosufloxacin from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of tosufloxacin are less than 10,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tosufloxacin to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

**Tosufloxacin Tosilate Tablets**

Tosufloxacin Tosilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tosufloxacin tosilate hydrate (C₁₉H₁₅F₃N₄O₃.C₇H₈O₃S.H₂O: 594.56).

**Method of preparation**—Prepare as directed under Tablets, with Tosufloxacin Tosilate Hydrate.

**Identification**—To a quantity of powdered Tosufloxacin Tosilate Tablets, equivalent to 75 mg of Tosufloxacin Tosilate Hydrate according to the labeled amount, add 200 mL of a mixture of methanol and sodium hydroxide TS (49:1), shake well, and centrifuge. To 2 mL of the supernatant liquid add 100 mL of a mixture of methanol and sodium hydroxide TS (49:1). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 260 nm and 264 nm, between 341 nm and 345 nm, and between 356 nm and 360 nm.

**Uniformity of dosage units**—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tosufloxacin Tosilate Tablets add V/10 mL of water and shake until the tablet is disintegrated. Add methanol to make exactly V mL so that each mL contains about 1.5 mg of tosufloxacin tosilate hydrate (C₁₉H₁₅F₃N₄O₃.C₇H₈O₃S.H₂O). Shake this solution for 10 minutes, and centrifuge. Pipet 4 mL of the supernatant liquid, and add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.
Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800).

Dissolution 6.10 When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Tosufloxacin Tosilate Tablets is not less than 65%.

Start the test with 1 tablet of Tosufloxacin Tosilate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, to make exactly 10 mL so that each mL contains about 17 μg of Tosufloxacin tosilate hydrate (C19H15F3N4O3.C7H8O3S.H2O) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of Tosufloxacin Tosilate RS (separately determine the water content, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Liquid Chromatography.


dissolve in N,N-dimethylformamide to make exactly 25 mL. Pipet 2 mL of this solution, add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the blank, and determine the absorbances, A1 and A3, at 346 nm.

Dissolution rate (%) with respect to the labeled amount of Tosufloxacin tosilate hydrate (C19H15F3N4O3.C7H8O3S.H2O) to that of the internal standard.

\[
M_S = M_S \times \frac{Q_T}{Q_S} \times \frac{V}{V'} \times 1.031
\]

M_S: Amount (mg) of Tosufloxacin Tosilate RS, calculated on the anhydrous basis

C: Labeled amount (mg) of Tosufloxacin tosilate hydrate (C19H15F3N4O3.C7H8O3S.H2O) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Tosufloxacin Tosilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of Tosufloxacin tosilate hydrate (C19H15F3N4O3.C7H8O3S.H2O), add 10 mL of water and methanol to make exactly 100 mL, shake for 10 minutes, and centrifuge. Pipet 4 mL of the supernatant liquid, add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Tosufloxacin Tosilate RS (separately determine the water content, and use this solution as the standard solution. Perform the test with the 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography, and calculate the ratios, Q_T and Q_S, of the peak area of Tosufloxacin to that of the internal standard.

\[
M_S = M_S \times \frac{Q_T}{Q_S} \times 5 \times 1.031
\]

M_S: Amount (mg) of Tosufloxacin Tosilate RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800).

Operating conditions—Proceed as directed in the operating conditions in the Assay under Tosufloxacin Tosilate Hydrate.

System suitability—Proceed as directed in the system suitability in the Assay under Tosufloxacin Tosilate Hydrate.

Containers and storage Containers—Well-closed containers.

Tranexamic Acid トランネキサム酸

\[
C_9H_{15}NO_2: 157.21
\]

trans-4-(Aminomethyl)cyclohexanecarboxylic acid [1197-18-8]

Tranexamic Acid, when dried, contains not less than 98.0% and not more than 101.0% of C9H15NO2.

Description Tranexamic Acid occurs as white crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification Determine the infrared absorption spectrum of Tranexamic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Tranexamic Acid RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.5> The pH of a solution prepared by dissolving 1.0 g of Tranexamic Acid in 20 mL of water is between 7.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Tranexamic Acid in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.0%>—Perform the test with 1.0 g of Tranexamic Acid. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Heavy metals—Dissolve 2.0 g of Tranexamic Acid in water to make 20 mL, and use this solution as the sample stock solution. To 12 mL of the sample stock solution add 2 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, mix, add 1.2 mL of thioacetamide TS, mix immediately, and use this solution as the sample solution. Sepa-
rately, proceed in the same manner as above with a mixture of 1 mL of Standard Lead Solution, 2 mL of the sample stock solution and 9 mL of water, and use the solution so obtained as the standard solution. Separately, proceed in the same manner with a mixture of 10 mL of water and 2 mL of the sample stock solution, and use the solution so obtained as the control solution. Conform that the color of the standard solution is slightly darker than that of the control solution. Compare the sample solution and the standard solution 2 minutes after they are prepared: the color of the sample solution is not more intense than that of the standard solution (not more than 10 ppm).

(4) Arsenic $<1.11>$ — Prepare the test solution by dissolving 1.0 g of Tranexamic Acid in 10 mL of water, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Tranexamic Acid in 20 mL of water, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and determine each peak area by the automatic integration method: the area multiplied by relative response factor 1.2 of the peak, having the relative retention time of about 1.5 with respect to tranexamic acid obtained from sample solution, is not larger than 2/5 times the peak area of tranexamic acid from the standard solution, and the area of the peak, having the relative retention time of about 2.1, is not larger than 1/5 times the peak area of tranexamic acid from the standard solution. The area of each peak other than tranexamic acid and other than the peaks mentioned above is not larger than 5. The total area of the peaks other than tranexamic acid are eluted in this order with the resolution between these peaks being not less than 5.

System suitability—

System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with 20 $\mu$L of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 0.6%.

Containers and storage—Containers—Well-closed containers.

Tranexamic Acid Capsules

トラネキサム酸カプセル

Tranexamic Acid Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid (C$_8$H$_{15}$NO$_2$: 157.21).

Method of preparation—Prepare as directed under Capsules, with Tranexamic Acid.

Identification—Take an amount of powdered contents of Tranexamic Acid Capsules, equivalent to 0.5 g of Tranexamic Acid according to the labeled amount, add 50 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and heat for 3 minutes: a dark purple color develops.

Uniformity of dosage units $<6.02>$ — It meets the requirement.
of the Mass variation test.

**Dissolution**<sup>6.10</sup> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Tranexamic Acid Capsules is not less than 80%.

Start the test with 1 tablet of Tranexamic Acid Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly ν mL so that each mL contains about 0.28 mg of tranexamic acid (C₈H₁₅NO₂) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Tranexamic Acid RS, previously dried at 105°C for 2 hours, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A<sub>T</sub> and A<sub>S</sub>, of tranexamic acid.

\[
\text{Dissolution rate (％) with respect to the labeled amount of tranexamic acid (C₈H₁₅NO₂) = } \frac{M_S \times A_T}{A_S} \times \frac{V}{V} \times \frac{1}{C} \times 900
\]

M<sub>S</sub>: Amount (mg) of Tranexamic Acid RS
C: Labeled amount (mg) of tranexamic acid (C₈H₁₅NO₂) in 1 capsule

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 11.0 g of anhydrous sodium dihydrogen phosphate in 500 mL of water, and add 10 mL of triethylamine and 1.4 g of sodium lauryl sulfate. Adjust the pH to 2.5 with phosphoric acid, add water to make 600 mL, and add 400 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 8 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of tranexamic acid are not less than 4000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 2.0%.

**Assay** Weigh accurately the mass of the contents of not less than 20 Tranexamic Acid Capsules, and powder. Weigh accurately an amount of the powder, equivalent to about 0.1 g of tranexamic acid (C₈H₁₅NO₂), add 30 mL of water, shake well, and add water to make exactly 50 mL. Centrifuge, filter the supernatant liquid through a membrane filter with pore size of not more than 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid RS, previously dried at 105°C for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A<sub>T</sub> and A<sub>S</sub>, of tranexamic acid.

\[
\text{Amount (mg) of tranexamic acid (C₈H₁₅NO₂) = } M_S \times \frac{A_T}{A_S} \times 2
\]

M<sub>S</sub>: Amount (mg) of Tranexamic Acid RS

**Operating conditions**—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.
Column temperature: A constant temperature of about 35°C.
Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 minutes.

**System suitability**—

System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with 30 μL of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

**Tranexamic Acid Injection**

**Tranexamic Acid Injection** トランニキサム酸注射液

Tranexamic Acid Injection is an aqueous injection. It contains not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid (C₈H₁₅NO₂: 157.21).

**Method of preparation** Prepare as directed under Injections, with Tranexamic Acid.

**Description** Tranexamic Acid Injection is a clear and colorless liquid.

**Identification** To a volume of Tranexamic Acid Injection, equivalent to 50 mg of Tranexamic Acid according to the labeled amount, add water to make 5 mL, add 1 mL of ninhydrin TS, and heat: a dark purple color develops.

**pH**<sup>2.54</sup>: 7.0 – 8.0

**Bacterial endotoxins**<sup>4.01</sup>: Not more than 0.12 EU/mg.

**Extractable volume**<sup>6.05</sup>: It meets the requirement.

**Foreign insoluble matter**<sup>6.06</sup>: Perform the test according to Method 1: it meets the requirement.
Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take accurately a volume of Tranexamic Acid Injection, equivalent to about 0.1 g of tranexamic acid (C₈H₁₅NO₂), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid RS, previously dried at 105 °C for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, A₁ and A₅, of tranexamic acid.

\[
\text{Amount (mg) of tranexamic acid (C₈H₁₅NO₂)} = M₅ \times \frac{A₁}{A₅} \times \frac{1}{2}
\]

M₅: Amount (mg) of Tranexamic Acid RS

Operating conditions—
Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

Column temperature: A constant temperature of about 35°C.

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 minutes.

System suitability—
System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with 30 μL of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

**Tranexamic Acid Tablets**

トラネキサム酸錠

Tranexamic Acid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid (C₈H₁₅NO₂: 157.21).

**Method of preparation** Prepare as directed under Tablets, with Tranexamic Acid.

**Identification** To an amount of powdered Tranexamic Acid Tablets, equivalent to 0.5 g of Tranexamic Acid according to the labeled amount, add 50 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and heat for 3 minutes: a dark purple color develops.

**Uniformity of dosage units <6.02>** It meets the requirement of the Mass variation test.

Dissolution Being specified separately.

**Assay** Weigh accurately the mass of not less than 20 Tranexamic Acid Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 5 g of tranexamic acid (C₈H₁₅NO₂), add 150 mL of water, disintegrate the tablets completely with the aid of ultrasonic waves, and add water to make exactly 200 mL. Centrifuge, pipet 4 mL of the supernatant liquid, and add water to make exactly 50 mL. Filter through a membrane filter with pore size of not more than 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, A₁ and A₅, of tranexamic acid.

\[
\text{Amount (mg) of tranexamic acid (C₈H₁₅NO₂)} = M₅ \times \frac{A₁}{A₅} \times \frac{1}{100}
\]

M₅: Amount (mg) of Tranexamic Acid RS

Operating conditions—
Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

Column temperature: A constant temperature of about 35°C.

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 minutes.

System suitability—
System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with 30 μL of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

**Trapidil**

トラピジル

\[
C_{10}H_{15}N_5: 205.26
\]

7-Diethylamino-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine [15421-84-8]

Trapidil, when dried, contains not less than 98.5% of C₁₀H₁₅N₅.

**Description** Trapidil occurs as a white to pale yellowish
white, crystalline powder.

It is very soluble in water and in methanol, freely soluble in ethanol (95), in acetic anhydride and in acetic acid (100), and sparingly soluble in diethyl ether.

The pH of a solution of Trapidil (1 in 100) is between 6.5 and 7.5.

**Identification**

1. **To 5 mL of a solution of Trapidil (1 in 50) add 3 drops of Dragendorff’s TS**; an orange color develops.

2. **Determine the absorption spectrum of a solution of Trapidil (1 in 125,000)** as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum; both spectra exhibit similar intensities of absorption at the same wavelengths.

**Absorbance** <2.24> \(E_1^1_{10} (307 \text{ nm}): 860 - 892\) (after drying, 20 mg, water, 2500 mL).

**Melting point** <2.60> 101 – 105°C

**Purity**

1. **Clarity and color of solution**—Dissolve 2.5 g of Trapidil in 10 mL of water: the solution is clear and colorless to pale yellow.

2. **Chloride** <1.07>—Perform the test with 0.5 g of Trapidil. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

3. **Ammonium**—Place 0.05 g of Trapidil in a glass-stoppered conical flask, thoroughly moisten with 10 drops of sodium hydroxide TS, and stopper the flask. Allow it to stand at 37°C for 15 minutes: the gas evolved does not change moistened red litmus paper to blue.

4. **Heavy metals** <1.07>—Dissolve 1.0 g of Trapidil in 40 mL of water, and add 1.5 mL of dilute hydrochloric acid, 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

5. **Arsenic** <1.11>—Prepare the test solution with 1.0 g of Trapidil according to Method 1, and perform the test (not more than 2 ppm).

6. **Related substances**—Dissolve 0.10 g of Trapidil in 4 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 20 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and acetic acid (100) (85:13:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 60 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.47> Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Trapidil, previously dried, dissolve in 20 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 20.53 mg of C\(_{6}\)H\(_{5}\)N\(_{3}\)

**Containers and storage** Containers—Tight containers.

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**Trehalose Hydrate**

トレハロース水和物

\(\text{C}_{12}\text{H}_{22}\text{O}_{11}\cdot\text{H}_{2}\text{O} \quad 378.33\)

\(\alpha\)-D-Glucopyranosyl \(\alpha\)-D-glucopyranoside dihydrate [6138-23-4]

Trehalose Hydrate contains not less than 98.0% and not more than 101.0% of trehalose (C\(_{12}\)H\(_{22}\)O\(_{11}\): 342.30), calculated on the anhydrous basis.

**Description** Trehalose Hydrate occurs as white crystals or a white crystalline powder.

It is freely soluble in water, and slightly soluble in methanol and in ethanol (99.5).

**Identification**

1. **To 1 mL of a solution of Trehalose Hydrate (2 in 5) add 5 – 6 drops of a solution of 1-naphthol in ethanol (95) (1 in 20), shake thoroughly, and add gently 2 mL of sulfuric acid: a purple color appears at the zone of contact.

2. **Mix 2 mL of a solution of Trehalose Hydrate (1 in 25) with 1 mL of dilute hydrochloric acid, and allow standing for 20 minutes at room temperature. Then add 4 mL of sodium hydroxide TS and 2 mL of a solution of glycine (1 in 25), and heat in a water bath for 10 minutes: no brown color appears.

3. **Determine the infrared absorption spectrum of Trehalose Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Trehalose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> \([\alpha]_{D}^{20} +197 \pm 201°\) (10 g, calculated on the anhydrous basis, water, 100 mL, 100 mm).

**pH** <2.54> The pH of a solution of 1 g of Trehalose Hydrate in 10 mL of water is between 4.5 and 6.5.

**Purity**

1. **Chloride** <1.07>—Perform the test with 2.0 g of Trehalose Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

2. **Sulfate** <1.14>—Perform the test with 2.0 g of Trehalose Hydrate. Prepare the control solution with 1.0 mL of
0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals CL1.07>—Proceed with 5.0 g of Trehalose Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(4) Related substances—Dissolve 0.5 g of Trehalose Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography CL2.01> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks which are eluted before the peak of trehalose and the total area of the peaks which are eluted after the peak of trehalose obtained from the sample solution are both not larger than 1/2 times the peak area of trehalose from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions under the Assay.

Time span of measurement: About 2 times as long as the retention time of trehalose.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add water to make exactly 10 mL. Confirm that the peak area of trehalose obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: To exactly 5 mL of the standard solution add water to make exactly 10 mL. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of trehalose is not more than 1.0%.

(5) Dextrin, soluble starch, and sulfite—Dissolve 1.0 g of Trehalose Hydrate in 10 mL of water and add 1 drop of iodine TS: a yellow color appears, which is changed to blue on addition of 1 drop of starch TS.

(6) Nitrogen—Perform the test with accurately weighed Trehalose Hydrate of about 5 g as directed under Nitrogen Determination CL1.08>, using 30 mL of sulfuric acid for the degradation and adding 45 mL of sodium hydroxide solution (2 in 5): the amount of nitrogen (N: 14.01) is not more than 0.005%.

Water CL2.04> not less than 9.0% and not more than 11.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition CL2.44> Not more than 0.1% (2 g).

Assay Weigh accurately about 0.2 g each of Trehalose Hydrate and Trehalose RS (separately determine the water CL2.48> in the same manner as Trehalose Hydrate), dissolve each in 6 mL of water, add exactly 2 mL each of the internal standard solution, add water to make them 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography CL2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of trehalose to that of the internal standard.

Amount (mg) of trehalose (C_{12}H_{22}O_{11}) = M_S × Q_T/Q_S

M_S: Amount (mg) of Trehalose RS, calculated on the anhydrous basis

Internal standard solution—A solution of glycerin (1 in 10).

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column 8 mm in inside diameter and 30 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography consist of styrene-divinylbenzene copolymer carrying sulfonic acid groups (6 μm in particle diameter).

Column temperature: A constant temperature of about 80°C.

Mobile phase: Water.

Flow rate: Adjust the flow rate so that the retention time of trehalose is about 15 minutes.

System suitability—

System performance: Dissolve 0.1 g each of maltotriose and glucose in 10 mL of the standard solution, add 1 mL of the internal standard solution, and add water to make 20 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, maltotriose, trehalose, glucose and the internal standard are eluted in this order, and the resolution between the peaks of maltotriose and trehalose is not less than 1.5, the resolution between the peaks of trehalose and glucose is not less than 4, and the resolution between the peaks of glucose and the internal standard is not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trehalose to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

**Trepibutone**

トレピブトン

C_{16}H_{22}O_{6}: 310.34

4-Oxo-4-(2,4,5-triethoxyphenyl)butanoic acid [41826-92-0]

Trepibutone, when dried, contains not less than 98.5% of C_{16}H_{22}O_{6}.

Description Trepibutone occurs as white to yellowish white crystals or crystalline powder. It is odorless, and is tasteless or has a slight, characteristic aftertaste.

It is soluble in acetone, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Trepibutone in diluted dilute sodium hydroxide TS (1 in 10) (1 in 100,000) as directed under Ultraviolet-
Triamcinolone

Description

Triamcinolone occurs as a white, crystalline powder. It is odorless.

It is freely soluble in N,N-dimethylformamide, slightly soluble in methanol, in ethanol (95%) and in acetone, and practically insoluble in water, in 2-propanol and in diethyl ether.

Melting point: about 264°C (with decomposition).

Identification

(1) Dissolve 1 mg of Triamcinolone in 6 mL of ethanol (95%), add 5 mL of 2,6-di-tert-butylcresol TS and 5 mL of sodium hydroxide TS, and heat on a water bath for 30 minutes under a reflux condenser: a red-purple color develops.

(2) Add 5 mL of water and 1 mL of Fehling's TS to 0.01 g of Triamcinolone, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Triamcinolone as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution responds to the Qualitative Tests <1.09> for fluoride.

(4) Determine the infrared absorption spectrum of Triamcinolone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Triamcinolone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 0.1 g each of Triamcinolone and Triamcinolone RS in 7 mL of a mixture of 2-propanol and water (2:1), respectively, by warming. Allow the solutions to cool in ice to effect crystals, filter, then wash the formed crystals with two 10-mL portions of water, and repeat the test on the dried crystals.

Optical rotation <2.49> [α]D20: +65° – +71° (after drying, 0.1 g, N,N-dimethylformamide, 10 mL, 100 mm).

Purity

Heavy metals <1.07>—Proceed with 0.5 g of Triamcinolone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Identification

(1) Dissolve 1 mg of Triamcinolone in 6 mL of ethanol (95%), add 5 mL of 2,6-di-tert-butylcresol TS and 5 mL of sodium hydroxide TS, and heat on a water bath for 30 minutes under a reflux condenser: a red-purple color develops.

(2) Add 5 mL of water and 1 mL of Fehling's TS to 0.01 g of Triamcinolone, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Triamcinolone as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution responds to the Qualitative Tests <1.09> for fluoride.

(4) Determine the infrared absorption spectrum of Triamcinolone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Triamcinolone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 0.1 g each of Triamcinolone and Triamcinolone RS in 7 mL of a mixture of 2-propanol and water (2:1), respectively, by warming. Allow the solutions to cool in ice to effect crystals, filter, then wash the formed crystals with two 10-mL portions of water, and repeat the test on the dried crystals.

Optical rotation <2.49> [α]D20: +65° – +71° (after drying, 0.1 g, N,N-dimethylformamide, 10 mL, 100 mm).

Purity

Heavy metals <1.07>—Proceed with 0.5 g of Triamcinolone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 30 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay

Weigh accurately about 0.5 g of Trebutinone, previously dried, dissolve in 50 mL of ethanol (95%), add 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 31.03 mg of C12H22O6

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.
Liquid Chromatography

Sample solution and standard solution as directed under respectively.

height of triamcinolone to that of the internal standard, nol (1 in 1000) to make 100 mL.

Containers and storage

Containers—Tight containers.

Internal standard solution—Dissolve 15 mg of methyl par-hydroxybenzoate in a solution of L-ascorbic acid in methanol (1 in 1000) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, add a solution of L-ascorbic acid in methanol (1 in 1000) to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Qt and Qs, of the peak height of triamcinolone to that of the internal standard, respectively.

Amount (mg) of C21H27FO6 = Ms × Qt/Qs

Ms: Amount (mg) of Triamcinolone RS

Internal standard solution—Dissolve 15 mg of methyl par-hydroxybenzoate in a solution of L-ascorbic acid in methanol (1 in 1000) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:1).

Flow rate: Adjust the flow rate so that the retention time of triamcinolone is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, triamcinolone and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of triamcinolone to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Triamcinolone Acetonide

スリアミシノロンアセトニド

C24H31FO6: 434.50

9-Fluoro-11β,21-dihydroxy-16α,17-β-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione [76-25-5]

Triamcinolone Acetonide, when dried, contains not less than 97.0% and not more than 103.0% of C21H27FO6.

Description Triamcinolone Acetonide occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in ethanol (99.5), in acetone, and in 1,4-dioxane, slightly soluble in methanol and in ethanol (95), and practically insoluble in water and in diethyl ether.

Melting point: about 290°C (with decomposition).

Identification (1) Dissolve 2 mg of Triamcinolone Acetonide in 40 mL of ethanol (95), add 5 mL of 2,6-di-tert-butylcresol TS and 5 mL of sodium hydroxide TS, and heat on a water bath under a reflux condenser for 20 minutes: a green color develops.

(2) Add 5 mL of water and 1 mL of Fehling’s TS to 0.01 g of Triamcinolone Acetonide, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Triamcinolone Acetonide as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution responds to the Qualitative Tests <1.09> for fluoride.

(4) Determine the absorption spectrum of a solution of Triamcinolone Acetonide in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Triamcinolone Acetonide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the infrared absorption spectrum of Triamcinolone Acetonide, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Triamcinolone Acetonide RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 0.1 g each of Triamcinolone Acetonide and Triamcinolone Acetonide RS in 20 mL of ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the dried residue.

Optical rotation <2.49> [α]20D: +100 to +107° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 0.5 g of
Triamcinolone Acetonide according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 40 mg of Triamcinolone Acetonide in 4 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (93:7) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.44> Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.2% (0.5 g, platinum crucible).

**Assay** Dissolve about 20 mg each of Triamcinolone Acetonide and Triamcinolone Acetonide RS, previously dried and accurately weighed, in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL each of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 20 \( \mu L \) each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak height of triamcinolone acetonide to that of the internal standard, respectively.

\[
M_S = \frac{M_T}{Q_T/Q_S}
\]

**Internal standard solution**—A solution of prednisolone in methanol (1 in 5000).

**Operating conditions**—
- Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with octadecylsilylated silica gel (10 \( \mu m \) in particle diameter).
- Column temperature: A constant temperature of about 98.5°C, 3 hours.
- Mobile phase: A mixture of water and acetonitrile (3:1).
- Flow rate: Adjust the flow rate so that the retention time of triamcinolone acetonide is about 13 minutes.

**System suitability**—
- System performance: When the procedure is run with 10 \( \mu L \) of the standard solution under the above operating conditions, the internal standard and triamcinolone acetonide are eluted in this order with the resolution between these peaks being not less than 6.
- System repeatability: When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of triamcinolone acetonide to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

**Storage**—Light-resistant.

**Triamterene**

トリアムテレン

C\(_{12}\)H\(_{11}\)N\(_{7}\): 253.26
6-Phenylpteridine-2,4,7-triamine [396-01-0]

Triamterene, when dried, contains not less than 98.5% of C\(_{12}\)H\(_{11}\)N\(_{7}\).

**Description** Triamterene occurs as a yellow, crystalline powder. It is odorless, and tasteless.

It is sparingly soluble in dimethylsulfoxide, very slightly soluble in acetic acid (100), and practically insoluble in water, in ethanol (95), and in diethyl ether.

It dissolves in nitric acid and in sulfuric acid, but does not dissolve in dilute nitric acid, in dilute sulfuric acid and in dilute hydrochloric acid.

**Identification** (1) To 0.01 g of Triamterene add 10 mL of water, heat, and filter after cooling: the filtrate shows a purple fluorescence. To 2 mL of the filtrate add 0.5 mL of hydrochloric acid: the fluorescence disappears.

(2) The filtrate obtained in (1) responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) Dissolve 0.01 g of Triamterene in 100 mL of acetic acid (100), and add 10 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Triamterene according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Proceed with the test solution with 1.0 g of Triamterene according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Triamterene in 20 mL of dimethylsulfoxide. To 2 mL of this solution add methanol to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28) and methanol (9:1:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.
Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.10% (1 g).

Assay Weigh accurately about 0.15 g of Triamterene, previously dried, and dissolve in 100 mL of acetic acid (100) by warming. Titrate <2.50> with 0.05 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 12.66 mg of C₁₂H₁₇N₂.

Containers and storage Containers—Well-closed containers.

### Trichlormethiazide

トリクロルメチアジド

C₈H₈Cl₃N₃O₄S₂: 380.66

(3RS)-6-Chloro-3-dichloromethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [133-67-5]

Trichlormethiazide, when dried, contains not less than 97.5% and not more than 102.0% of C₈H₈Cl₃N₃O₄S₂.

Description Trichlormethiazide occurs as a white powder.

It is freely soluble in N,N-dimethylformamide and in acetone, slightly soluble in acetonitrile and in ethanol (95), and practically insoluble in water.

A solution of Trichlormethiazide in acetone (1 in 50) shows no optical rotation.

Melting point: about 270°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Trichlormethiazide in ethanol (95) (in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Trichlormethiazide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trichlormethiazide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Trichlormethiazide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Trichlormethiazide as directed under Flame Coloration Test <1.04> (2): a green color appears.

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Trichlormethiazide in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetic acid, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.036%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Trichlormethiazide in 30 mL of acetone, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.005 mol/L sulfuric acid VS add 30 mL of acetic acid, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Trichlormethiazide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 0.6 g of Trichlormethiazide according to Method 5, using 20 mL of N,N-dimethylformamide, and perform the test (not more than 3.3 ppm).

(5) Related substances—Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile, and use the solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of related substances by the area percentage method: the amount of 4-amino-6-chlorobenzene-1,3-disulfonamide, having the relative retention time of about 0.3 with respect to trichlormethiazide, is not more than 2.0%, and the total amount of the related substances is not more than 2.5%.

Operating conditions—Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Mobile phase B: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (3:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>100</td>
<td>0</td>
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<tr>
<td>10 – 20</td>
<td>100 → 0</td>
<td>0 → 100</td>
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</tbody>
</table>

Flow rate: 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of trichlormethiazide beginning after the solvent peak.

System suitability—Test for required detectability: To exactly 1 mL of the sample solution add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 20 mL. Confirm that the
peak area of trichlormethiazide obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that from 10 μL of the solution for system suitability test.

System performance: To 5 mL of the solution for system suitability test add 5 mL of water, and warm in a water bath at 60°C for 30 minutes. When the procedure is run with 10 μL of this solution, after cooling, under the above operating conditions, 4-amino-6-chlorobenzene-1,3-disulfonamide and trichlormethiazide are eluted in this order, the relative retention time of 4-amino-6-chlorobenzene-1,3-disulfonamide to trichlormethiazide is about 0.3, and the number of theoretical plates and the symmetry factor of the peak of trichlormethiazide are not less than 5000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 20%.

Loss on drying <2.4% Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.4% Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg of Trichlormethiazide and Trichlormethiazide RS, previously dried, and dissolve separately in exactly 20 mL of the internal standard solution. To 1 mL of these solutions add acetonitrile to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the means, Q1 and Q2, of the peak area of trichlormethiazide to that of the internal standard.

\[ M_5 = \frac{m_{5}}{Q_1/Q_2} \]

Internal standard solution—A solution of 3-nitrophenol in acetonitrile (1 in 800).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Flow rate: Adjust the flow rate so that the retention time of trichlormethiazide is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and trichlormethiazide are eluted in this order with the resolution between these peaks not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trichlormethiazide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Trichlormethiazide Tablets

Trichlormethiazide Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of trichlormethiazide (C₈H₈Cl₃N₃O₄S₂: 380.66).

Method of preparation Prepare as directed under Tablets, with Trichlormethiazide.

Identification To an amount of pulverized Trichlormethiazide Tablets, equivalent to 4 mg of Trichlormethiazide according to the labeled amount, add 10 mL of acetonitrile, shake vigorously for 5 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 4 mg of Trichlormethiazide RS in 10 mL of acetonitrile, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and methanol (10:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spots from the sample solution and the standard solution show the same Rf value.

Purity Related substances—Pulverize a suitable amount of Trichlormethiazide Tablets in an agate mortar. Take an amount of pulverized Trichlormethiazide tablets, equivalent to 4 mg of Trichlormethiazide according to the labeled amount, add 20 mL of acetone, shake vigorously for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of each related substance by the area percentage method: the amount of 4-amino-6-chlorobenzene-1,3-disulfonamide, having the relative retention time of about 0.3 with respect to trichlormethiazide, is not more than 4.0%, and the total amount of the peaks other than trichlormethiazide is not more than 5.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Mobile phase B: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (3:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.
Flow rate: 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of trichlormethiazide beginning after the solvent peak.

**System suitability**—

Test for required detectability: Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile. To 1 mL of this solution add acetonitrile to make 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of trichlormethiazide obtained from 10 μL of this solution is equivalent to about 3.5 to 6.5% of that obtained from 10 μL of the solution for system suitability test.

System performance: To 5 mL of the solution for system suitability test add 5 mL of water, and warm in a water bath of 60°C for 30 minutes. When the procedure is run with 10 μL of this solution, after cooling, under the above operating conditions, 4-amino-6-chlorobenzene-1,3-disulfonamide and trichlormethiazide are eluted in this order, the relative retention time of 4-amino-6-chlorobenzene-1,3-disulfonamide to trichlormethiazide is about 0.3, and the number of theoretical plates and the symmetry factor of the peak of trichlormethiazide are not less than 5000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

**Uniformity of dosage units**<6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Trichlormethiazide Tablets add 5 mL of diluted phosphoric acid (1 in 50) to disintegrate. Add exactly an amount of the internal standard solution, equivalent to 10 mL per 2 mg of trichlormethiazide \((C_8H_8Cl_3N_3O_4S_2)\), add acetonitrile to make 25 mL, shake vigorously for 15 minutes, and centrifuge. To an amount of the supernatant liquid add the mobile phase to make a solution so that it contains about 40 μg of trichlormethiazide \((C_8H_8Cl_3N_3O_4S_2)\) in each mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Trichlormethiazide RS, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 200 mL. Pipet 2 mL of this solution, add diluted phosphoric acid (1 in 50) to make exactly 200 mL, and use this solution as the standard solution. Per- form the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \(A_{ts}\) and \(A_{s}\), of trichlormethiazide obtained with the sample solution and standard solution, and the area, \(A_{tr}\), of the peak, having the relative retention time of about 0.3 to trichlormethiazide, obtained with the sample solution.

Dissolution rate (%) with respect to the labeled amount of trichlormethiazide \((C_8H_7Cl_3N_3O_4S_2)\) = \(M_S \times (A_{ts} + 0.95A_{tr})/A_S \times V/V \times 1/C \times 9/2\)

\(M_S\): Amount (mg) of Trichlormethiazide RS
\(C\): Labeled amount (mg) of trichlormethiazide \((C_8H_7Cl_3N_3O_4S_2)\) in 1 tablet

**Operating conditions**—

Proceed as directed in the operating conditions in the Assay.

**System suitability**—

System performance: Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile. To 1 mL of this solution add acetonitrile to make 50 mL. To 5 mL of this solution add 5 mL of water, and heat at 60°C in a water bath for 30 minutes. After cooling, when the procedure is run with 10 μL of this solution under the above operating conditions, 4-amino-6-chlorobenzene-1,3-disulfonamide and trichlormethiazide are eluted in this order, the relative retention time of 4-amino-6-chlorobenzene-1,3-disulfonamide to trichlormethiazide is about 0.3, and the number of theoretical plates and the symmetry factor of the peak of trichlormethiazide are not less than 5000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

**Assay** Weigh accurately the mass of not less than 20 Trichlormethiazide Tablets, and pulverize the tablets in

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</table>
an agate mortar. Weigh accurately an amount of the powder, equivalent to about 2 mg of trichlormethiazide (C₈H₇Cl₂N₂O₄S₂), add 5 mL of diluted phosphoric acid (1 in 50) and exactly 10 mL of the internal standard solution, add 10 mL of acetonitrile, shake vigorously for 15 minutes, and centrifuge. To 2 mL of the supernatant liquid add 2 mL of the mobile phase, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Trichlormethiazide RS, previously dried at 105 °C for 3 hours, and dissolve in acetonitrile to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard and 5 mL of diluted phosphoric acid (1 in 50), and use this solution as the standard solution. Perform the test with 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of trichlormethiazide to that of the internal standard.

\[
M_5 = \frac{M_S \times Q_1}{Q_2} 	imes \frac{1}{20}
\]

where \( M_5 \) is the amount (mg) of trichlormethiazide RS.

**Internal standard solution**—A solution of 3-nitrophenol in acetonitrile (1 in 5000).

**Operating conditions**—

- **Detector**: An ultraviolet absorption photometer (wavelength: 268 nm).
- **Column**: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature**: A constant temperature of about 25°C.
- **Mobile phase**: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).
- **Flow rate**: Adjust the flow rate so that the retention time of trichlormethiazide is about 8 minutes.

**System suitability**—

- **System performance**: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and trichlormethiazide are eluted in this order with the resolution between these peaks being not less than 2.0.
- **System repeatability**: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trichlormethiazide to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

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**Trichomycin**

Trichomycin A
33-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-17-[6-(4-aminophenyl)-4-hydroxy-1-methyl-6-oxohexyl]-1,3,5,9,11,37-hexahydroxy-18-methyl-13,15-dioxo-16,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid
[12698-99-6]

Trichomycin B
33-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-17-[6-(4-aminophenyl)-4-hydroxy-1-methyl-6-oxohexyl]-1,3,5,7,9,37-hexahydroxy-18-methyl-13,15-dioxo-16,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid
[12699-00-2]

Trichomycin is a mixture of polyene macrolide substances having antifungal and antiprotozoal activities produced by the growth of *Streptomyces hachijoensis*.

It contains not less than 7000 Units per mg, calculated on the dried basis. The potency of Trichomycin is expressed as unit based on the amount of trichomycin. One unit of Trichomycin is equivalent to 0.05 μg of trichomycin.

**Description**—Trichomycin occurs as a yellow to yellow-brown powder.

- It is practically insoluble in water, in ethanol (99.5) and in tetrahydrofuran.
- It dissolves in dilute sodium hydroxide TS.
- It is hygroscopic.

**Identification** (1) To 2 mg of Trichomycin add 2 mL of sulfuric acid: a blue color appears, and the color is changed to a blue-purple after allowing to stand.

(2) Dissolve 1 mg of Trichomycin in 50 mL of a solution of sodium hydroxide (1 in 200). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 359 nm and 365 nm, between 378 nm and 384 nm, and between 400 nm and 406 nm.

**Content ratio of the active principle**—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve about 10 mg of Trichomycin in 50 mL of a mixture of tetrahydrofuran for liquid chromatography and water (3:1), and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amount of trichomycin A and trichomycin B by the area percentage.
method: the amount of trichomycin A is between 20% and 40%, and that of trichomycin B is between 15% and 25%. The relative retention time of trichomycin B with respect to trichomycin A is about 1.2.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 360 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in a mixture of 600 mL of water and 400 mL of acetonitrile for liquid chromatography.
Flow rate: Adjust the flow rate so that the retention time of trichomycin A is about 8 minutes.
Time span of measurement: About 4 times as long as the retention time of trichomycin A.
System suitability—
Test for required detectability: Measure 5 mL of the sample solution, add a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 30 mL. Confirm that the peak area of trichomycin A is about 1.2.
The relative retention time of trichomycin B with respect to trichomycin A is about 1.2. The relative standard deviation of the peak area of trichomycin A is not more than 2.0.
System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, trichomycin A and trichomycin B are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trichomycin is not more than 2.0%.

Containers and storage—Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Triclofos Sodium

Monosodium Trichloroethyl Phosphate

トリクロホスナトリウム

C₃H₃Cl₃NaO₄P: 251.37
Monosodium 2,2,2-trichloroethyl monohydrogenphosphate [7246-20-0]

Triclofos Sodium, when dried, contains not less than 97.0% and not more than 102.0% of C₃H₃Cl₃NaO₄P, and not less than 41.0% and not more than 43.2% of chlorine (Cl: 35.45).

Description
Triclofos Sodium is a white, crystalline powder.
It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.
It is hygroscopic.

Identification
(1) Determine the infrared absorption spectrum of Triclofos Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 0.5 g of Triclofos Sodium add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite further over a flame. Dissolve the residue in 5 mL of water, and filter it necessary: the filtrate responds to Qualitative Tests <1.09> for sodium salt.

(3) To 0.1 g of Triclofos Sodium add 1 g of anhydrous sodium carbonate, and heat for 10 minutes. After cooling, dissolve the residue in 40 mL of water, filter if necessary, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.
Determine the absorbances, not more than 1.0 solution at 740 nm: the content of the free phosphoric acid is a blank determination.

\[ \text{pH} < 2.54 \] Dissolve 1.0 g of Triclofos Sodium in 50 mL of water: the pH of this solution is between 3.0 and 4.5.

**Purity**
1. Clarity and color of solution—Dissolve 1.0 g of Triclofos Sodium in 50 mL of water: the solution is clear and colorless.
2. Chloride \(<1.0\) Perform the test with 0.2 g of Triclofos Sodium. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.178%).
3. Heavy metals \(<1.0\) Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
4. Arsenic \(<1.1\) Prepare the test solution with 1.0 g of Triclofos Sodium according to Method 1, and perform the test (not more than 2 ppm).
5. Free phosphoric acid—Weigh accurately about 0.3 g of Triclofos Sodium, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Standard Phosphoric Acid Solution, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20°C for 30 minutes. Perform the test with these solutions, using a solution obtained in the same manner with 5 mL of water as the blank, as directed under Ultraviolet-visible Spectrophotometry \(<2.24\). Determine the absorbances, \(A_T\) and \(A_S\), of each from the sample solution and Standard Phosphoric Acid Solution at 740 nm: the content of the free phosphoric acid is not more than 1.0%.

\[ \text{Content} (\%) \text{of the free phosphoric acid} (\text{H}_3\text{PO}_4) = \frac{1}{M} \times \frac{A_T}{A_S} \times 258.0 \]

\(M: \text{Amount (mg)} \text{ of the sample}\)

\[ \text{Loss on drying} \text{ < 2.41} \] Not more than 5.0% (1 g, in vacuum, 100°C, 3 hours).

**Assay**
1. Triclofos sodium—Weigh accurately about 0.2 g of Triclofos Sodium, previously dried, place in a Kjeldhal flask, add 2 mL of sulfuric acid and 2.5 mL of nitric acid, and heat until brown gas are not evolved. After cooling, add 1 mL of nitric acid, heat until white fumes are produced, and cool. Repeat this procedure until the solution becomes colorless. Transfer this solution to a flask using 150 mL of water, add 50 mL of molybdemum (III) oxide-citric acid TS, heat gently to boil, add gradually 25 mL of quinoline TS with stirring, and heat on a water bath for 5 minutes. After cooling, filter the precipitate, and wash repeatedly with water until the washing does not indicate acidity. Transfer the precipitate to a flask using 100 mL of water, add exactly 50 mL of 0.5 mol/L sodium hydroxide VS, dissolve, and titrate \(<2.50\) with 0.5 mol/L hydrochloric acid VS until the color of the solution changes from purple to yellow (indicator: 3 drops of phenolphthalein-thymol blue TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS = 4.834 mg of \(\text{C}_2\text{H}_3\text{Cl}_3\text{NaO}_4\text{P}\)

2. Chlorine—Weigh accurately about 10 mg of Triclofos Sodium, previously dried, perform the test according to the procedure of determination for chlorine as directed under Oxygen Flask Combustion Method \(<1.06\), using 1 mL of sodium hydroxide TS and 20 mL of water as the absorbing liquid.

**Containers and storage** Containers—Tight containers.

### Triclofos Sodium Syrup

**Monosodium Trichloroethyl Phosphate Syrup**

トリクロホスナトリウムシロップ

Triclofos Sodium Syrup contains not less than 90.0% and not more than 110.0% of the labeled amount of triclofos sodium (\(\text{C}_2\text{H}_3\text{Cl}_3\text{NaO}_4\text{P}\): 251.37).

**Method of preparation** Prepare as directed under Syrups, with Triclofos Sodium.

**Identification**
1. Weigh a portion of Triclofos Sodium Syrup, equivalent to 0.25 g of Triclofos Sodium according to the labeled amount, add 40 mL of water, shake well, add 5 mL of diluted sulfuric acid (3 in 50), and extract with 25 mL of 3-methyl-1-butanol. Take 5 mL of the extract, evaporate on a water bath to dryness, and add 1 mL of diluted sulfuric acid (1 in 2) and 1 mL of a solution of potassium permanganate (1 in 20) to the residue. Heat in a water bath for 5 minutes, add 7 mL of water, and then add a solution of oxalic acid dihydrate (1 in 20) until the color of the solution disappears. To 1 mL of this solution add 1 mL of pyridine and 1 mL of a solution of sodium hydroxide (1 in 5), and heat in a water bath while shaking, for 1 minute: a light red color develops in the pyridine layer.

2. Take 10 mL of the extract obtained in (1), evaporate on a water bath to dryness, add 1 g of anhydrous sodium carbonate to the residue, and heat for 10 minutes. After cooling, dissolve the residue in 40 mL of water, filter if necessary, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests \(<1.0\) for chloride. The remainder of the filtrate responds to the Qualitative Tests \(<1.0\) for chloride and to the Qualitative Tests \(<1.0\) for phosphate.

\[ \text{pH} < 2.54 \] 6.0 – 6.5

**Assay** Weigh accurately a portion of Triclofos Sodium Syrup, equivalent to 0.13 g of Triclofos Sodium according to the labeled amount, add 15 mL of water, 1 mL of sodium hydroxide TS and 15 mL of diethyl ether, shake for 1 minute, and separate the water layer. Wash the diethyl ether layer with 1 mL of water, and combine the washing with above water layer. To this solution add 2.5 mL of diluted sulfuric acid (3 in 50), and extract with four 10-mL portions of 3-methyl-1-butanol. Combine the 3-methyl-1-butanol extracts, and add 3-methyl-1-butanol to make exactly 50 mL. Measure exactly 10 mL of this solution, and dilute with potassium hydroxide-ethanol TS. Place in a glass ampule, fire-seal, mix, and heat at 120°C for 2 hours in an autoclave. After cooling, transfer the contents to a flask, add 20 mL of diluted nitric acid (63 in 500) and exactly 25 mL of 0.02 mol/L silver nitrate VS, shake well, and titrate \(<2.50\) the
Containers and storage  Containers—Tight containers.

Storage—In a cold place.

Trihexyphenidyl Hydrochloride

トリヘキシフェニジル塩酸塩

C₂₀H₃₁NO.HCl: 337.93
(1R5)-1-Cyclohexyl-1-phenyl-3-(pipiderin-1-yl)propan-1-ol monohydrochloride

[52-49-3]

Trihexyphenidyl Hydrochloride, when dried, contains not less than 98.5% of C₂₀H₃₁NO.HCl.

Description Trihexyphenidyl Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is soluble in ethanol (95%), sparingly soluble in acetic acid (100), slightly soluble in water, very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 1 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming, and cool. Use this solution as the sample solution. To 5 mL of the sample solution add 1 mL of a solution of 2,4,6-trinitrophenol in chloroform (1 in 50), and shake vigorously: a yellow precipitate is formed.

(2) To 20 mL of the sample solution obtained in (1) add 2 mL of sodium hydroxide TS: a white precipitate is formed. Collect the precipitate, wash with a small amount of water, recrystallize from methanol, and dry in a desiccator (in vacuum, silica gel) for 2 hours: the crystals so obtained melt between 113°C and 117°C.

(3) The sample solution obtained in (1) responds to the Qualitative Tests 〈1.09〉 (2) for chloride.

pH 〈2.54〉 Dissolve 1.0 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming, and cool: the pH of this solution is between 5.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming: the solution is clear and colorless.

(2) Heavy metals 〈1.07〉—Dissolve 1.5 g of Trihexyphenidyl Hydrochloride in 60 mL of water by warming on a water bath at 80°C, cool, and filter. To 40 mL of the filtrate add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) Piperidylpropophenone—Dissolve 0.10 g of Trihexyphenidyl Hydrochloride in 40 mL of water and 1 mL of 1 mol/L hydrochloric acid VS by warming, cool, and add water to make 100 mL. Determine the absorbance of this solution at 247 nm as directed under Ultraviolet-visible Spectrophotometry 〈2.24〉: the absorbance is not more than 0.50.

Loss on drying 〈2.41〉 Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition 〈2.44〉 Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Trihexyphenidyl Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate 〈2.50〉 with 0.1 mol/L perchloric acid-dioxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS = 33.79 mg of C₂₀H₃₁NO.HCl

Containers and storage  Containers—Tight containers.

Trihexyphenidyl Hydrochloride Tablets

トリヘキシフェニジル塩酸塩錠

Trihexyphenidyl Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of trihexyphenidyl hydrochloride (C₂₀H₃₁NO.HCl: 337.93).

Method of preparation Prepare as directed under Tablets, with Trihexyphenidyl Hydrochloride.

Identification (1) Weigh a quantity of powdered Trihexyphenidyl Hydrochloride Tablets, equivalent to 0.1 g of Trihexyphenidyl Hydrochloride according to the labeled amount, with 5 mL of chloroform, shake, and filter. Evaporate the filtrate on a water bath to dryness. Dissolve the residue in 10 mL of water by warming, cool, and use this solution as the sample solution. With 5 mL of the sample solution, proceed as directed in the Identification (1) under Trihexyphenidyl Hydrochloride.

(2) Shake a quantity of powdered Trihexyphenidyl Hydrochloride Tablets, equivalent to 0.01 g of Trihexyphenidyl Hydrochloride according to the labeled amount, with 5 mL of chloroform, filter, and use the filtrate as the sample solution. Dissolve 0.02 g of Trihexyphenidyl Hydrochloride RS in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 〈2.03〉. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots from the sample solution and the standard solution show a blue-purple color and the same RF value.

(3) The sample solution obtained in (1) responds to the Qualitative Tests 〈1.09〉 (2) for chloride.
Uniformity of dosage units \(<6.02\) Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Trihexyphenidyl Hydrochloride Tablets add 2 mL of dilute hydrochloric acid and 60 mL of water, disintegrate by vigorous shaking for 10 minutes, and warm on a water bath with occasional shaking for 10 minutes. Cool, add 2 mL of methanol, and add water to make exactly \(V\) mL of the solution contains about 20 \(\mu\)g of trihexyphenidyl hydrochloride \((C_{20}H_{31}NO\cdot HCl)\) per mL. Centrifuge, if necessary, and use the supernatant liquid as the sample solution. Separately, dissolve about 20 mg of Trihexyphenidyl Hydrochloride RS (determine previously its loss on drying \(<2.4\)\%) in the same conditions as Trihexyphenidyl Hydrochloride in methanol to make exactly 20 mL. Pipet 2 mL of this solution, and add 2 mL of dilute hydrochloric acid and water to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, transfer to glass-stoppered centrifuge tubes, add exactly 10 mL of bromocresol purple-dipotassium hydrogenphosphate-citric acid TS and 15 mL of chloroform, stopper tightly, shake well, and centrifuge. Pipet 10 mL each of the chloroform layers, add chloroform to make exactly 50 mL. Determine the absorbances, \(A_1\) and \(A_2\), of the subsequent solutions of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24\)

\[
\text{Amount (mg) of trihexyphenidyl hydrochloride (C}_{20}\text{H}_{31}\text{NO\cdot HCl)} = M_5 \times A_1/ A_2 \times V/1000
\]

\(M_5\): Amount (mg) of Trihexyphenidyl Hydrochloride RS, calculated on the dried basis

Dissolution \(<6.10\) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Trihexyphenidyl Hydrochloride Tablets is not less than 70\%.

Start the test with 1 tablet of Trihexyphenidyl Hydrochloride Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \(\mu\)m. Discard the first 10 mL of the filtrate, pipet \(V\) mL of the subsequent filtrate, add the dissolution medium to make exactly \(V\) mL so that each mL contains about 2.2 \(\mu\g of trihexyphenidyl hydrochloride \((C_{20}H_{31}NO\cdot HCl)\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Trihexyphenidyl Hydrochloride RS, previously dried at 105\°C for 3 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 mL each of the sample solution, the standard solution and the dissolution medium add exactly 1 mL of dilute acetic acid (31) (1 in 10), and immediately add 5 mL of bromocresol green-sodium hydroxide-acetic acid-sodium acetate TS, and shake. Then, add exactly 10 mL each of dichloromethane, shake well, centrifuge, and take the dichloromethane layer. Determine the absorbances, \(A_1\), \(A_2\) and \(A_3\), of these dichloromethane layers at 415 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24\), using dichloromethane as the blank.

Dissolution rate (\%) with respect to the labeled amount of trihexyphenidyl hydrochloride \((C_{20}H_{31}NO\cdot HCl)\)

\[
M_5 \times (A_1 - A_3)/(A_3 - A_0) \times V/V \times 1/C \times 18
\]

\(M_5\): Amount (mg) of Trihexyphenidyl Hydrochloride RS

C: Labeled amount (mg) of trihexyphenidyl hydrochloride \((C_{20}H_{31}NO\cdot HCl)\) in 1 tablet

Assay Weigh accurately and powder not less than 20 Trihexyphenidyl Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of trihexyphenidyl hydrochloride \((C_{20}H_{31}NO\cdot HCl)\), dissolve in 2 mL of dilute hydrochloric acid and 60 mL of water by warming on a water bath for 10 minutes with occasional shaking. After cooling, add 2 mL of methanol and water to make exactly 100 mL, and use this solution as the sample solution. Dissolve about 50 mg of Trihexyphenidyl Hydrochloride RS (determine previously its loss on drying \(<2.4\)\%) in the same conditions as Trihexyphenidyl Hydrochloride, weighed accurately, in methanol, add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add 2 mL of dilute hydrochloric acid and water to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution into glass-stoppered centrifuge tubes, add exactly 10 mL each of bromocresol purple-dipotassium hydrogenphosphate-citric acid TS and 15 mL each of chloroform, stopper tightly, shake thoroughly, and centrifuge. Pipet 10 mL each of the chloroform layers, and add chloroform to make exactly 50 mL. Determine the absorbances, \(A_1\) and \(A_4\), of the subsequent solutions of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24\)

\[
\text{Amount (mg) of trihexyphenidyl hydrochloride (C}_{20}\text{H}_{31}\text{NO\cdot HCl)} = M_5 \times A_1/ A_4 \times 1/10
\]

\(M_5\): Amount (mg) of Trihexyphenidyl Hydrochloride RS, calculated on the dried basis

Containers and storage Containers—Tight containers.

**Trimebutine Maleate**

トリメブチンマレイン酸塩

\[
C_{22}H_{29}NO_5\cdot C_4H_4O_4 = 503.54
\]

(2R5)-2-Dimethylamino-2-phenylbutyl 3,4,5-trimethoxybenzoate monomaleate

\(34140-59-5\)

Trimebutine Maleate, when dried, contains not less than 98.5\% and not more than 101.0\% of \(C_{22}H_{29}NO_5\cdot C_4H_4O_4\).
**Description**  Trimebutine Maleate occurs as white, crystals or crystalline powder. It is freely soluble in N,N-dimethylformamide and in acetic acid (100), solubile in acetonitrile, and slightly soluble in water and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS. A solution of it in N,N-dimethylformamide (1 in 20) shows no optical rotation.

**Identification (1)**  Determine the absorption spectrum of a solution of Trimebutine Maleate in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)  Determine the infrared absorption spectrum of Trimebutine Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point**  <2.60>  131 – 135°C

**Purity (1)**  Heavy metals <1.07>—Proceed with 2.0 g of Trimebutine Maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2)  Arsenic <1.11>—Prepare the test solution with 2.0 g of Trimebutine Maleate according to Method 3, and perform the test (not more than 1 ppm).

(3)  Related substances—Dissolve 0.10 g of Trimebutine Maleate in 100 mL of a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7) to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than maleic acid and trimebutine from the sample solution is not larger than 1/2 times the peak area of trimebutine from the standard solution, and the total area of the peaks other than maleic acid and trimebutine is not larger than the peak area of trimebutine from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 650 mL of diluted perchloric acid (17 in 20,000), previously adjusted the pH to 3.0 with a solution of ammonium acetate (1 in 1000), add 1 g of sodium 1-pentanesulfonate to dissolve. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of trimebutine is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of trimebutine beginning after the peak of maleic acid.

**System suitability**—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7) to make exactly 20 mL. Confirm that the peak area of trimebutine obtained from 20 μL of this solution is equivalent to 20 to 30% of that from 20 μL of the standard solution.

System performance: Dissolve 40 mg of Trimebutine Maleate and 20 mg of imipramine hydrochloride in 100 mL of a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7). When the procedure is run with 20 μL of this solution under the above operating conditions, trimebutine and imipramine are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimebutine is not more than 5%.

**Loss on drying**  <2.41>  Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition**  <2.44>  Not more than 0.1% (1 g).

**Assay**  Weigh accurately about 0.8 g of Trimebutine Maleate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 50.35 mg of C14H22N2O3.2HCl.

**Containers and storage**  Containers—Well-closed containers.

**Trimetazidine Hydrochloride**

Trimetazidine Hydrochloride contains not less than 98.0% and not more than 101.0% of C14H22N2O3.2HCl, calculated on the anhydrous basis.

**Description**  Trimetazidine Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water and in formic acid, sparingly soluble in methanol, and slightly soluble in ethanol (99.5).

The pH of a solution of Trimetazidine Hydrochloride (1 in 20) is between 2.3 and 3.3.

Melting point: about 227°C (with decomposition).

**Identification (1)**  Determine the absorption spectrum of a solution of Trimetazidine Hydrochloride in 0.1 mol/L hy-
Purity (1)

Heavy metals under Liquid Chromatography of Trimetazidine Hydrochloride as directed in the potassium tensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimetazidine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Trimetazidine Hydrochloride (1 in 50) responds to the Qualitative Tests 1.09% for chloride.

Purity (1) Heavy metals 1.07—Proceed with 2.0 g of Trimetazidine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.2 g of Trimetazidine Hydrochloride in 50 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than trimetazidine obtained from the sample solution is not larger than 15 times the area of trimetazidine obtained from the sample solution, and the total area of the peaks other than trimetazidine from the sample solution is not larger than 2.5 times the peak area of trimetazidine from the standard solution. Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 2.87 g of sodium 1-heptanesulfonate in water to make 1000 mL, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10). Mix 3 volumes of this solution and 2 volumes of methanol.

Mobile phase B: Methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 50</td>
<td>95 → 75</td>
<td>5 → 25</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of trimetazidine is about 25 minutes.

Time span of measurement: About 2 times as long as the retention time of trimetazidine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of trimetazidine obtained from 10 μL of this solution is equivalent to 18 to 32% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of trimetazidine are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetazidine is not more than 2.0%.

Water 2.48 Not more than 1.5% (2 g, volumetric titration, direct titration).

Residue on ignition 2.44 Not more than 0.1% (1 g).

Assay Weigh accurately about 0.12 g of Trimetazidine Hydrochloride, dissolve in 5 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat at 90 – 100°C for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate 2.5, the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS = 16.96 mg of C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>·2HCl

Containers and storage Containers—Tight containers.

**Trimetazidine Hydrochloride Tablets**

トリメタジジン塩酸塩

Trimetazidine Hydrochloride Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of trimetazidine hydrochloride (C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>·2HCl: 339.26).

Method of preparation Prepare as directed under Tablets, with Trimetazidine Hydrochloride.

Identification Shake a quantity of powered Trimetazidine Hydrochloride Tablets, equivalent to 10 mg of Trimetazidine Hydrochloride according to the labeled amount, with 10 mL of a mixture of ethanol (95) and water (3:1), and filter. Evaporate the filtrate on a water bath, add 2 mL of water to the residue, and shake. To 1 mL of this solution add 1 mL of p-benzoquinone TS, boil gently for 2 to 3 minutes, and cool: a red color develops.

Uniformity of dosage units 2.02 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Trimetazidine Hydrochloride Tablets add 15 mL of a mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to disintegrate the tablet, and treat with ultrasonic waves for 10 minutes. Shake the solution for 10 minutes, and add the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 20 mL. Centrifuge, pipet 2 mL of the supernatant liquid, equivalent to about 0.75 mg of trimetazidine hydrochloride (C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>·2HCl), add exactly 2.5 mL of the internal solution, and add water to make exactly 10 mL. Perform the test with exactly 2.0 mL of the internal solution, direct titration, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
standard solution, add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of trimetazidine hydrochloride for assay (separately determine the water content in the same manner as Trimetazidine Hydrochloride), and dissolve in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Amount (mg) of trimetazidine hydrochloride
(C₁₄H₂₂N₂O₃·2HCl) = Mₛ × Qₛ/Qₛ × 1/21V

Mₛ: Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of parahydroxybenzoic acid in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) (7 in 40,000).

Dissolution <6.10>—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Trimetazidine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Trimetazidine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V mL so that each mL contains about 3.3 μg of trimetazidine hydrochloride (C₁₄H₂₂N₂O₃·2HCl) according to the labeled amount. Pipet 3 mL of this solution, add exactly 3 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of trimetazidine hydrochloride for assay (separately determine the water content in the same manner as Trimetazidine Hydrochloride), and dissolve in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) (7 in 40,000).

Operating conditions—When the procedure is run with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetazidine is not more than 1.5%.

Dissolution rate (%) with respect to the labeled amount of trimetazidine hydrochloride (C₁₄H₂₂N₂O₃·2HCl)

= Mₛ × Aₛ × V'/V × 1/C × 18

Mₛ: Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of trimetazidine hydrochloride (C₁₄H₂₂N₂O₃·2HCl) in 1 tablet

System suitability—When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of trimetazidine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetazidine is not more than 1.5%.

Dissolution rate (%) with respect to the labeled amount of trimetazidine hydrochloride (C₁₄H₂₂N₂O₃·2HCl)

= Mₛ × Qₛ/Qₛ × 1/10

Mₛ: Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of parahydroxybenzoic acid in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) (7 in 40,000).

Assay—When the test is performed at 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetazidine is not more than 1.5%.

Dissolution rate (%) with respect to the labeled amount of trimetazidine hydrochloride (C₁₄H₂₂N₂O₃·2HCl)

= Mₛ × Qₛ/Qₛ × 1/10

Mₛ: Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of trimetazidine hydrochloride (C₁₄H₂₂N₂O₃·2HCl) in 1 tablet

Operating conditions—Proceed as directed in the operating conditions in the Assay.

System suitability—When the procedure is run with 10 μL of the standard solution under the above operating conditions, trimetazidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trimetazidine to that of the internal standard is not more than 1.5%.
Trimethadione

トリメタジオン

Trimethadione, when dried, contains not less than 98.0% of \( \text{C}_6\text{H}_9\text{NO}_3 \).

Description Trimethadione occurs as white crystals or crystalline powder. It has a camphor-like odor. It is very soluble in ethanol (95) and in chloroform, freely soluble in diethyl ether, and soluble in water.

Identification (1) To 5 mL of a solution of Trimethadione (1 in 50) add 2 mL of barium hydroxide TS: a precipitate is formed immediately.

(2) Determine the infrared absorption spectrum of a solution of Trimethadione in chloroform (1 in 50) as directed in the solution method under Infrared Spectrophotometry \(<2.25>\), using a 0.1-mm fixed sodium chloride cell, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melt point \(<2.60>\ 45 - 47^\circ\text{C}\)

Purity Heavy metals \(<1.0>\)—Proceed with 2.0 g of Trimethadione according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying \(<2.41>\ Not more than 0.5% (1 g, silica gel, 6 hours).

Residue on ignition \(<2.44>\ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Trimethadione, previously dried, in a glass-stoppered conical flask, dissolve in 5 mL of ethanol (95), add exactly measured 50 mL of 0.1 mol/L sodium hydroxide VS, stopper, and allow to stand for 15 minutes with occasional shaking. Titrate \(<2.50>\) the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 4 drops of cresol red TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS = 14.31 mg of \( \text{C}_6\text{H}_9\text{NO}_3 \)

Containers and storage Containers—Tight containers.

Storage—Not exceeding 30°C.

Trimethadione Tablets

トリメタジオン錠

Trimethadione Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of trimethadione (\( \text{C}_6\text{H}_9\text{NO}_3 \); 143.14).

Method of preparation Prepare as directed under Tablets, with Trimethadione.

Identification (1) Weigh a portion of powdered Trimethadione Tablets, equivalent to 1 g of Trimethadione according to the labeled amount, add 10 mL of petroleum benzine, and shake frequently for 15 minutes. Decant, remove the petroleum benzine, add another 10 mL of petroleum benzine, and repeat the extraction in the same manner. To the residue add 25 mL of diethyl ether, allow to stand for 20 minutes with occasional shaking, filter, evaporate the filtrate at room temperature, and dry the residue in a desiccator (silica gel) for 6 hours: the residue melts \(<2.60>\) between 44°C and 47°C. Proceed with this residue as directed in the Identification (1) under Trimethadione.

(2) Determine the infrared absorption spectrum of a solution of the residue obtained in (1) in chloroform (1 in 50) in a 0.1-mm fixed sodium chloride cell, as directed in the solution method under Infrared Spectrophotometry \(<2.25>\); it exhibits absorption at the wave numbers of about 2960 cm\(^{-1}\), 1814 cm\(^{-1}\), 1735 cm\(^{-1}\), 1445 cm\(^{-1}\), 1394 cm\(^{-1}\), 1290 cm\(^{-1}\), 1100 cm\(^{-1}\) and 1055 cm\(^{-1}\).

Assay Weigh accurately and powder not less than 20 Trimethadione Tablets. Weigh accurately a portion of the powder, equivalent to about 1 g of trimethadione (\( \text{C}_6\text{H}_9\text{NO}_3 \)), add 50 mL of ethanol (95), and boil gently for 15 minutes under a reflux condenser. Filter the warm ethanol (95) solution into a 100-mL volumetric flask through a glass filter (G4), and wash the residue with three 10-mL portions of warm ethanol (95). Combine the washings with the filtrate in the flask, cool, and add ethanol (95) to make exactly 100 mL. Pipet 25 mL of the solution into a glass-stoppered conical flask, add 25 mL of water and exactly 30 mL of 0.1 mol/L sodium hydroxide VS, stopper, allow to stand for 15 minutes with occasional shaking, and titrate \(<2.50>\) the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 4 drops of cresol red TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS = 14.31 mg of \( \text{C}_6\text{H}_9\text{NO}_3 \)

Containers and storage Containers—Tight containers.

Storage—Not exceeding 30°C.
Trimetoquinol Hydrochloride Hydrate

Trimetoquinol Hydrochloride

トリメトキノール塩酸塩水和物

Trimetoquinol Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of trimetoquinol hydrochloride (C₁₉H₂₃NO₅.HCl: 381.85), calculated on the anhydrous basis.

Description Trimetoquinol Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5).

Melting point: about 151°C (with decomposition, after drying in vacuum, 105°C, 4 hours).

Identification (1) Determine the absorption spectrum of a solution of Trimetoquinol Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry.<2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimetoquinol Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry.<2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than trimetoquinol from the sample solution is not larger than the peak area of trimetoquinol from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 283 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 2 g of potassium dihydrogen phosphate and 2 g of sodium 1-pentane sulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH between 2.8 and 3.2, and filter through a membrane filter with a pore size of 0.4 μm. Add 200 mL of acetonitrile to 800 mL of the filtrate.
Flow rate: Adjust the flow rate so that the retention time of trimetoquinol is about 7 minutes.

Time span of measurement: About twice as long as the retention time of trimetoquinol beginning after the solvent peak.

System suitability—
Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of trimetoquinol obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: Dissolve 5 mg of Trimetoquinol Hydrochloride Hydrate and 1 mg of procaine hydrochloride in 50 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, procaine and trimetoquinol are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetoquinol is not more than 2.0%.

Water<2.48> 3.5 – 5.5% (0.3 g, volumetric titration, direct titration).

Residue on ignition<2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Trimetoquinol Hydrochloride Hydrate, dissolve in 2 mL of 0.1 mol/L hydrochloric acid VS and 70 mL of ethanol (99.5) with thorough shaking, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Calculate the consumed volume of 0.1 mol/L potassium hydroxide-
ethanol VS between the first inflection point and of the second inflection point.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 38.19 mg of C$_{19}$H$_{23}$NO$_5$.HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Dental Triozinc Paste

歯科用トリオジンクパスタ

Dental Triozinc Paste consists of a powder containing Paraformaldehyde, Thymol, anhydrous zinc sulfate and Zinc Oxide, and a solution containing Cresol, Potash Soap and Glycerin. Suitable amounts of the two components are triturated before use.

Method of preparation

(1) The powder
Paraformaldehyde, finely powdered 10 g
Thymol, finely powdered 3 g
Zinc Sulfate Hydrate 9 g
Zinc Oxide 82 g

To make about 100 g

Heat Zinc Sulfate Hydrate at about 250°C to obtain anhydrous zinc sulfate, cool, and pulverize to a fine powder. Mix homogeneously this powder with Thymol, Paraformaldehyde, and Zinc Oxide.

(2) The solution
Cresol 40 g
Potash Soap 40 g
Glycerin 20 g

To make 100 g

Dissolve Potash Soap in a mixture of Cresol and Glycerin.

Description The powder occurs as a fine, white powder, having a characteristic odor. The solution is a clear, yellow-brown to red-brown, viscous liquid, having the odor of cresol.

Containers and storage Containers—Tight containers.

Tropicamide

トロピカミド

C$_{17}$H$_{20}$N$_2$O$_2$: 284.35
(2RS)-N-Ethyl-3-hydroxy-2-phenyl-N-(pyridin-4-ylmethyl)propanamide
[1508-75-4]

Tropicamide, when dried, contains not less than 98.5% of C$_{17}$H$_{30}$N$_2$O$_2$.

Description Tropicamide occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in ethanol (95) and in chloroform, slightly soluble in water and in diethyl ether, and practically insoluble in petroleum ether.

It dissolves in dilute hydrochloric acid.

The pH of a solution of Tropicamide (1 in 500) is between 6.5 and 8.0.

Identification (1) To 5 mg of Tropicamide add 0.5 mL of a solution of ammonium vanadate (V) in sulfuric acid, (1 in 200), and heat: a blue-purple color develops.

(2) Dissolve 5 mg of Tropicamide in 1 mL of ethanol (95) and 1 mL of water, add 0.1 g of 1-chloro-2,4-dinitrobenzene, and heat on a water bath for 5 minutes. Cool, and add 2 to 3 drops of a solution of sodium hydroxide (1 in 10) and 3 mL of ethanol (95): a red-purple color develops.

Absorbance $<2.24 > E_{1}% (255$ nm): 166 – 180 (after drying, 5 mg, 2 mol/L hydrochloric acid TS, 200 mL).

Melting point $<2.60 > 96–99°C$

Purity (1) Chloride $<1.0%>$—Dissolve 1.0 g of Tropicamide in 30 mL of ethanol (95), add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of ethanol (95), 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.016%).

(2) Heavy metals $<1.07>$—Dissolve 1.0 g of Tropicamide in 30 mL of ethanol (95), add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 30 mL of ethanol (95), 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) $N$-Ethyl-$\gamma$-picolyamine—Dissolve 0.10 g of Tropicamide in 5 mL of water by heating, add 1 mL of a solution of acetaldehyde (1 in 20), and shake well. Add 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS and 1 to 2 drops of sodium hydrogen carbonate TS, and shake: no blue color develops.

(4) Tropic acid—To 10 mg of Tropicamide add 5 mg of sodium borate and 7 drops of 4-dimethylaminobenzaldehyde TS, and heat in a water bath for 3 minutes. Cool in ice water, and add 5 mL of acetic anhydride: no red-purple
Troxipide occurs as a white, crystalline powder.

It is freely soluble in acetone (100), soluble in methanol, sparingly soluble in ethanol (99.5) and slightly soluble in water.

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Troxipide in 1 mol/L hydrochloric acid TS (1 in 5) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Troxipide in 0.1 mol/L hydrochloric acid TS (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Troxipide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Troxipide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Troxipide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 177 – 181°C

Purity (1) Chloride <1.07>—Dissolve 1.0 g of Troxipide in 30 mL of methanol, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.009%).

(2) Heavy metals <1.07>—Moisten 2.0 mg of Troxipide with 1 mL of sulfuric acid, and gently heat until charred. After cooling, add 2 mL of nitric acid, carefully heat until white fumes are no longer evolved, and perform the test according to Method 2. Prepare the control solution as follows: evaporate 1 mL of sulfuric acid, 2 mL of nitric acid and 2 mL of hydrochloric acid on a water bath and then on a sand bath to dryness, and moisten the residue with 3 drops of hydrochloric acid. Proceed in the same manner for the preparation of the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 10 ppm).

(3) Related substances—Dissolve 0.20 g of Troxipide in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, ethyl acetate, water, hexane and ammonia water (28:20:5:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spots other than the principal spot obtained from the sample solution is not more than three, and they are not more intense than the spot from the standard solution.

(4) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tropicamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 29.44 mg of C15H22N2O4.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.
chloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.247: it exhibits a maximum between 256 nm and 260 nm.

**Uniformity of dosage units 6.02** Perform the test according to the following method: Troxipide Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Troxipide Fine Granules, add 80 mL of 0.1 mol/L hydrochloric acid TS, stir for 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL so that each mL contains about 1 mg of troxipide (C15H22N2O4). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 3 mL of the internal standard solution, and water to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

**Amount (mg) of troxipide (C15H22N2O4)**

\[
M_s = \frac{M_t \times Q_t \times Q_s \times V}{6.02}
\]

Thus, the amount (mg) of troxipide (C15H22N2O4) in a single unit is about

\[
M_s \times A_1 \times A_2 \times 1 / C \times 450
\]

**Dissolution 6.10** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Troxipide Fine Granules is not less than 85%.

Weigh accurately an amount of Troxipide Fine Granules, equivalent to about 0.1 g of troxipide (C15H22N2O4) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Troxipide RS, previously dried at 105 °C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 25 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution, and water to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q1 and Qs, of the peak area of troxipide to that of the internal standard.

**Amount (mg) of troxipide (C15H22N2O4)**

\[
M_s = \frac{M_t \times Q_1}{Q_s} \times 20
\]

**Internal standard solution**—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 258 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: To diluted phosphoric acid (1 in 500) add diethylylamidine to adjust the pH to 3.0. To 1500 mL of this solution add 100 mL of methanol and 50 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of troxipide is about 7 minutes.

**System suitability**—

System performance: When the procedure is run with 20 μL of standard solution under the above operating conditions, troxipide and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of troxipide to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

**Troxipide Tablets**

**トロキシピド錠**

Troxipide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of troxipide (C15H22N2O4); 294.35.

**Method of preparation**—Prepare as directed under Tablets, with Troxipide.

**Identification**—Weigh accurately an amount of powdered Troxipide Tablets, equivalent to 0.1 g of Troxipide according to the labeled amount, add 250 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 4 mL of the filtrate add...
0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24); it exhibits maximum between 256 nm and 260 nm.

Uniformity of dosage units (6.02) Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Troxipide Tablets add 90 mL of 0.1 mol/L hydrochloric acid TS, shake well to disintegrate, shake for another 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 1 mg of troxipide (C₁₁H₁₂N₂O₂). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 3 mL of the internal standard solution, add water to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of troxipide (C₁₁H₁₂N₂O₂)} = M_s \times \frac{Q_1}{Q_2} \times V / 1000 \\
M_s: \text{Amount (mg) of Troxipide RS}
\]

Internal standard solution—A solution of 4-aminoacetoephone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Dissolution (6.10) When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Troxipide Tablets is not less than 70%.

Start the test with 1 tablet of Troxipide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 22 μg of troxipide (C₁₁H₁₂N₂O₂) according to the labeled amount, and use this solution as the sample solution. Separately weigh accurately about 20 mg of Troxipide RS, previously dried at 105°C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 25 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution and water to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of troxipide to that of the internal standard.

\[
\text{Amount (mg) of troxipide (C₁₁H₁₂N₂O₂)} = M_s \times \frac{Q_1}{Q_2} \times V / 25 \\
M_s: \text{Amount (mg) of Troxipide RS}
\]

Internal standard solution—A solution of 4-aminoacetoephone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 258 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: To 1500 mL of dilute phosphoric acid (1 in 500) add diethylamine to adjust the pH to 3.0. To 1500 mL of this solution add 100 mL of methanol and 50 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of troxipide is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, troxipide and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of troxipide to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

L-Tryptophan

L-トリプトファン

\[
\begin{align*}
\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2: & \quad 204.23 \\
(25)-2\text{-Amino-3-(indol-3-yl)}\text{propanoic acid} & \quad [73-22-3]
\end{align*}
\]

L-Tryptophan, when dried, contains not less than 98.5% of C₁₁H₁₂N₂O₂.

Description—L-Tryptophan occurs as white to yellowish white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in formic acid, slightly soluble in water,
and very slightly soluble in ethanol (95). It dissolves in dilute hydrochloric acid.

**Identification** Determine the infrared absorption spectrum of L-Tryptophan, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** <2.49> [α]D = −30.0° to −33.0°: Weigh accurately about 0.25 g of L-Tryptophan, previously dried, and dissolve in 20 mL of water by warming. After cooling, add water to make exactly 25 mL, and determine the optical rotation of the solution in a 100-mm cell.

**pH** <2.54> Dissolve 1.0 g in 100 mL of water by warming, and cool: the pH of this solution is between 5.4 and 6.4.

**Purity** (1) Clarity of solution—Dissolve 0.20 g of L-Tryptophan in 10 mL of 2 mol/L hydrochloric acid TS: the solution is clear.

(2) Chloride <1.05>—Dissolve 0.5 g of L-Tryptophan in 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.6 g of L-Tryptophan in 40 mL of water and 1 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Tryptophan. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Tryptophan according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Dissolve 1.0 g of L-Tryptophan in 3 mL of 1 mol/L hydrochloric acid TS and 2 mL of water by heating, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.30 g of L-Tryptophan in 1 mL of 1 mol/L hydrochloric acid TS, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of L-Tryptophan, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 20.42 mg of C12H12ClNO.HCl.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Tulobuterol Hydrochloride**

ツロブテロール塩酸塩

C12H18ClNO.HCl: 264.19
(1RS)-1-(2-Chlorophenyl)-2-(1,1-dimethylethyl)aminoethanol monohydrochloride [56776-01-3]

Tulobuterol Hydrochloride, when dried, contains not less than 98.5% of C12H18ClNO.HCl.

**Description** Tulobuterol Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in methanol, freely soluble in water, in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and very slightly soluble in diethyl ether.

A solution of Tulobuterol Hydrochloride (1 in 20) shows no optical rotation.

Melting point: about 163°C.

**Identification** (1) Determine the absorption spectrum of a solution of Tulobuterol Hydrochloride (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tulobuterol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tulobuterol Hydrochloride (1 in 20) responds to the Qualitative Tests <1.00> for chloride.

**Purity** (1) Clarity of solution—Dissolve 1.0 g of Tulobuterol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Tulobuterol Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.30 g of Tulobuterol Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Use a
plate previously developed with the upper-layer of a mixture of ethyl acetate and ammonia solution (28) (200:9) to the top of the plate and air-dried. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of a mixture of ethyl acetate and ammonia solution (28) (200:9) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of starting point from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.4\)% Not more than 0.5% (0.5 g in vacuum, 60°C, 4 hours).

**Residue on ignition** \(<2.4\)% Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Tulobuterol Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate between 60°C dropwise 25 mL of fuming sulfuric acid while shaking, warm Containers and storage

Containers—Tight containers.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

### L-Tyrosine

L-チロシン

C₉H₆NO₃: 181.19

(2S)-2-Amino-3-(4-hydroxyphenyl)propanoic acid [60-18-4]

L-Tyrosine, when dried, contains not less than 99.0% and not more than 101.0% of C₉H₆NO₃.

**Description** L-Tyrosine occurs as white crystals or a crystalline powder.

It is freely soluble in formic acid, and practically insoluble in water and in ethanol (99.5).

It dissolves in dilute hydrochloric acid and in ammonia TS.

**Identification** (1) Determine the absorption spectrum of a solution of L-Tyrosine in 0.1 mol/L hydrochloric acid (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.5\)%, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of L-Tyrosine as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.2\)%), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \(<2.4\)° \([\alpha]_D^20: -10.5 \text{ to } -12.5^\circ \) (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of L-Tyrosine in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride \(<1.0\)%—Dissolve 0.5 g of L-Tyrosine in 12 mL of dilute nitric acid and 20 mL of water, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 12 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(3) Sulfate \(<1.14\)%—Dissolve 0.6 g of L-Tyrosine in 5 mL of dilute hydrochloric acid, and add water to make 45 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 5 mL of dilute hydrochloric acid and water to make 45 mL. To the test solution and the control solution add 5 mL of barium chloride TS (not more than 0.028%).

(4) Ammonium \(<1.02\)%—Perform the test with 0.25 g of L-Tyrosine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals \(<1.07\)%—Proceed with 1.0 g of L-Tyrosine according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

### Turpentine Oil

*Oleum Terebinthinae*

テレビン油

Turpentine Oil is the essential oil distilled with steam from the wood or balsam of *Pinus* species (*Pinaceae*).

**Description** Turpentine Oil is a clear, colorless to pale yellow liquid. It has a characteristic odor and a pungent, bitter taste.

Turpentine Oil (1 mL) is miscible with 5 mL of ethanol (95) and this solution is neutral.

**Refractive index** \(<2.45\%> n_D^20: 1.465 – 1.478\)

**Specific gravity** \(<1.13\%> d_20^0: 0.860 – 0.875\)

**Purity** (1) Foreign matter—Turpentine Oil has no offensive odor. Shake 5 mL of Turpentine Oil with 5 mL of a solution of potassium hydroxide (1 in 6): the aqueous layer does not show a yellow-brown to dark brown color.

(2) Hydrochloric acid-coloring substances—Shake 5 mL of Turpentine Oil with 5 mL of hydrochloric acid, and allow to stand for 5 minutes: the hydrochloric acid layer is light yellow and not brown in color.

(3) Mineral oil—Place 5 mL of Turpentine Oil in a Cassia flask, cool to a temperature not exceeding 15°C, add dropwise 25 mL of fuming sulfuric acid while shaking, warm between 60°C and 65°C for 10 minutes, and add sulfuric acid to raise the lower level of the oily layer to the graduated portion of the neck: not more than 0.1 mL of oil separates.

**Distilling range** \(<2.57\%> 150 – 170°C, not less than 90 vol%.

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.
(6) Iron \( <1.0 \text{D} \)—Prepare the test solution with 1.0 g of L-Tyrosine according to Method 3, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.20 g of L-Tyrosine in 10 mL of diluted ammonia solution (28) (1 in 2), add water to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL, pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.07 \). Spot 5 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then develop with a mixture of l-propanol and ammonia solution (28) (67:33) to a distance of about 10 cm, and dry the plate at 80 °C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and then heat at 80 °C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying \( <2.4 \% \) Not more than 0.3% (1 g, 105 °C, 3 hours).

Residue on ignition \( <2.4 \% \) Not more than 0.1% (1 g).

Assay Weigh accurately about 0.18 g of L-Tyrosine previously dried, dissolve in 6 mL of formic acid, add 50 mL of acetic acid (100), and titrate \( <2.5 \text{D} \) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 18.12 mg of \( \text{C}_9\text{H}_6\text{N}_2\text{O}_3 \)

Containers and storage Containers—Tight containers.

Ubenimex

ウベニメクス

\[
\begin{align*}
\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_4: & \quad 308.37 \\
(2S)-2-[(2S,3R)-3-\text{Amino}-2-\text{hydroxy}-4-\text{phenylbutanoylamino}]4-\text{methylpentanoic acid} & \quad [58970-76-6]
\end{align*}
\]

Ubenimex, when dried, contains not less than 98.5% and not more than 101.0% of \( \text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_4 \).

Description Ubenimex occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in ethanol (99.5).

It dissolves in 1 mol/L hydrochloric acid TS.

Melting point: about 230 °C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ubenimex (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry \( <2.24 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation \( <2.4 \) \( \alpha \) \( [\alpha]_D^2 \) \( -15.5 \) to \( -17.5 \)° (after drying, 0.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity (1) Heavy metals \( <0.07 \) —Proceed with 2.0 g of Ubenimex according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 30 mg of Ubenimex in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase A to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.07 \) according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than ubenimex obtained from the sample solution is not larger than 1/2 times the peak area of ubenimex from the standard solution. Furthermore, the total area of the peaks other than ubenimex is not larger than the peak area of ubenimex from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \text{m} \) in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase A: A mixture of diluted 0.1 mol/L potassium dihydrogen phosphate TS (13 in 20) and acetonitrile for liquid chromatography (17:3).

Mobile phase B: A mixture of acetonitrile for liquid chromatography and diluted 0.1 mol/L potassium dihydrogen phosphate TS (13 in 20) (2:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 20</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>20 – 60</td>
<td>100 ( \rightarrow ) 0</td>
<td>0 ( \rightarrow ) 100</td>
</tr>
<tr>
<td>60 – 70</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of ubenimex is about 14 minutes.

Time span of measurement: About 5 times as long as the retention time of ubenimex, beginning after the solvent peak.

System suitability—

Test for required detectability—Pipet 1 mL of the standard
solution, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of ubenimex obtained from 20 mL of this solution is equivalent to 7 to 13% of that from 20 mL of the standard solution.

System performance: When the procedure is run with 20 mL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ubenimex are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 mL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ubenimex is not more than 2.0%.

**Loss on drying** (2.4) Not more than 0.5% (0.5 g, in vacuum, 80°C, 4 hours).

**Residue on ignition** (2.44) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Ubenimex, previously dried, dissolve in 60 mL of acetic acid (100), and titrate proceeding as directed in the operating conditions in the Assay.

**Systems suitability**—Perform the test with 20 mL of the standard solution under the above operating conditions, ubenimex and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System performance: When the procedure is run with 20 mL of the standard solution under the above operating conditions, ubenimex and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 mL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ubenimex to that of the internal standard is not more than 20%.

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in a mixture of water and acetonitrile (7:3) (1 in 2000).

**Operating conditions**—Proceed as directed in the operating conditions in the Assay.

**Containers and storage**—Tight containers.

## Ubenimex Capsules

ウベンメクスカプセル

Ubenimex Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of ubenimex (C₁₆H₂₄N₂O₄: 308.37).

**Method of preparation** Prepare as directed under Capsules, with Ubenimex.

**Identification** To a quantity of the contents of Ubenimex Capsules, equivalent to 25 mg of Ubenimex according to the labeled amount, add water to make 50 mL, shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

**Uniformity of dosage units** (6.02) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Ubenimex Capsules add 30 mL of a mixture of water and acetonitrile (7:3), shake well for 30 minutes, and add a mixture of water and acetonitrile (7:3) to make exactly 50 mL. Centrifuge this solution and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, equivalent to about 3 mg of ubenimex (C₁₆H₂₄N₂O₄), add exactly 4 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of ubenimex for assay, previously dried in vacuo at 80°C for 4 hours under reduced pressure, and dissolve in a mixture of water and acetonitrile (7:3) to make exactly 100 mL. Pipet 15 mL of this solution, add exactly 4 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.21D) according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of ubenimex to that of the internal standard.

\[
\text{Amount (mg) of ubenimex (C}_{16}\text{H}_{24}\text{N}_{2}\text{O}_{4}) = M_S \times Q_1/Q_2 \times 1/V \times 15/2
\]

\[
M_S: \text{ Amount (mg) of ubenimex for assay}
\]

**Dissolution** (6.10D) When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Ubenimex Capsules is not less than 70%.

Start the test with 1 capsule of Ubenimex Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add a mixture of water and acetonitrile (7:3) to make exactly V mL so that each mL contains about 11 μg of ubenimex (C₁₆H₂₄N₂O₄) according to labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of ubenimex for assay, previously dried in vacuo at 80°C for 4 hours, and dissolve in a mixture of water and acetonitrile (7:3) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of water and acetonitrile (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.21D) according to the following conditions, and determine the peak areas, A₁ and A₅, of ubenimex in each solution.

Dissolution rate (%) with respect to the labeled amount of ubenimex (C₁₆H₂₄N₂O₄) = \[ M_5 \times A_1/A_5 \times V/V \times 1/C \times 45 \]

\[
M_5: \text{ Amount (mg) of ubenimex for assay}
\]

C: Labeled amount (mg) of ubenimex (C₁₆H₂₄N₂O₄) in 1 capsule
Operating conditions—
Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 µL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of Ubenimex are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of Ubenimex is not more than 2.0%.

Assay To 10 Ubenimex capsules add 140 mL of a mixture of water and acetonitrile (7:3), shake well for 30 minutes, and add a mixture of water and acetonitrile (7:3) to make exactly 200 mL. Centrifuge this solution, and filter. Discard the first 20 mL of the filtrate, pipet a volume of the subsequent filtrate, equivalent to about 7.5 mg of ubenimex (C₁₆H₂₄N₂O₄), add exactly 10 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of ubenimex for assay, previously dried at 80°C for 4 hours under reduced pressure, dissolve in a mixture of water and acetonitrile (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, and a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of ubenimex to that of the internal standard.

Amount (mg) of ubenimex (C₁₆H₂₄N₂O₄)

\[ M_S = \frac{S \times Q_1}{Q_2} \times \frac{1}{4} \]

M₅: Amount (mg) of ubenimex for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in mixture of water and acetonitrile (7:3) (1 in 2000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 200 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 100) and acetonitrile for liquid chromatography (83:17).
Flow rate: Adjust the flow rate so that the retention time of ubenimex is about 8 minutes.

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, ubenimex and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ubenimex to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ubidecarenone

\[ \text{Ubidecarenone contains not less than 98.0\% of C}_{99}\text{H}_{90}\text{O}_{4}, \text{calculated on the anhydrous basis.} \]

Description Ubidecarenone occurs as a yellow to orange crystalline powder. It is odorless and has no taste.

It is soluble in diethyl ether, very slightly soluble in ethanol (99.5), and practically insoluble in water.

It is gradually decomposed and colored by light.

Melting point: about 48°C.

Identification (1) Dissolve 0.05 g of Ubidecarenone in 1 mL of diethyl ether, and add 10 mL of ethanol (99.5). To 2 mL of this solution add 3 mL of ethanol (99.5) and 2 mL of dimethyl malonate, then add dropwise 1 mL of a solution of potassium hydroxide (1 in 5), and mix: a blue color appears.

(2) Determine the infrared absorption spectrum of Ubidecarenone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ubidecarenone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Ubidecarenone according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.05 g of Ubidecarenone in 50 mL of ethanol (99.5) by warming at about 50°C for 2 minutes, and after cooling use this solution as the sample solution. To exactly 1 mL of the sample solution add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of ubidecarenone from the sample solution is not larger than the peak area of ubidecarenone from the standard solution.

Operating conditions—
Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.
Detection sensitivity: Adjust the detection sensitivity so that the peak height of ubidecarenone obtained from 5 \(\mu L\) of the standard solution is between 20 mm and 40 mm.

Time span of measurement: About 2 times of the retention time of ubidecarenone beginning after the solvent peak.

**Water** <2.48> Not more than 0.20% (1 g, volumetric titration, direct titration).

**Residue on ignition** <2.48> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 50 mg each of Ubidecarenone and Ubidecarenone RS (separately determined the Assay).

Weigh accurately about 50 mg each of Ubidecarenone and Ubidecarenone RS (separately determined the Assay). Weigh accurately about 50 mg each of Ubidecarenone and Ubidecarenone RS (separately determined the Assay). Weigh accurately about 50 mg each of Ubidecarenone and Ubidecarenone RS (separately determined the Assay). Weigh accurately about 50 mg each of Ubidecarenone and Ubidecarenone RS (separately determined the Assay).

Add each in 40 mL of ethanol (99.5) by warming at about 50°C for 2 minutes, and after cooling add ethanol (99.5) to make exactly 50 mL each, and use these solutions as the sample solution and standard solution. Perform the test with exact 5 \(\mu L\) of each of the sample solution and standard solution as directed under Liquid Chromatography <2.00> according to the following conditions, and determine peak areas, \(A_T\) and \(A_S\), of ubidecarenone of these solutions.

\[
\text{Amount (mg) of } C_{30}H_{36}O_4 = M_S \times A_T / A_S
\]

M_

Magnitude (mg) of Ubidecarenone RS, calculated on the anhydrous basis.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wave-length: 275 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu m\) in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of methanol and ethanol (99.5) (13:7).

Flow rate: Adjust the flow rate so that the retention time of ubidecarenone is about 10 minutes.

Selection of column: Dissolve 0.01 g each of Ubidecarenone and ubiquinone-9 in 20 mL of ethanol (99.5) by warming at about 50°C for 2 minutes. After cooling, proceed with 5 \(\mu L\) of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of ubiquinone-9 and ubidecarenone in this order with the resolution between these peaks being not less than 4.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions: the relative standard deviation of the peak areas of ubidecarenone is not more than 0.8%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Ulinastatin**

ウリナスタチン

Ulinastatin is a solution of a glycoprotein having trypsin inhibiting activity, which is separated and purified from human urine. It contains ulinastatin of not less than 45,000 Units per mL and not less than 2500 Units per mg protein.

**Description** Ulinastatin occurs as a light brown to brown, clear liquid.

**Identification** (1) Dilute a suitable amount of Ulinastatin with water to make a solution containing 4000 Units of ulinastatin per mL. To 1 mL of this solution add 1 mL of a solution of phenol (1 in 20), then carefully add 5 mL of sulfuric acid, and mix: an orange to red-orange color develops.

(2) Dilute a suitable quantity of Ulinastatin with water to make a solution containing 2000 units per mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Dilute a suitable amount of Ulinastatin with pH 7.8 2,2,2'-nitrotritolisethanol buffer solution to make a solution containing 500 Units of ulinastatin per mL, and use this solution as the sample solution. Use the same buffer solution as the control solution. To 0.1 mL each of the sample solution and the control solution add 1.6 mL of the buffer solution and 0.2 mL of trypsin TS for test of ulinastatin, mix, and allow them to stand in a water bath at 25°C for 1 minute. Then add 1 mL of \(N\)-\(\alpha\)-benzoyl-L-arginine-4-nitroanilide TS, mix, and allow them to stand at 25°C for 2 minutes: the solution obtained with the sample solution develops no color while that obtained with the control solution develops a yellow color.

(4) To 1.5 g of Powdered Agar add 100 mL of pH 8.4 boric acid-sodium hydroxide buffer solution, dissolve by warming in a water bath, then pour immediately into a Petri dish placed horizontally so that the agar layer is about 2 mm thick. After the agar becomes hard, bore two wells about 2.5 mm in diameter with a separation of 6 mm from each other. In one of the wells place 10 \(\mu L\) of a solution of Ulinastatin containing 500 Units per mL in pH 8.4 boric acid-sodium hydroxide buffer solution, and in the other well place 10 \(\mu L\) of anti-ulnastatin rabbit serum, cover the dish to avoid drying of the agar, and allow to stand for overnight at a room temperature: a clear precipitin line appears between the wells.

**pH** <2.54> 6.0 – 8.0

**Specific activity** When calculated from the results obtained by the Assay and the following method, the specific activity is not less than 2500 Units per 1 mg protein.

(i) Sample solution—To an exactly measured volume of Ulinastatin, equivalent to about 10,000 Units according to the labeled amount, add water to make exactly 20 mL.

(ii) Standard solutions—Weigh accurately about 10 mg of bovine serum albumin for test of ulinastatin, and dissolve in water to make exactly 20 mL. To a suitable volume of this solution add water to make four solutions containing exactly 300, 200, 100 and 50 \(\mu L\) of the albumin per mL, respectively.

(iii) Procedure—Pipet 0.5 \(\mu L\) each of the sample solution and standard solutions, put them in glass test tubes about 18 mm in internal diameter and about 130 mm in length, add exactly 5 mL of alkaline copper TS, mix, and allow the tubes to stand in a water bath at 30°C for 10 minutes. Then add exactly 0.5 mL of diluted Folin’s TS (1 in 2), mix, and warm in the water bath for 20 minutes. Determine the absorbances of these solutions at 750 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution obtained
in the same manner with 0.5 mL of water as the blank.

Plot the absorbances of the standard solutions on the vertical axis and their protein concentrations on the horizontal axis to prepare a calibration curve, and calculate the protein content of the sample solution from its absorbance by using this curve. Then calculate the amount of protein per mL of Ulinastatin.

**Purity**

(1) Heavy metals $<1.07$—Proceed with 10 mL of Ulinastatin according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 1 ppm).

(2) Related substances—To a suitable volume of Ulinastatin add water to make a solution containing exactly 12,500 Units per mL, and use this solution as the sample stock solution. To exactly 0.25 mL of the sample stock solution add exactly 0.2 mL of 0.2% bromophenol blue TS, mix, and use this solution as the sample solution. Separately, to exactly 1 mL of the sample stock solution add water to make exactly 100 mL. To exactly 0.25 mL of this solution add exactly 0.2 mL of glycerin and exactly 0.05 mL of 0.05% bromophenol blue TS, mix, and use this solution as the standard solution. Perform the following test with the sample solution and the standard solution: the bands other than the principal band obtained from the sample solution are not more intense than the band from the standard solution in the electrophoretogram.

(i) Tris buffer solution for polyacrylamide gel electrophoresis A Dissolve 18.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 80 mL of water, adjust to pH 8.8 with 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

(ii) Tris buffer solution for polyacrylamide gel electrophoresis B Dissolve 6.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 80 mL of water, adjust to pH 8.8 with 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

(iii) Tris buffer solution for polyacrylamide gel electrophoresis C Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.4 g of glycine in water to make 1000 mL.

(iv) Acrylamide solution for polyacrylamide gel electrophoresis Dissolve 30 g of acrylamide and 0.8 g of N,N′-methylenebisacrylamide in water to make 100 mL.

(v) Gel for separation Mix gently 15 mL of tris buffer solution for polyacrylamide gel electrophoresis A, 20 mL of acrylamide solution for polyacrylamide gel electrophoresis, 24.5 mL of water, 0.022 mL of N,N,N′,N′-tetramethylethlenediamine, 0.52 mL of 10% ammonium peroxodisulfate TS and 0.3 mL of 1 mol/L sodium sulfite TS, pour into a plate for slab gel preparation, then cover the gel mixture with a layer of water, and allow to set for 1 hour.

(vi) Gel for concentration Remove the water layer on the gel for separation, then pour a mixture of 2.5 mL of tris buffer solution for polyacrylamide gel electrophoresis B, 2.66 mL of acrylamide solution for polyacrylamide gel electrophoresis, 14.6 mL of water, 0.01 mL of N,N,N′,N′-tetramethylethlenediamine, 0.2 mL of 10% ammonium peroxodisulfate TS and 0.04 mL of 1 mol/L sodium sulfite TS on the gel. Then position a plastic sample well former so that the height of the gel for concentration is about 15 mm, and allow to set for 2 hours.

(vii) Procedure

Electrophoresis—Set the gel in an apparatus for slab gel electrophoresis, and fill the upper and lower reservoirs with tris buffer solution for polyacrylamide gel electrophoresis C. Introduce carefully 10 μL each of the sample solution and standard solution into the wells using a different well for each solution, and allow electrophoresis to proceed using the electrode of the lower reservoir as the anode. Switch off the power supply when the bromophenol blue band has migrated to about 10 mm from the bottom of the gel.

Staining—Dissolve 2.0 g of bromophenol blue R-250 in a mixture of 400 mL of methanol and 100 mL of acetic acid (100), add water to make 1000 mL, and use this solution as the staining solution. Stain the gel for 2 hours in the staining solution warmed to 40°C.

Decolorization—To 100 mL of methanol and 75 mL of acetic acid (100) add water to make 1000 mL, and use this solution as the rinsing solution. Immerse the gel removed from the staining solution in the rinsing solution to decolorise.

(3) Kallidinogenase—Dilute a suitable volume of Ulinastatin with water so that each mL of the solution contains about 50,000 Units, and use this solution as the sample solution. Take exactly 0.4 mL of the sample solution into a test tube, add exactly 0.5 mL of pH 8.2 tris buffer solution, mix, and allow the tube to stand in a water bath at 37 ± 0.2°C for 5 minutes. Add exactly 0.1 mL of substrate TS for kallidinogenase assay (4), mix, allow the tube to stand in the water bath at 37 ± 0.2°C for exactly 30 minutes, then add exactly 0.1 mL of diluted acetic acid (100) (1 in 2), mix, and use this solution as the test solution. Separately, take exactly 0.4 mL of the sample solution in a test tube, add exactly 0.5 mL of pH 8.2 tris buffer solution, mix, and allow the tube to stand in the water bath of 37 ± 0.2°C for 35 minutes. Then add exactly 0.1 mL of diluted acetic acid (100) (1 in 2), mix, add exactly 0.1 mL of substrate TS for kallidinogenase assay (4), mix, and use this solution as the control solution. Determine the absorbances of the test solution and the control solution at 405 nm as directed under Ultraviolet-visible Spectrophotometry $<2.24>$ using water as the blank, and calculate the difference between them: the difference is not more than 0.050.

**Molecular mass** Dilute a suitable volume of Ulinastatin with the mobile phase so that each mL of the solution contains about 6500 Units, and use this solution as the sample solution. Separately, dissolve 1.0 mg each of γ-globulin (mol. mass: 160,000), bovine serum albumin for test of ulinastatin (mol. mass: 67,000), and myoglobin (mol. mass: 17,000) in about 1 mL of the mobile phase, and use this solution as the molecular mass reference solution. Perform the test with 50 μL each of the sample solution and molecular mass reference solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions. Prepare a calibration curve by plotting the logarithm of molecular masses on the vertical axis and the retention times (min) of the molecular mass reference substances on the horizontal axis, and determine the molecular mass of the sample using the calibration curve and the retention time obtained with the sample solution: the molecular mass is 67,000 ± 5000.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 7 mm in inside diameter and about 60 cm in length, packed with porous silica
gel for liquid chromatography (10 – 12 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 16.33 g of potassium dihydrogenphosphate and 124.15 g of ethylene glycol in water to make 1000 mL. If necessary, adjust to pH 4.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of bovine serum albumin is about 36 minutes.

Selection of column: Proceed with 50 μL of the molecular mass reference solution according to the above operating conditions, and calculate the resolution. Use a column from which γ-globulin, bovine serum albumin, and myoglobin are eluted in this order with the resolution between their peaks being not less than 1.5, respectively.

**Antigenicity** Dilute a suitable volume of Ulinastatin with isotonic sodium chloride solution so that each mL of the solution contains 15,000 Units, and use this solution as the sample solution. Inject 0.10 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously into each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit none of the signs mentioned above, and all the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

**Toxicity** Inject intravenously 0.50 mL of Ulinastatin into each of five well-fed, healthy albino mice weighing 18 to 25 g: no mouse dies within 48 hours after injection. If any mouse dies within 48 hours, repeat the test using 5 albino mice weighing 19 to 21 g: all the animals survive for 48 hours.

**Assay** Measure exactly a suitable volume of Ulinastatin, dilute with 2,2',2''-nitrilotriethanol buffer solution, pH 7.8 so that each mL of the solution contains about 150 Units according to the labeled amount, and use this solution as the sample solution. Separately, dilute a suitable volume of Ulinastatin RS with 2,2',2''-nitrilotriethanol buffer solution, pH 7.8 so that each mL of the solution contains exactly 300, 200, 100, 50 or 0 Units, and use these solutions as the standard solutions. 2,2',2''-Nitrilotriethanol buffer solution, pH 7.8 and N-α-benzoyl-L-arginine-4-nitroanilide TS are warmed in a water bath of 25 ± 1°C for use as described below. Take exactly 0.1 mL each of the sample solution and the standard solutions in test tubes, add exactly 1.6 mL of 2,2',2''-nitrilotriethanol buffer solution, pH 7.8 mix, and put the tubes in the water bath of 25 ± 1°C. One minute after addition of the buffer solution add exactly 0.2 mL of ice-cooled trypsin TS for test of ulinastatin, mix, and put the tubes again in the water bath. One minute later add exactly 1 mL of N-α-benzoyl-L-arginine-4-nitroanilide TS, mix, and then put the tubes in the water bath. Exactly 2 minutes later add exactly 0.1 mL of diluted acetic acid (100) (1 in 2) to stop the enzyme reaction, and determine the absorbances of the solutions so obtained at 405 nm as directed under Ultraviolet-visible Spectrophotometry <\textit{2.24}>, using water as the blank. Prepare a calibration curve using the absorbances obtained with the standard solutions, and calculate ulinastatin Units in the sample solution from its absorbance by using this curve.

**Containers and storage** Containers—Tight containers. Storage—Not exceeding at −20°C.

**Urapidil**

Urapidil, when dried, contains not less than 98.0% and not more than 101.0% of C_{20}H_{28}N_{2}O_{3}.

**Description** Urapidil occurs as white to pale yellowish, white, crystals or crystalline powder. It has a bitter taste.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (95) and in acetone, and very slightly soluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Urapidil in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <\textit{2.24}>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Urapidil as directed in the potassium bromide disk method under Infrared Spectrophotometry <\textit{2.25}>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <\textit{2.60}> 156 – 161°C

**Purity** (1) Chloride <\textit{1.07}>—Dissolve 3.0 g of Urapidil in 40 mL of acetonitrile and make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of acetonitrile, 6 mL of dilute nitric acid, and water to make 50 mL (not more than 0.003%).

(2) Heavy metals <\textit{1.07}>—Proceed with 1.0 g of Urapidil according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 40 mg of Urapidil in 5
mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.00>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, ethanol (95) and ammonia water (28:22:13:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light and ammonia water (28) (22:13:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot appears not more than one and it is not more intense than the spot from the standard solution.

**Loss on drying** <2.00> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.41> Not more than 0.2% (1 g).

**Assay** Weigh accurately about 70 mg of Urapidil, previously dried, dissolve in 80 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 12.92 \text{ mg of C}_2\text{H}_3\text{N}_5\text{O}_3
\]

**Containers and storage** Containers—Well-closed containers.

**Urea**

尿素

![Urea structure](image)

\[\text{CH}_2\text{N}_2\text{O}: 60.06\]

**Urea**

Urea occurs as colorless to white crystals or crystalline powder. It is odorless, and has a cooling, saline taste. It is very soluble in water, freely soluble in boiling ethanol (95), soluble in ethanol (95), and very slightly soluble in diethyl ether.

A solution of Urea (1 in 100) is neutral.

**Identification** (1) Dissolve 0.5 g of Urea: it liquefies and the odor of ammonia is perceptible. Continue heating until the liquid becomes turbid, then cool. Dissolve the resulting lump in a mixture of 10 mL of water and 2 mL of sodium hydroxide TS, and add 1 drop of copper (II) sulfate TS: a reddish purple color develops.

(2) Dissolve 0.1 g of Urea in 1 mL of water, and add 1 mL of nitric acid: a white, crystalline precipitate is formed.

**Melting point** <2.60> 132.5 – 134.5°C

**Purity** (1) Chloride <1.00>—Perform the test with 2.0 g of Urea. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(2) Sulfate <1.14>—Perform the test with 2.0 g of Urea. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Urea according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Urea, dissolve in water, and make exactly 200 mL. Measure exactly 5 mL of this solution into a Kjeldahl flask, and proceed as directed under Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS = 0.3003 mg of CH\textsubscript{3}N\textsubscript{2}O

**Containers and storage** Containers—Well-closed containers.

**Urokinase**

ウロキナーゼ

[Urokinase](9010-53-1]

Urokinase is an enzyme, obtained from human urine, that activates plasminogen, and has the molecular mass of about 54,000.

It is a solution using a suitable buffer solution as the solvent.

It contains not less than 60,000 Units per mL, and not less than 120,000 Units per mg of protein.

**Identification** (1) Dissolve 0.07 g of fibrinogen in 10 mL of phosphate buffer solution, pH 7.4. To this solution add 1 mL of a solution of thrombin containing 10 Units per mL in isotonic sodium chloride solution, mix, place in a Petri dish about 90 mm in inside diameter, and keep horizontally until the solution is coagulated. On the surface drop 10 μL of a solution of Urokinase containing 100 Units per mL in gelatin-tris buffer solution, and stand for overnight: lysis circle is appeared.

(2) Dissolve 1.0 g of Powdered Agar in 100 mL of boric acid-sodium hydroxide buffer solution, pH 8.4, by warming, and pour the solution into a Petri dish until the height come to about 2 mm. After cooling, make two wells of 2.5 mm in diameter with the space of 6 mm. To each well place separately 10 μL of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution and 10 μL of anti-urokinase serum, and stand for overnight: a clear precipitin line is appeared.

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 mL of Urokinase according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Blood group substances—Dilute Urokinase with iso-
tonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. To anti-A type antibody for blood typing add isotonic sodium chloride solution to dilute each 32, 64, 128, 256, 512 and 1024 times, place separately 25 μL each of these solutions in six wells on the first and second lane of a V-shaped 96-wells microplate. Next, add 25 μL of the sample solution into the six wells on the first lane and 25 μL of isotonic sodium chloride solution into the six wells of the second lane, mix, and allow to stand for 30 min. To each well add 50 μL of A-type erythrocyte suspension, mix, allow to stand for 2 hours, and compare the agglutination of erythrocyte in both lanes: dilution factor of anti-A type antibody of the wells which show the agglutination is equal in both lanes.

Perform the same test by using anti-B type antibody for blood typing and B-type erythrocyte suspension.

Abnormal toxicity Dilute Urokinase with isotonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g. Observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

High-molecular mass urokinase Dilute Urokinase with gelatin-phosphate buffer solution so that each mL of the solution contains 10,000 Units, and use this solution as the sample solution. Perform the test with 100 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas of two peaks eluted closely at about 35 minutes having smaller retention time, \( A_a \), and larger retention time, \( A_b \), by the automatic integration method: the value, \( A_a/(A_a + A_b) \), is not less than 0.85.

Operating conditions—

Apparatus: Use a pumping system for the mobile phase, a sample injection port, a column, a pumping system for the reaction reagent, a reaction coil, a reaction chamber, a spectrofluorometer and a recorder. Attach a 3-way tube to the outlet for the mobile phase of the column, connect the pumping system for the reaction reagent and the reaction coil, and join outlet of the reaction coil to the spectrofluorometer.


Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with porous silica gel for liquid chromatography (10 to 12 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Reaction coil: A stainless steel column 0.25 mm in inside diameter and 150 cm in length.

Reaction coil temperature: 37°C.

Mobile phase: Gelatin-phosphate buffer solution.

Flow rate of mobile phase: 0.5 mL per minute.

Reaction reagent: 7-(Glycyl-L-arginylaminomethylcoumarin TS).

Flow rate of reaction reagent: 0.75 mL per minute.

Selection of column: Adjust the pH of Urokinase to 7.5 with sodium hydroxide TS, allow to stand at 37°C for over 24 hours, and add gelatin-phosphate buffer solution to make the solution containing 20,000 Units per mL. Proceed with 100 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of high molecular mass urokinase (mol. wt.: 54,000) and low molecular mass urokinase (mol. wt.: 33,000) in this order with the resolution between these peaks being not less than 1.0.

Assay (1) Urokinase—Pipet 1 mL of Urokinase, dilute exactly with gelatin-tris buffer solution so that each mL of the solution contains about 30 Units, and use this solution as the sample solution. Add exactly 2 mL of gelatin-tris buffer solution to contents of one ampoule of High-Molecular Mass Urokinase RS to dissolve, pipet 1 mL of this solution, dilute exactly with gelatin-tris buffer solution so that each mL of the solution contains about 30 Units, and use this solution as the standard solution. Place 1.0 mL of L-pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride TS in two silicon-coated test tubes about 10 mm in inside diameter, warm them in a water bath at 35 ± 0.2°C for 5 minutes, add separately 0.50 mL each of the sample solution and the standard solution, warm in a water bath at 35 ± 0.2°C for exactly 30 minutes, then add 0.50 mL of diluted acetic acid (100) (2 in 5). Determine the absorbances, \( A_T \) and \( A_S \), of these solutions at 405 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank. Separately place 1.0 mL of L-pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride TS in two test tubes, add 0.50 mL of diluted acetic acid (100) (2 in 5), and 0.50 mL each of the sample solution and the standard solution. Determine the absorbances, \( A_{T_0} \) and \( A_{S_0} \), of these solutions at 405 nm as the same manner, using water as the blank.

\[
\text{Amount (Units) of Urokinase} = \frac{(A_T - A_{T_0})}{(A_S - A_{S_0})} \times a \times b
\]

\( a \): Amount (Units) of urokinase in 1 mL of the standard solution

\( b \): Total volume (mL) of the sample solution

(2) Protein—Measure exactly a volume of Urokinase, equivalent to about 15 mg of protein, and perform the test as directed under Nitrogen Determination <1.00>.

Each mL of 0.005 mol/L sulfuric acid VS = 0.8754 mg of protein

Containers and storage Containers—Tight containers.

Storage—Not exceeding −20°C.
Ursodeoxycholic Acid

Ursodeoxycholic Acid

ウルソデオキシコール酸

\[
\text{C}_{24}\text{H}_{40}\text{O}_{4} \quad 392.57
\]
3α,7β-Dihydroxy-5β-cholan-24-oic acid

[128-13-2]

Ursodeoxycholic Acid, when dried, contains not less than 98.5% and not more than 101.0% of \( \text{C}_{24}\text{H}_{40}\text{O}_{4} \).

**Description** Ursodeoxycholic Acid occurs as a white crystal or powder, with bitter taste.

It is freely soluble in me thanol, in ethanol (99.5) and in acetic acid (100), and practically insoluble in water.

**Identification** Determine the infrared absorption spectrum of Ursodeoxycholic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> \([\alpha]_{D}^{20} + 59.0 – + 62.0^\circ\) (after drying, 1.0 g, ethanol (99.5), 25 mL, 100 mm).

**Melting point** <2.60> 200 – 204°C

**Purity (1)** Sulfate <1.14>—Dissolve 2.0 g of Ursodeoxycholic Acid in 20 mL of acetic acid (100), add water to make 200 mL, and allow to stand for 10 minutes. Filter this solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. To 40 mL of the sample solution add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS by adding 4 mL of acetic acid (100), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

**(2)** Heavy metal <1.07>—Proceed with 1.0 g of Ursodeoxycholic Acid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.5 g of Ursodeoxycholic Acid, previously dried, dissolve in 40 mL of ethanol (95) and 20 mL of water, and titrate <2.5d> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 39.26 mg of \( \text{C}_{24}\text{H}_{40}\text{O}_{4} \).

**Containers and storage** Containers—Well-closed containers.

Ursodeoxycholic Acid Granules

ウルソデオキシコール酸顆粒

Ursodeoxycholic Acid Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of ursodeoxycholic acid (\( \text{C}_{24}\text{H}_{40}\text{O}_{4} \): 392.57).

**Method of preparation** Prepare as directed under Granules, with Ursodeoxycholic Acid.

**Identification** To a quantity of powdered Ursodeoxycholic
Acid Granules, equivalent to 20 mg of Ursodeoxycholic Acid according to the labeled amount, add 10 mL of methanol, and shake for 20 minutes. Centrifuge this solution, pipet 4 mL of the supernatant liquid, and evaporate the methanol under reduced pressure. To the residue add 4 mL of acetone, disperse with ultrasonic waves, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of ursodeoxycholic acid in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, immediately splay evenly a solution of phosphomolybdic acid n-hydrate in ethanol (99.5) (1 in 5), and heat at 120°C for 3 to 5 minutes: the principle spot obtained from the sample solution and the spot from the standard solution show a blue color and the same Rf value.

Dissolution 6.10 When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Ursodeoxycholic Acid Granules is not less than 80%.

Start the test with an accurately weigh amount of Ursodeoxycholic Acid Granules, equivalent to about 50 mg of ursodeoxycholic acid (C₂₄H₄₀O₄) according to labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, use the subsequent filtrate as the sample solution. Separately, weigh accurately about 22 mg of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL of each sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of ursodeoxycholic acid of each solution.

Dissolution rate (%) with respect to the labeled amount of ursodeoxycholic acid (C₂₄H₄₀O₄)

\[ M_5 = \frac{M_5}{M_1} \times \frac{A_1}{A_5} \times \frac{1}{C} \times 225 \]

M₅: Amount (mg) of Ursodeoxycholic Acid Granules
M₁: Amount (g) of Ursodeoxycholic Acid Granules
C: Labeled amount (mg) of ursodeoxycholic acid (C₂₄H₄₀O₄) in 1 g

Operating conditions—
Proceed as directed in the operating conditions in the Assay.

System suitability—
System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of ursodeoxycholic acid are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0%.

Assay Weigh accurately an amount of powdered Ursodeoxycholic Acid Granules, equivalent to about 0.1 g of ursodeoxycholic acid (C₂₄H₄₀O₄), add exactly 20 mL of the internal standard solution, shake for 10 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, dissolve in exactly 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL of each sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₅, of the peak area of ursodeoxycholic acid to that of the internal standard.

\[ M_5 = \frac{M_5}{M_1} \times \frac{A_1}{A_5} \times \frac{1}{C} \times 225 \]

M₅: Amount (mg) of Ursodeoxycholic Acid for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile for liquid chromatography (11:9).
Flow rate: Adjust the flow rate so that the retention time of ursodeoxycholic acid is about 6 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ursodeoxycholic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ursodeoxycholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ursodeoxycholic Acid Tablets

ウルソデオキシコール酸錠

Ursodeoxycholic Acid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of Ursodeoxycholic Acid (C₂₄H₄₀O₄; 392.57).

Method of preparation Prepare as directed under Tablets, with Ursodeoxycholic Acid.

Identification To a quantity of powdered Ursodeoxycholic Acid Tablets, equivalent to 20 mg of Ursodeoxycholic Acid
according to the labeled amount, add 10 mL of methanol, and shake for 20 minutes. Centrifuge this solution, pipet 4 mL of the supernatant liquid, and evaporate the methanol under reduced pressure. To the residue add 4 mL of acetone, disperse with ultrasonic waves, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of ursodeoxycholic acid in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.01 > \). Spot 10 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isocyanate, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, and immediately spray evenly a solution of phosphomolybdic acid \( n \)-hydrate in ethanol (95) (1 in 5) on the plate, and heat at 120°C for 3 to 5 minutes; the principal spot obtained from the sample solution and the spot from the standard solution show a blue color and the same \( R_f \) value.

**Uniformity of dosage units** \( <6.02 > \) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Ursodeoxycholic Acid Tablets and add exactly \( V \) mL of the internal standard solution so that each mL contains about 5 mg of ursodeoxycholic acid (C\(_{24}\)H\(_{40}\)O\(_{4}\)), disperse it with ultrasonic waves, then agitate to mix for 10 minutes and then centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 \( \mu m \), and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of ursodeoxycholic acid (C\(_{24}\)H\(_{40}\)O\(_{4}\))

\[
M_s = \frac{M_i \times Q_s \times V}{20}
\]

\( M_s \): Amount (mg) of ursodeoxycholic acid for assay

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200,000).

**Dissolution** \( <6.10 > \) When the test is performed at 30 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rates in 30 minutes of a 50-mg tablet and in 45 minutes of a 100-mg tablet are not less than 80% and not less than 70%, respectively.

Start the test with 1 tablet of Ursodeoxycholic Acid Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu m \). Discard the first 10 mL of the filtrate and pipet \( V \) mL of the subsequent filtrate. Add the dissolution medium to make exactly \( V \) mL so that each mL contains about 56 \( \mu g \) of ursodeoxycholic acid (C\(_{24}\)H\(_{40}\)O\(_{4}\)) according to the labeled amount, and use the solution as the sample solution. Separately weigh accurately about 22 mg of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 100 \( \mu L \) each of sample solution and standard solution as directed under Liquid Chromatography \( <2.07 > \) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of ursodeoxycholic acid in each solution.

Dissolution rate (%) with respect to the labeled amount of ursodeoxycholic acid (C\(_{24}\)H\(_{40}\)O\(_{4}\))

\[
M_s = \frac{M_i \times Q_s \times V}{20}
\]

\( M_s \): Amount (mg) of ursodeoxycholic acid for assay

\( C \): Labeled amount (mg) of ursodeoxycholic acid in 1 tablet (C\(_{24}\)H\(_{40}\)O\(_{4}\))

**Operating conditions**—
Proceed as directed in the operating conditions in the Assay.

**System suitability**—
System performance: When the procedure is run with 100 \( \mu L \) of the standard solution under the above operating condition, the number of theoretical plates and symmetry factor of the peak of ursodeoxycholic acid are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0%.

**Assay** Weigh accurately the mass of not less than 20 Ursodeoxycholic Acid Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of ursodeoxycholic acid (C\(_{24}\)H\(_{40}\)O\(_{4}\)), add exactly 20 mL of the internal standard solution, shake for 10 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 \( \mu m \), and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, dissolve in exactly 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.07 > \) according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of ursodeoxycholic acid to that of the internal standard.

Amount (mg) of ursodeoxycholic acid (C\(_{24}\)H\(_{40}\)O\(_{4}\))

\[
M_s = \frac{M_i \times Q_s \times V}{20}
\]

\( M_s \): Amount (mg) of ursodeoxycholic acid for assay

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200,000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecysilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile for liquid chromatography (11:9).

Flow rate: Adjust the flow rate so that the retention time of ursodeoxycholic acid is about 6 minutes.

**System suitability**—
System performance: When the procedure is run with 10 \( \mu L \) of the standard solution according to the above operating conditions, ursodeoxycholic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ursodeoxycholic acid to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

**L-Valine**

\[ \text{C}_5\text{H}_{11}\text{NO}_2: 117.15 \]

(2S)-2-Amino-3-methylbutanoic acid [72-18-4]

L-Valine, when dried, contains not less than 98.5% of C5H11NO2.

**Description** L-Valine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly sweet taste, which becomes bitter.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (95). It dissolves in dilute hydrochloric acid.

**Identification** Determine the infrared absorption spectrum of L-Valine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \( \left[ \alpha \right]_{D}^{20} \): +26.5 – +29.0° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** <2.54> Dissolve 0.5 g of L-Valine in 20 mL of water: the pH of this solution is between 5.5 and 6.5.

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of L-Valine in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of L-Valine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Valine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Valine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Valine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 2 ppm).

(6) Arsenic <1.17>—Proceed with 1.0 g of L-Valine, prepare the test solution according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Valine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution.

Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.12 g of L-Valine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 11.72 mg of C5H11NO2

**Containers and storage** Containers—Tight containers.
Vancomycin Hydrochloride

パンコマイシン塩酸塩

Vancomycin Hydrochloride is the hydrochloride of a glycopeptide substance having antibacterial activity produced by the growth of *Streptomyces orientalis*.

It contains not less than 1000 μg (potency) and not more than 1200 μg (potency) per mg, calculated on the anhydrous basis. The potency of Vancomycin Hydrochloride is expressed as mass (potency) per mg, calculated on the anhydrous basis, water, 20 mL, 100 mm).

**Description** Vancomycin Hydrochloride occurs as a white powder. It is freely soluble in water, soluble in formamide, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile. It is hygroscopic.

**Identification (1)** Determine the absorption spectrum of a solution of Vancomycin Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.4>, and compare the spectrum with the Reference Spectrum of Vancomycin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 20 mg of Vancomycin Hydrochloride in 10 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

**Optical rotation** <2.4> [α]D<sub>20</sub>: −30° to −40° (0.2 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

**pH** <2.5> The pH of a solution obtained by dissolving 0.25 g of Vancomycin Hydrochloride in 5 mL of water is between 2.5 and 4.5.

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Vancomycin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Related substances—Dissolve 0.10 g of Vancomycin Hydrochloride in 10 mL of the mobile phase A, and use this solution as the standard solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under the Liquid Chromatography <2.3> according to the following conditions. If necessary, proceed with 20 μL of the mobile phase A in the same manner to compensate for the base line. Determine each peak area by the automatic integration method: the area of each peak other than vancomycin from the sample solution is not larger than the peak area of vancomycin from the standard solution, and the total area of the peaks other than vancomycin is not larger than 3 times of the peak area of vancomycin from the standard solution.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wave-length: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of triethylamine buffer solution, pH 3.2, acetonitrile and tetrahydrofuran (92:7:1). Adjust the amount of acetonitrile so that the retention time of vancomycin is 7.5 to 10.5 minutes.

Mobile phase B: A mixture of triethylamine buffer solution, pH 3.2, acetonitrile and tetrahydrofuran (70:29:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 12</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>12 – 20</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>20 – 22</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 1.5 mL per minute.

Time span of measurement: As long as about 2.5 times of the retention time of vancomycin beginning after the solvent peak.
System suitability—

Test for required detectability: Confirm that the peak area of vancomycin obtained from 20 \( \mu L \) of the standard solution is equivalent to 3 to 5% of that from 20 \( \mu L \) of the sample solution.

System performance: Dissolve 5 mg of Vancomycin Hydrochloride in 10 mL of water, heat at 65°C for 48 hours, and cool to the ordinal temperature. When the procedure is run with 20 \( \mu L \) of this solution under the above operating conditions, related substance 1, vancomycin and related substance 2 are eluted in this order, the resolution between the peaks of the related substance 1 and vancomycin is not less than 3, the number of theoretical plates of the peak of vancomycin is not less than 1500, and the related substance 2 is eluted between 15 and 18 minutes.

System repeatability: When the test is repeated 5 times with 20 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vancomycin is not more than 2.0%.

**Water** \( \text{<2.48} \) Not more than 5.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for Karl Fisher method (3:1)).

**Residue on ignition** \( \text{<2.44} \) Not more than 1.0% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics \( \text{<4.02} \) according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.2 to 6.4 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Vancomycin Hydrochloride RS, equivalent to about 25 mg (potency), dissolve in water to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 100 \( \mu g \) (potency) and 25 \( \mu g \) (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Vancomycin Hydrochloride, equivalent to about 25 mg (potency), and dissolve in water to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 100 \( \mu g \) (potency) and 25 \( \mu g \) (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

## Vancomycin Hydrochloride for Injection

注射用バンコマイシン塩酸塩

Vancomycin Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled amount of vancomycin (\( \text{C}_{66}\text{H}_{75}\text{Cl}_{2}\text{N}_{9}\text{O}_{24} \): 1449.25).

**Method of preparation** Prepare as directed under Injections, with Vancomycin Hydrochloride.

**Description** Vancomycin Hydrochloride for Injection occurs as white masses or a white powder.

**Identification** (1) Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 5 mg (potency) of Vancomycin Hydrochloride, in 50 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \( \text{<2.24} \); it exhibits a maximum between 279 and 283 nm.

(2) Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 20 mg (potency) of Vancomycin Hydrochloride, in 10 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

**pH** \( \text{<2.54} \) The pH of a solution prepared by dissolving an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.5 g (potency) of Vancomycin Hydrochloride according to the labeled amount, in 10 mL of water is between 2.5 and 4.5.

**Purity** (1) Clarity and color of solution—Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.5 g (potency) of Vancomycin Hydrochloride according to the labeled amount, in 10 mL of water: the solution is clear and colorless to pale yellow, and the absorbance of the solution, determined at 465 nm as directed under Ultraviolet-visible Spectrophotometry \( \text{<2.24} \), is not more than 0.05.

(2) Related substances—Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.1 g (potency) of Vancomycin Hydrochloride according to the labeled amount, in 10 mL of the mobile phase A, and use this solution as the sample solution.

Proceed as directed in the Purity (2) under Vancomycin Hydrochloride.

**Water** \( \text{<2.48} \) Not more than 5.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for Karl Fisher method and methanol for Karl Fisher method (3:1)).

**Bacterial endotoxins** \( \text{<4.01} \) Less than 0.25 EU/mg (potency).

**Uniformity of dosage unit** \( \text{<6.02} \) It meets the requirement of the Mass variation test.

**Foreign insoluble matter** \( \text{<6.06} \) Perform the test according to the Method 2: it meets the requirement.

**Insoluble particulate matter** \( \text{<6.07} \) It meets the requirement.
Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Vancomycin Hydrochloride.

(ii) Sample solutions—Weigh accurately the contents of not less than 10 Vancomycin Hydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 25 mg (potency) of Vancomycin Hydrochloride according to the labeled amount, and dissolve in water to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Vasopressin Injection

バソプレシン注射液

Vasopressin Injection is an aqueous solution for injection.

It contains synthetic vasopressin or the pressor principle, vasopressin, obtained from the posterior lobe of the pituitary of healthy cattle and pigs, from which the majority of the oxytocic principle, oxytocin, has been removed.

It contains not less than 85% and not more than 120% of the labeled vasopressin Units.

Method of preparation Prepare as directed under Injections, with vasopressin prepared by synthesis or obtained from the posterior lobe of the pituitary.

Description Vasopressin Injection is a clear and colorless liquid. It is odorless or has a slight, characteristic odor. pH: 3.0 – 4.0

Purity Oxytocic principle—When tested by the following procedure, Vasopressin Injection contains not more than 0.6 oxytocin Units for each determined 10 vasopressin Units.

(i) Standard stock solution: Dissolve 2000 Units of Oxytocin RS, according to the labeled Units, in exactly 10 mL of diluted acetic acid (100) (1 in 400). Pipet 1 mL of this solution, and add diluted acetic acid (100) (1 in 400) to make exactly 10 mL. Store in a cold place, avoiding freezing. Use within 6 months from the date of preparation.

(ii) Standard solution: Dilute the standard stock solution with isotonic sodium chloride solution so that each mL of the solution contains 0.02 oxytocin Units.

(iii) Sample solution: Assume oxytocin Units as equivalent to 6/100 of the determined vasopressin Units. Dilute Vasopressin Injection with isotonic sodium chloride solution so that each mL of the resulting solution is expected to contain 0.02 oxytocin Unit.

(iv) Apparatus: Use the apparatus for the uterus contraction test, equipped with a thermostatic bath. Maintain a temperature of the bath at 37°C to 38°C with a variation of not more than 0.1°C during the course of the test. Use a 100-mL Magnus’ chamber for suspending the uterus.

(v) Test animal: Use healthy, virgin and metestrus guinea pigs weighing between 175 g and 350 g. They have been bred under conditions where they have been completely isolated from the sight and smell of males since the time of weaning.

(vi) Procedure: Immerse the Magnus’ chamber in the bath maintained at a constant temperature, add Locke-Ringer’s solution to the chamber, and introduce oxygen into the solution at a moderate rate. Sacrifice a guinea pig by means of a blow on the head, immediately remove the uterus from the body, suspend it in the chamber, and connect one horn of the uterus to the lever with a thread. If necessary, weigh the lever provided that the mass is not changed throughout the assay. Start the assay after 15 to 30 minutes when the uterus is completely relaxed. Administer the same quantities, 0.1 to 0.5 mL, of the standard solution and the sample solution to the Magnus’ chamber alternately twice with regular intervals of between 10 and 20 minutes to contract the uterus, finally administer the standard solution in a quantity which is 25% larger than the preceding doses, and measure the height of every contraction. The mean height of uterus contraction caused by the standard solution is equal to or higher than that caused by the sample solution. The height of contraction caused by the increased dose of the standard solution is distinctly higher than those caused by the preceding doses of the standard solution.

Bacterial endotoxins <4.01> Less than 15 EU/vasopressin Unit.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (i) Test animals: Use healthy male rats weighing between 200 g and 300 g.

(ii) Standard stock solution: Dissolve 2000 Units of Vasopressin RS, according to the labeled Units, in exactly 100 mL of diluted acetic acid (100) (1 in 400). Pipet 1 mL of this solution, and add diluted acetic acid (100) (1 in 400) to make exactly 10 mL. Store in a cold place, avoiding freezing. Use within 6 months from the date of preparation.

(iii) Standard solution: Dilute the standard stock solution with isotonic sodium chloride solution so that 0.2 mL of the obtained solution causes blood pressure increases of between 35 mmHg and 60 mmHg in test animals when injected according to (vi), and designate this solution as the high-dose standard solution (S_h). Then dilute this solution with isotonic sodium chloride solution 1.5 to 2.0 times by volume, and designate it as the low-dose standard solution (S_l).

(iv) Sample solution: Dilute an accurately measured volume of Vasopressin Injection with isotonic sodium chloride solution so that the obtained solution contains the same concentration in Units as the high-dose standard solution based on the labeled Units, and designate it as the high-dose sample solution (T_h). Then dilute this solution with isotonic sodium chloride solution 1.5 to 2.0 times by volume, and
 designate it as the low-dose sample solution (T1). Make the concentration ratio of S1 to S4 equal to the ratio of T1 to T4. When the sensitivity of an animal is changed, adjust the concentration of S1 and T1 before the next set of assay is started. However, keep the same ratio of S1 to S4 and T1 to T4, as in the primary set.

(v) Dose of injection: Although 0.2 mL of each solution is usually injected, the dose of injection can be determined based from preliminary tests or experiences. Inject the same volume throughout a set of tests.

(vi) Procedure: Inject subcutaneously 0.7 mL of a solution of ethyl carbamate (1 in 4) per 100 g of body mass to anesthetize the test animals and cannulate the trachea. Under artificial respiration (about 60 strokes per minute), remove a part of the second cervical vertebra, cut off the spinal cord and destroy the brain through the foramen magnum. Insert a cannula filled with isotonic sodium chloride solution into a carotid artery, and connect the cannula to a manometer for blood pressure measurement with a vinyl tube. The cannula and the vinyl tube have previously been filled with isotonic sodium chloride solution. Inject the standard and the sample solutions at regular intervals of 10 to 15 minutes into the femoral vein through the cannula followed by 0.3 mL of the isotonic solution when the blood pressure increases caused by each solution returns to the original level. Measure the height of blood pressure increases within 1 mmHg on the kymogram. Maintain a constant temperature between 20°C and 25°C during the assay. In advance, make four pairs from S1, S2, T1, T2 as follows. Randomize the order of injection for pairs, but keep the order of injection within pairs as indicated.

Pair 1: S1, T1 Pair 2: S2, T2 Pair 3: T1, S1 Pair 4: T2, S1

Carry out this assay using the same animals throughout a set of four pairs of observations. Perform this assay with two sets. If necessary, however, use the different animals for both sets of tests.

(vii) Calculation: Subtract increases of blood pressure caused by the low dose from those caused by the high dose in the Pair 1, 2, 3 and 4 of each set, and obtain the responses \( y_1, y_2, y_3 \) and \( y_4 \), respectively. Sum up \( y_i \) for each set to obtain \( Y_1 \) and \( Y_2, Y_3 \) and \( Y_4 \) in the same way.

Units in each mL of Vasopressin Injection

\[ M = \log (M) \times b/a \]

\[ I = \log (S)/Y_0 \]

\[ Y_0 = Y_1 + Y_2 + Y_3 - Y_4 \]

\[ Y_5 = Y_1 + Y_2 + Y_3 + Y_4 \]

\( a \): Volume (mL) of Vasopressin Injection sampled.

\( b \): Total volume (mL) of the high-dose sample solution prepared by diluting with isotonic sodium chloride solution.

Compute \( L \) (P = 0.95) by the following equation, and confirm \( L \) to be 0.15 or less. If \( L \) exceeds 0.15, repeat the test, improving the conditions of the assay or increasing the number of sets until \( L \) reaches 0.15 or less.

\[ L = 2\sqrt{(C - 1)(CM^2 + F)} \]

\( C \): Number of sets

\[ s^2 = \frac{(\Sigma y_1 - (Y_1/y_0) - (Y_2/y_0) + (Y_3/y_0) + (Y_4/y_0))/n}{n} \]

\( \Sigma y_2 \): The sum of the squares of \( y_1 \), \( y_2 \), \( y_3 \) and \( y_4 \).

\( Y = Y_1 + Y_2 + Y_3 + Y_4 \)

\( Y' \): The sum of the squares of the sum of \( y_1 \), \( y_2 \), \( y_3 \) and \( y_4 \) in each set.

\[ n = 3(f - 1) \]

\( t^2 \): Value shown in the following table against \( n \) for which \( s^2 \) is calculated.

<table>
<thead>
<tr>
<th>( n )</th>
<th>( t^2 = F_1 )</th>
<th>( n )</th>
<th>( t^2 = F_1 )</th>
<th>( n )</th>
<th>( t^2 = F_1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>161.45</td>
<td>13</td>
<td>4.667</td>
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<tr>
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<tr>
<td>12</td>
<td>4.747</td>
<td>24</td>
<td>4.260</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Containers and storage Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Expiration date 36 months after preparation.

Verapamil Hydrochloride

Iproveratril Hydrochloride

ベラパミル塩酸塩

\[
\text{C}_{27}\text{H}_{38}\text{N}_2\text{O}_4\cdot\text{HCl}: 491.06
\]

\[ \text{2(2RS)-3-[3,4-Dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-(1-methylethyl)pentanenitrile monohydrochloride} \]

[152-11-4]

Verapamil Hydrochloride, when dried, contains not less than 98.5% of \( \text{C}_{27}\text{H}_{38}\text{N}_2\text{O}_4\cdot\text{HCl} \).

Description Verapamil Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in methanol, in acetic acid (100) and in chloroform, soluble in ethanol (95) and in acetic anhydride, sparingly soluble in water, and practically insoluble in

\[ \text{acetone}, \text{ether}, \text{and chloroform} \]
diethyl ether.

**Identification (1)** To 2 mL of a solution of Verapamil Hydrochloride (1 in 50) add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Verapamil Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Verapamil Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Verapamil Hydrochloride (1 in 50) responds to the Qualitative Tests C<1.09> for chloride.

**Melting point** C<2.60> 141 - 145°C.

**pH** C<2.54> Dissolve 1.0 g of Verapamil Hydrochloride in 20 mL of freshly boiled and cooled water by warming, and cool: the pH of this solution is between 4.5 and 6.5.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Verapamil Hydrochloride in 20 mL of water by warming: the solution is clear and colorless.

(2) Heavy metals C<1.07>—Proceed with 1.0 g of Verapamil Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic C<1.11>—Prepare the test solution with 1.0 g of Verapamil Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Verapamil Hydrochloride in exactly 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution (1). Separately, pipet 5 mL of standard stock solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography C<2.07>. Spot 10 µL each of the sample solution and standard solutions (1) and (2) on two plates of silica gel for thin-layer chromatography. With the one plate, develop the plate with a mixture of cyclohexane and diethylamine (17:3) to a distance of about 15 cm, air-dry the plate, heat at 110°C for 1 hour, and cool.

Examine immediately after spraying even iron (III) chloride-iodine TS on the plate: the three spots, having more intense color in the spots other than the principal spot and the original point from the sample solution, are not more intense than the spot from the standard solution (2) in color.

The remaining spots from the sample solution are not more intense than the spot from the standard solution (1) in color. With another plate, develop the plate with a mixture of toluene, methanol, acetone and acetic acid (100) (14:4:1:1), and perform the test in the same manner.

**Loss on drying** C<2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition** C<2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.7 g of Verapamil Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate C<2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 49.11 mg of C_{27}H_{35}N_{2}O_{4}.HCl

**Containers and storage** Containers—Well-closed containers. Storage—Light-resistant.

**Verapamil Hydrochloride Tablets**

Verapamil Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of verapamil hydrochloride (C_{27}H_{35}N_{2}O_{4}.HCl: 491.06).

**Method of preparation** Prepare as directed under Tablets, with Verapamil Hydrochloride.

**Identification (1)** To a quantity of pulverized Verapamil Hydrochloride Tablets, equivalent to 0.2 g of Verapamil Hydrochloride according to the labeled amount, add 70 mL of 0.02 mol/L hydrochloric acid TS, and shake occasionally in a water bath at 60°C. After cooling, add 0.02 mol/L hydrochloric acid TS to make 100 mL, and filter. To 3 mL of the filtrate add several drops of Reinecke's salt TS: a light red precipitate is formed.

(2) To 2 mL of the filtrate obtained in (1) add 0.02 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry C<2.25>: it exhibits maxima between 227 nm and 231 nm, and between 276 nm and 280 nm.

**Uniformity of dosage unit** C<6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Verapamil Hydrochloride Tablets add 70 mL of 0.02 mol/L hydrochloric acid TS, disintegrate the tablet by occasional shaking in a water bath at 60°C for about 30 minutes, and then shake for 5 minutes. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, take exactly V mL of the subsequent filtrate, add 0.02 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 40 μg of verapamil hydrochloride (C_{27}H_{35}N_{2}O_{4}.HCl), and use this solution as the sample solution. Hereafter, proceed as directed in the Assay.

**Amount (mg) of verapamil hydrochloride (C_{27}H_{35}N_{2}O_{4}.HCl)**

\[ M_S = \frac{M_5 \times A_T \times A_S \times \sqrt{V/V \times 1/25}} {} \]

**Assay** To 10 tablets of Verapamil Hydrochloride Tablets add 140 mL of 0.02 mol/L hydrochloric acid TS, disintegrate the tablets by occasional shaking in a water bath at
60°C for about 30 minutes, and then shake for 5 minutes. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 200 mL, and filter. Discard the first 20 mL of the filtrate, take an exact volume of the subsequent filtrate, equivalent to about 4 mg of verapamil hydrochloride (C₂₇H₃₈N₂O₄·HCl), add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of verapamil hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in 70 mL of 0.02 mol/L hydrochloric acid TS by occasional shaking in a water bath at 60°C. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₅, of the sample solution and standard solution at 278 nm as directed under Ultraviolet-visible Spectrophotometry §2.24.1.

Amount (mg) of verapamil hydrochloride (C₂₇H₃₈N₂O₄·HCl) = Mₛ × A₁/₅ × 1/25

Mₛ: Amount (mg) of verapamil hydrochloride for assay

Containers and storage: Containers—Tight containers.

**Vinblastine Sulfate**

ビンブラスチン硫酸塩

![Chemical Structure](image)

C₄₆H₅₸N₄O₉·H₂SO₄: 909.05
Methyl (3aR,4R,5S,6aR,10bR,13aR)-4-acetoxy-3a-ethyl-9-[(5S,7S,9S)-5-ethyl-5-hydroxy-9-methoxy carbonyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3azacycloundecino[5,4-b]indol-9-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,6a,7,11,12,13a-octahydro-1H-indolizin[8,1-cf]carbazole-5-carboxylate monosulfate [143-67-9]

Vinblastine Sulfate contains not less than 96.0% and not more than 102.0% of C₄₆H₅₸N₄O₉·H₂SO₄, calculated on the dried basis.

**Description** Vinblastine Sulfate occurs as a white to pale yellow powder.

- It is soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95.9%).
- It is hygroscopic.
- Optical rotation [α]₂₀ = -28 to -35° (0.20 g calculated on the dried basis, methanol, 10 mL, 100 mm).

**Identification** (1) Determine the absorption spectrum of a solution of Vinblastine Sulfate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry §2.24.1, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vinblastine Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Vinblastine Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry §2.25.1, and compare the spectrum with the Reference Spectrum or the spectrum of Vinblastine Sulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Vinblastine Sulfate (1 in 100) responds to the Qualitative Tests §1.092 for sulfate.

**pH** §2.54.2: Dissolve 15 mg of Vinblastine Sulfate in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

**Purity** (1) Clarity and color of solution—Dissolve 50 mg of Vinblastine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve about 4 mg of Vinblastine Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 200 µL each of the sample solution and standard solution as directed under Liquid Chromatography §2.01, according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of peak other than the main peak obtained from sample solution is not larger than 1/4 times the peak area of vinblastine from the standard solution, and the total area of the peaks other than the main peak is not larger than 3/4 times the peak area of vinblastine from the standard solution.

**Operating conditions**

- Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
- Time span of measurement: About 4 times as long as the retention time of vinblastine beginning after the solvent peak.

**System suitability**

- Test for required detectability: To exactly 2.5 mL of the standard solution add water to make exactly 100 mL. Confirm that the peak area of vinblastine obtained from 200 µL of this solution is equivalent to 1.7 to 3.3% of that from 200 µL of the standard solution.
- System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 200 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vinblastine is not more than 1.5%.

**Loss on drying** Perform the test with about 10 mg of Vinblastine Sulfate as directed in Method 2 under the Thermal Analysis §2.52.2 according to the following conditions: not more than 15.0%.

**Operating conditions**

- Heating rate: 5°C per minute.
- Temperature range: room temperature to 200°C.
- Atmospheric gas: dried Nitrogen.
- Flow rate of atmospheric gas: 40 mL per minute.

**Assay** Weigh accurately about 10 mg each of Vinblastine Sulfate and Vinblastine Sulfate RS (previously determine the loss on drying in the same conditions as Vinblastine Sulfate),
dissolve in water to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography (2.07) according to the following conditions, and determine the peak areas, \( A_1 \) and \( A_s \), of vinblastine.

\[
\text{Amount (mg)} \times \frac{C_{46}H_{58}N_4O_9.H_2SO_4}{M_s} = M_s \times \frac{A_1}{A_s}
\]

\( M_s: \) Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis

**Operating conditions**

- **Detector:** An ultraviolet absorption photometer (wavelength: 262 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature:** A constant temperature of about 25°C.
- **Mobile phase:** To 7 mL of diethylamine add water to make 500 mL, and adjust the pH to 7.5 with phosphoric acid. To 380 mL of this solution add 620 mL of a mixture of methanol and acetonitrile (4:1).
- **Flow rate:** Adjust the flow rate so that the retention time of vinblastine is about 8 minutes.

**System suitability**

- **System performance:** Dissolve 10 mg each of Vinblastine Sulfate and vincristine in 25 mL of water. When the procedure is run with 20 μL of this solution under the above operating conditions, vincristine and vinblastine are eluted in this order with the resolution between these peaks being not less than 4.
- **System repeatability:** When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vinblastine is not more than 1.0%.

**Containers and storage**

- **Containers—**Tight containers.
- **Storage—**Light-resistant, at not exceeding –20°C.

**Vinblastine Sulfate for Injection**

注射用ビンブラスチン硫酸塩

Vinblastine Sulfate for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of vinblastine sulfate (\( \text{C}_{46}\text{H}_{58}\text{N}_4\text{O}_9\cdot \text{H}_2\text{SO}_4: \text{909.05} \)).

**Method of preparation**

Prepare as directed under Injections, with Vinblastine Sulfate.

**Description**

Vinblastine Sulfate for Injection occurs as white to pale yellow, light masses or powder. It is freely soluble in water.

The pH of a solution (1 in 1000) is 3.5 – 5.0.

**Identification**

Proceed as directed in the Identification (1) under Vinblastine Sulfate.

**Purity**

Related substances—Dissolve 4 mg of Vinblastine Sulfate for Injection in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 200 μL of the sample solution and standard solution as directed under Liquid Chromatography (2.07) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than the main peak from the sample solution is not larger than 1/2 times the peak area of vinblastine from the standard solution, and the total area of the peaks other than the main peak is not larger than 2 times the peak area of vinblastine from the standard solution.

**Operating conditions**

- Perform as directed in the operating conditions in Purity (2) under Vinblastine Sulfate.

**System suitability**

- Perform as directed in the system suitability in Purity (2) under Vinblastine Sulfate.

**Bacterial endotoxins (4.01)**

Less than 10 EU/mg.

**Uniformity of dosage units (6.02)**

Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Dissolve 1 Vinblastine Sulfate for Injection in water to make exactly 4 mL so that each mL contains about 0.4 mg of vinblastine sulfate (\( \text{C}_{46}\text{H}_{58}\text{N}_4\text{O}_9\cdot \text{H}_2\text{SO}_4 \)), and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Vinblastine Sulfate RS (previously determine the loss on drying in the same conditions as Vinblastine Sulfate), dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Vinblastine Sulfate.

\[
\text{Amount (mg)} \times \frac{\text{C}_{46}\text{H}_{58}\text{N}_4\text{O}_9\cdot \text{H}_2\text{SO}_4}{M_s} = M_s \times \frac{A_1}{A_s} \times 25/V
\]

\( M_s: \) Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis

**Foreign insoluble matter (6.06)**

Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter (6.07)**

It meets the requirement.

**Sterility (4.06)**

Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay**

Take an amount of Vinblastine Sulfate for Injection, equivalent to 0.10 g of vinblastine sulfate (\( \text{C}_{46}\text{H}_{58}\text{N}_4\text{O}_9\cdot \text{H}_2\text{SO}_4 \)), dissolve each content with a suitable amount of water, transfer into a 100-mL volumetric flask, wash each container with water, transfer the washings into the volumetric flask, and add water to make exactly 100 mL.

Pipet 10 mL of this solution, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Vinblastine Sulfate RS (previously determine the loss on drying in the same conditions as Vinblastine Sulfate), dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Vinblastine Sulfate.

\[
\text{Amount (mg)} \times \frac{\text{C}_{46}\text{H}_{58}\text{N}_4\text{O}_9\cdot \text{H}_2\text{SO}_4}{M_s} = M_s \times \frac{A_1}{A_s} \times 10
\]

\( M_s: \) Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis
Containers and storage  Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant, at 2 to 8°C.

Vincristine Sulfate

ビンクリスチン硫酸塩

C₄₆H₅₆N₄O₁₀.H₂SO₄: 923.04
Methyl (3aR,4R,5S,6aR,10bR,13aR)-4-acetoxy-3a-ethyl-9-[4S(5S,7S,9S)5-ethyl-5-hydroxy-9-methoxy-3a-ethyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3-azacycloundecino[5,4-b]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,6,7,8,9,10-octahydro-1H-indolizino[8,1-c]carbazole-5-carboxylate monosulfate [2068-78-2]

Vincristine Sulfate contains not less than 95.0% and not more than 105.0% of C₄₆H₅₆N₄O₁₀.H₂SO₄, calculated on the dried basis.

Description  Vincristine Sulfate occurs as a white to light yellowish white powder.
It is very soluble in water, and practically insoluble in ethanol (99.5).
It is hygroscopic.
Optical rotation $[\alpha]_{D}^{20}$: +28.5 to +35.5° (0.2 g, calculated on the dried basis, water, 10 mL, 100 mm).

Identification (1)  Determine the absorption spectrum of a solution of Vincristine Sulfate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\leq 2.25^a$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vincristine Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2)  Determine the infrared absorption spectrum of Vincristine Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\leq 2.25^c$, and compare the spectrum with the Reference Spectrum or the spectrum of Vincristine Sulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3)  A solution of Vincristine Sulfate (1 in 100) responds to the Qualitative Tests $\leq 1.09^d$ for sulfate.

pH $\leq 2.5^a$  Dissolve 10 mg of Vincristine Sulfate in 10 mL of water: the pH of this solution is between 3.5 and 4.5.

Purity (1)  Clarity and color of solution—Dissolve 50 mg of Vincristine Sulfate in 10 mL of water: the solution is clear and colorless.

(2)  Related substances—Dissolve 10 mg of Vincristine Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 200 µL each of the sample solution and standard solution as directed under Liquid Chromatography $\leq 2.0^d$ according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than vincristine, desacetyl vincristine and vinblastine from the sample solution is not larger than 1/4 times the peak area of vincristine from the standard solution. Furthermore, the total area of the peaks other than vincristine from the sample solution is not larger than the area of vincristine from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 297 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octysilsanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase A: methanol.
Mobile phase B: A mixture of water and diethylamine (197.3), adjusted the pH to 7.5 with phosphoric acid.
Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 12</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>12 - 27</td>
<td>62 → 92</td>
<td>38 → 8</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time of vincristine is about 15 minutes.
Time span of measurement: About 1.7 times as long as the retention time of vincristine, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 200 mL. Confirm that the peak area of vincristine obtained from 200 µL of this solution is equivalent to 1.75 to 3.25% of that from 200 µL of the standard solution.
System performance: Dissolve 15 mg each of Vincristine Sulfate and vinblastine sulfate in 100 mL of water. When the procedure is run with 200 µL of this solution under the above operating conditions, vincristine and vinblastine are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 6 times with 200 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vincristine is not more than 1.5%.

Loss on drying  Perform the test with about 10 mg of Vincristine Sulfate as directed in Method 2 under Thermal Analysis $\leq 2.5^a$ according to the following conditions: not
more than 12.0%.

Operating conditions—
Heating rate: 5°C per minute.
Temperature range: room temperature to 200°C.
Atmospheric gas: dried nitrogen.
Flow rate of atmospheric gas: 40 mL per minute.

Assay
Weigh accurately about 10 mg each of Vincristine Sulfate and Vincristine Sulfate RS (separately determine the loss on drying in the same conditions as Vincristine Sulfate), dissolve each in water to make exactly 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (<2.01>) according to the following conditions, and determine the vincristine peak areas, A₁ and Aₛ, of both solutions.

Amount (mg) of C₄₆H₅₆N₄O₁₀.H₂SO₄ = Mₛ × A₁/Aₛ

Mₛ: Amount (mg) of Vincristine Sulfate RS, calculated on the dried basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 297 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Adjust the pH to 7.5 of a mixture of water and diethylamine (59:1) with phosphoric acid. To 300 mL of this solution add 700 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of vincristine is about 7 minutes.

System suitability—
System performance: Dissolve 5 mg each of Vincristine Sulfate and vinblastine sulfate in 5 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, vinceristine and vinblastine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vincristine is not more than 1.0%.

Containers and storage
Containers—Tight containers.
Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere.

Vitamin A Oil Capsules

Vitamin A Oil

Vitamin A Oil is synthetic vitamin A esters diluted with fixed oils.
It contains not less than 30,000 vitamin A Units per g.
It may contain suitable antioxidants.
It contains not less than 90.0% and not more than 120.0% of the labeled amount of vitamin A.

Description
Vitamin A Oil is a yellow to yellow-brown, clear or slightly turbid oil. It is odorless or has a faint, characteristic odor.
It is decomposed upon exposure to air or light.

Identification
Dissolve Vitamin A Oil, Retinol Acetate RS and Retinol Palmitate RS, equivalent to 15,000 Units, in 5 mL of petroleum ether, and use these solutions as the sample solution, the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography (<2.05>). Spot 5 μL of each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution has the same color tone and the same RF value with the blue spot obtained from the standard solution (1) or the standard solution (2).

Purity
(1) Acidity—Dissolve 1.2 g of Vitamin A Oil in 30 mL of a mixture of neutralized ethanol and diethyl ether (1:1), boil gently for 10 minutes under a reflux condenser, cool, and add 5 drops of phenolphthalein TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.
(2) Rancidity—No unpleasant odor of rancid oil is perceptible by warming Vitamin A Oil.

Assay
Proceed as directed in Method 1-1 under Vitamin A Oil (<2.55>).

Containers and storage
Containers—Tight containers.
Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere.
Compound Vitamin B Powder

Method of preparation

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine Nitrate</td>
<td>10 g</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>10 g</td>
</tr>
<tr>
<td>Pyridoxine Hydrochloride</td>
<td>10 g</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>100 g</td>
</tr>
<tr>
<td>Starch, Lactose Hydrate or their mixture</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description

Compound Vitamin B Powder is orange-yellow in color. It has a slightly bitter taste.

Identification (1) Shake 2 g of Compound Vitamin B Powder with 100 mL of water, filter, and to 5 mL of the filtrate add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and observe under ultraviolet light: the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline (thiamine).

(2) Shake 0.1 g of Compound Vitamin B Powder with 100 mL of water, and filter. Perform the following tests with the filtrate (riboflavin).

(i) The filtrate is light yellow-green in color and has an intense yellow-green fluorescence. This color and fluorescence of the solution disappears upon the addition of 0.02 g of sodium hydrosulfito to 5 mL of the filtrate, and again appears by shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(ii) To 10 mL of the filtrate placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, after illuminating with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31), and shake thoroughly with 5 mL of chloroform: the chloroform layer shows yellow-green fluorescence.

(iii) To 10 mL of the filtrate placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS and 40 mg of manganese dioxide. Heat on a water bath for 30 minutes, cool, and filter. Add 5 mL of 2-propanol to 1 mL of the filtrate, and use the solution as the sample solution. To 3 mL of the sample solution add 2 mL of barbital buffer solution, 4 mL of 2-propanol and 2 mL of a freshly prepared solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monomine in ethanol (95) (1 in 4000) prepared when required for use: a blue color develops. To 1 mL of the sample solution add 1 mL of a saturated boric acid solution, and proceed as directed in the same manner as above: no blue color develops (pyridoxine).

(4) Shake 0.5 g of Compound Vitamin B Powder with 10 mL of ethanol (95), filter, and evaporate 1 mL of the filtrate on a water bath to dryness. Add 0.01 g of 2,4-dinitrochlorobenzen to the residue, heat gently for 5–6 seconds to fuse, and after cooling, add 4 mL of potassium hydroxide-ethanol TS: a red color develops (nicotinamide).

(5) Shake 1 g of Compound Vitamin B Powder with 5 mL of diluted ethanol (7 in 10), filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g each of thiamine mononitrate, riboflavin, pyridoxine hydrochloride and nicotinamide in 1 mL, 50 mL, 1 mL and 1 mL of water, respectively, and use these solutions as standard solutions (1), (2), (3) and (4). Perform the test with these solutions as directed under Thin-layer Chromatography <2.09>. Spot 2 µL each of the sample solution and standard solutions (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and acetic acid (100) (100:50:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): four spots from the sample solution show the same color tone and the same RF value as the corresponding spots from standard solutions (1), (2), (3) and (4).

Containers and storage

Containners—Well-closed containers.

Storage—Light-resistant.

Voglibose

ボグリボース

C_{10}H_{17}NO_7: 267.28
3,4-Dideoxy-4-[2-hydroxy-1-(hydroxymethyl)ethylamino]-2-(hydroxymethyl)-D-epi-inositol

[83480-29-9]

Voglibose contains not less than 99.5% and not more than 101.0% of C_{10}H_{17}NO_7, calculated on the anhydrous basis.

Description

Voglibose occurs as white crystals or crystalline powder.

It is very slightly soluble in water, freely soluble in acetic acid (100), slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the infrared absorption spectrum of Voglibose as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
Voglibose Tablets / Official Monographs

(2) Determine the 1H spectrum of a solution of Voglibose in heavy water for nuclear magnetic resonance spectroscopy (3 in 70) as directed under Nuclear Magnetic Resonance Spectroscopy <2.216>, using sodium 3-trimethylsilylpropionate-d₄ for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits 2 double signals A at about δ 1.5 ppm, 2 double signals B at about δ 2.1 ppm, a multiple signal C at about δ 2.9 ppm, and a multiple signal D between δ 3.4 ppm and δ 3.9 ppm. The area intensity ratio of each signal, A:B:C:D, is about 1:1:1:10.

Optical rotation <2.49> [α]D²⁰°⁺45°–48° (0.2 g calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Voglibose in 10 mL of water: the pH of the solution is between 9.8 and 10.4.

Melting point <2.60> 163–168°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Voglibose according to Method 1, and perform the test. Adjust the pH of the test solution between 3.0 and 3.5 with dilute hydrochloric acid instead of dilute acetic acid. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Voglibose in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.20> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than voglibose obtained from sample solution is not larger than 1/5 times the peak area of voglibose from the standard solution. For the calculate of the total area, use the area of the peaks, having the relative retention time of about 1.7, about 2.0 and about 2.3 to voglibose, after multiplying by their relative response factors, 2, 2 and 2.5, respectively.

Operating conditions—
Apparatus: Use an apparatus consisting of 2 pumps for the mobile phase and reaction reagent transportation, sample injection port, column, reaction coil, cooling coil, detector and recording device, and the reaction coil and cooling coil maintained at a constant temperature.
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with pentaethylenehexaaminated polyvinyl alcohol polymer bead for liquid chromatography.
Column temperature: A constant temperature of about 25°C.
Reaction coil: A polytetrafluoroethylene tube 0.5 mm in inside diameter and 20 m in length.
Cooling coil: A polytetrafluoroethylene tube 0.3 mm in inside diameter and 2 m in length.
Mobile phase: To 1.56 g of sodium dihydrogen phosphate dihydrate add water to make 500 mL. To this solution add a solution, prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL, to adjust to pH 6.5. To 370 mL of this solution add 630 mL of acetonitrile.

—Proceed with 1.0 g of Voglibose, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 26.73 mg of C₁₀H₂₁NO₇.

Containers and storage Containers—Tight containers.

Voglibose Tablets

ボグリボース錠

Voglibose Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of voglibose (C₁₀H₂₁NO₇: 267.28).

Method of preparation Prepare as directed under Tablets, with Voglibose.

Identification Shake vigorously an amount of pulverized Voglibose Tablets, equivalent to 5 mg of Voglibose according to the labeled amount, with 40 mL of water, and centrifuge. Transfer the supernatant liquid to a chromatographic column [prepared by pouring 1.0 mL of strongly acidic ion-exchange resin (H type) for column chromatography (100 to 200 µm in particle diameter) into a chromatographic column (8 mm in inside diameter and 130 mm in height), and allow to flow at a rate of about 5 mL per minute. Then wash the column with 200 mL of water, and allow to flow with 10 mL

Reaction reagent: Dissolve 6.25 g of taurine and 2.56 g of sodium periodate in water to make 1000 mL.

Assay Weigh accurately about 0.4 g of Voglibose, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Related substances—Dissolve 1.0 g of Voglibose in 10 mL of water: the pH of the solution is between 9.8 and 10.4.

Residue on ignition <2.44> Not more than 0.1% (0.5 g, coulometric titration).

Water <2.48> Not more than 0.2% (0.5 g, coulometric titration).

Cooling temperature: A constant temperature of about 15°C.

Flow rate of the mobile phase: Adjust the flow rate so that the retention time of voglibose is about 20 minutes.

Flow rate of the reaction reagent: Same as the flow rate of the mobile phase.

Time span of measurement: About 2.5 times as long as the retention time of voglibose, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make 100 mL. Confirm that the peak area of voglibose obtained from 50 µL of this solution is equivalent to 7 to 13% of that from 50 µL of the standard solution.

System performance: When the procedure is run with 50 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of voglibose are not less than 7000 and between 0.8 and 1.2, respectively.

System repeatability: When the test is repeated 6 times with 50 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of voglibose is not more than 3.0%.
of diluted ammonia TS (1 in 4) at a rate of about 5 mL per minute. Filter the effluent solution two times through a membrane filter with a pore size not exceeding 0.22 μm. Evaporate the filtrate to dryness at 50°C under reduced pressure, dissolve the residue with 0.5 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Separately, dissolve 20 mg of voglibose for assay in 2 mL of the mixture of water and methanol (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.01>\). Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ammonia water (28) and water (5:3:1) to a distance of about 12 cm, air-dry the plate, and allow to stand in iodine vapors: the principal spot from the sample solution and the spot from the standard solution show a yellow-brown color, and the same Rf value.

### Uniformity of dosage unit \(<6.02>\)
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Voglibose Tablets add exactly V mL of the mobile phase so that the solution contains about 40 μg of voglibose (C_{10}H_{21}NO_{7}) per mL, disintegrate the tablet completely by shaking, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Hereinafter, proceed as directed in the Assay.

\[
\text{Amount (g) of voglibose (C}_{10}\text{H}_{21}\text{NO}_{7}) = M_S \times A_T/A_S \times V/500
\]

\(M_S\): Amount (mg) of voglibose for assay, calculated on the anhydrous basis

### Assay
To 20 tablets of Voglibose Tablets add 80 mL of the mobile phase, and completely disintegrate by shaking. To an exact volume of the solution, equivalent to about 4 mg of voglibose (C_{10}H_{21}NO_{7}), add the mobile phase to make exactly 100 mL, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of voglibose for assay (previously determined the water \(<2.48>\) in the same manner as Voglibose), and dissolve in the mobile phase to make exactly 25 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and determine the peak areas, \(A_T\) and \(A_S\), of voglibose.

\[
\text{Amount (mg) of voglibose (C}_{10}\text{H}_{21}\text{NO}_{7}) = M_S \times A_T/A_S \times 1/500
\]

\(M_S\): Amount of voglibose for assay, calculated on the dried basis

### Operating conditions—
Apparatus: Use an apparatus consisting of 2 pumps for the mobile phase and reaction reagent transportation, sample injection port, column, reaction coil, cooling coil, detector and recording device, and the reaction coil and cooling coil maintained at a constant temperature.


Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Reaction coil: A polytetrafluoroethylene tube 0.5 mm in inside diameter and 20 m in length.

Cooling coil: A polytetrafluoroethylene tube 0.3 mm in inside diameter and 2 m in length.

Mobile phase: To 1.56 g of sodium dihydrogen phosphate dihydrate add water to make 500 mL. To this solution add a solution, prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL, to adjust to pH 6.5. To 300 mL of this solution add 600 mL of acetonitrile.

Reaction reagent: Dissolve 6.25 g of taurine and 2.56 g of sodium periodate in water to make 1000 mL.

Reaction temperature: A constant temperature of about 100°C.

Cooling temperature: A constant temperature of about 15°C.

Flow rate of mobile phase: Adjust the flow rate so that the retention time of voglibose is about 20 minutes.

Flow rate of reaction reagent: Same as the flow rate of the mobile phase.

### System suitability—
System performance: Dissolve 2 mg of voglibose for assay and 0.2 g of lactose monohydrate in 5 mL of water, and add the mobile phase to make 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, lactose and voglibose are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of voglibose is not more than 2.0%.

### Containers and storage
Containers—Tight containers.

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**Warfarin Potassium**

Warfarin Potassium, when dried, contains not less than 98.0% and not more than 102.0% of C_{19}H_{15}KO_{4}.

**Description**
Warfarin Potassium occurs as a white, crystalline powder.
It is very soluble in water, and freely soluble in ethanol.

It dissolves in sodium hydroxide TS.

The pH of a solution prepared by dissolving 1.0 g of Warfarin Potassium in 100 mL of water is 7.2–8.3.

It is colored to light yellow by light.

A solution of Warfarin Potassium (1 in 10) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Warfarin Potassium in 0.02 mol/L potassium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Warfarin Potassium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Warfarin Potassium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry.

(3) A solution of Warfarin Potassium (1 in 250) responds to the Qualitative Tests 1.09 (1) for potassium salt.

**Purity (1)** Alkaline colored substances—Dissolve 1.0 g of Warfarin Potassium in a solution of sodium hydroxide (1 in 20) to make exactly 10 mL, and determine the absorbance at 385 nm within 15 minutes as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum or the spectrum of Warfarin Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Heavy metals 1.07—Dissolve 2.0 g of Warfarin Potassium in 30 mL of ethanol (95), add 2 mL of dilute acetic acid and ethanol (95) to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid, and ethanol (95) to make 50 mL (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Warfarin Potassium in 100 mL of a mixture of water and methanol (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and methanol (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than warfarin obtained with the sample solution is not larger than 1/10 times the peak area of warfarin with the standard solution, and the total area of the peaks other than warfarin is not larger than 1/2 times the peak area of warfarin with the standard solution.

**Operating conditions**—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of warfarin beginning after the solvent peak.

**System suitability**—
Test for required detectability: To exactly 1 mL of the standard solution add the mixture of water and methanol (3:1) to make exactly 20 mL. Confirm that the peak area of warfarin obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that with 20 μL of the standard solution.

System performance: Dissolve 20 mg of propyl parahydroxybenzoate in 50 mL of methanol, and add water to make 200 mL. To 5 mL of this solution add 4 mL of a solution of Warfarin Potassium in the mixture of water and methanol (3:1) (1 in 2000), and add the mixture of water and methanol (3:1) to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, propyl parahydroxybenzoate and warfarin are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 2.0%.

**Loss on drying** 2.41 Not more than 4.5% (1 g, 105°C, 3 hours).

**Assay** Weigh accurately about 25 mg each of Warfarin Potassium and Warfarin Potassium RS, previously dried, and separately dissolve in the mixture of water and methanol (3:1) to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mixture of water and methanol (3:1) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the peak areas, A₇ and A₉, of warfarin.

Amount (mg) of C₁₉H₁₅KO₄ = Mₛ × A₇/A₉

Mₛ: Amount (mg) of Warfarin Potassium RS
Warfarin Potassium Tablets

Warfarin Potassium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of warfarin potassium (C_{19}H_{15}KO_{4}: 346.42).

Method of preparation Prepare as directed under Tablets, with Warfarin Potassium.

Identification (1) Determine the absorption spectrum of the solution T_{2} obtained in the Assay, using 0.02 mol/L potassium hydroxide TS as the blank, as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 226 nm and 230 nm, and a minimum between 281 nm and 285 nm. Separately, determine the absorption spectrum of the solution T_{1} obtained in the Assay, using 0.02 mol/L hydrochloric acid TS as the blank, as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 281 nm and 285 nm and between 303 nm and 307 nm, and a minimum between 234 nm and 247 nm.

(2) Weigh a quantity of Warfarin Potassium Tablets, equivalent to 0.01 g of Warfarin Potassium according to the labeled amount, add 10 mL of acetone, shake, and filter. Heat the filtrate on a water bath to evaporate the acetone. To the residue add 10 mL of diethyl ether and 2 mL of dilute hydrochloric acid, and shake: the aqueous layer responds to the Qualitative Tests <1.09> (1) for potassium salt.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Powder 1 tablet of Warfarin Potassium Tablets, add 40 mL of water, and shake vigorously for 30 minutes. Add water to make exactly V mL of this solution containing about 20 μg of warfarin potassium (C_{19}H_{15}KO_{4}) per mL. Filter this solution, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 80 mg of Warfarin Potassium RS, previously dried at 105°C, to make exactly 25 mL, and use these solutions as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, A_{T} and A_{S}, of warfarin from each solution.

Dissolution rate (% with respect to the labeled amount of warfarin potassium (C_{19}H_{15}KO_{4})

\[ M_{S} = \frac{A_{T} / A_{S}}{V / V} \times \frac{C}{V} \times 9 / 4 \]

\[ M_{S} = \text{Amount (mg) of Warfarin Potassium RS} \]

C: Labeled amount (mg) of warfarin potassium (C_{19}H_{15}KO_{4}) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of methanol, water and phosphoric acid (700:300:1).

Flow rate: Adjust the flow rate so that the retention time of warfarin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of warfarin are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 2.0%.

Assay Weigh accurately and powder not less than 20 Warfarin Potassium Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of warfarin potassium (C_{19}H_{15}KO_{4}), add 80 mL of water, shake vigorously for 15 minutes, and add water to make exactly 100 mL. Filter this solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 80 mg of Warfarin Potassium RS, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, A_{T} and A_{S}, of warfarin from each solution.

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 15 minutes of 0.5-mg, 1-mg and 2-mg tablet and in 30 minutes of 5-mg tablet of Warfarin Potassium Tablets are not less than 80%.

Start the test with 1 tablet of Warfarin Potassium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 0.56 μg of warfarin potassium (C_{19}H_{15}KO_{4}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Warfarin Potassium RS, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, A_{T} and A_{S}, of warfarin from each solution.

Amount (mg) of warfarin potassium (C_{19}H_{15}KO_{4})

\[ M_{S} = \frac{A_{T}}{A_{S}} \times \frac{V}{V} \times 9 / 4 \]

\[ M_{S} = \text{Amount (mg) of Warfarin Potassium RS} \]

C: Labeled amount (mg) of warfarin potassium (C_{19}H_{15}KO_{4}) in 1 tablet
make exactly 20 mL, and use these solutions as the solution T₁ and the solution S₁, respectively. Separately, pipet 10 mL each of the sample solution and standard solution, add 0.02 mol/L potassium hydroxide TS to make exactly 20 mL, and use these solutions as the solution T₂ and the solution S₂, respectively. Determine the absorbances, A₁ and A₂, of the solution T₁ and the solution S₁ at 272 nm as directed under Ultraviolet-visible Spectrophotometry <2.2>, using the solution T₂ and the solution S₂ as the blank, respectively.

Amount (mg) of warfarin potassium (C₁₉H₁₅KO₄) = Mₛ × A₁/ₐ₃ × 1/20

Mₛ: Amount (mg) of Warfarin Potassium RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Water

H₂O: 18.02

Water must meet the Quality Standards of Drinking water provided under the Article 4 of the Water Supply Law (the Ministry of Health, Labour and Welfare Ministerial Ordinance No.101, May 30, 2003). In the case that Water is prepared at individual facilities using well water or industrial water as source water, it must meet the following additional requirement as well as the Quality Standards of Drinking water:

Purity Ammonium <1.02>—Perform the test with 30 mL of Water as directed under Ammonium Limit Test. Prepare the control solution as follows: to 0.15 mL of Standard Ammonium Solution add water for ammonium limit test to make 30 mL (not more than 0.05 mg/L).

Water for Injection

注射用水

Water for Injection is prepared by distillation or by reverse osmosis and/or ultrafiltration, either from Water after applying appropriate pretreatments such as ion-exchange or reverse osmosis, or from Purified Water.

When Water for Injection is prepared by the reverse osmosis and/or ultrafiltration (methods for refining water by using a reverse osmosis membrane module, an ultrafiltration membrane module capable of removing substances having molecular masses of 6,000 and above, or a module using both types of membranes), care must be taken to avoid microbial contamination of the water processing system, and to provide water with equivalent quality to that prepared by distillation consistently.

Water for Injection must be used immediately after preparation. However, it may be stored temporarily, if adequate countermeasures able to prevent microbial proliferation stringently, such as circulating it in a loop at a high temperature, are established.

Description Water for Injection is a clear and colorless liquid, having no odor.

Purity Total organic carbon <2.59>—Not more than 0.50 mg/L.

Conductivity <2.51> When the test is performed according to the following method, the conductivity (25°C) is not more than 2.1 μS·cm⁻¹.

Transfer a suitable amount of Water for Injection to a beaker, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than 0.1 μS·cm⁻¹ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Bacterial endotoxins <4.01> Less than 0.25 EU/mL.

Purified Water

精製水

Purified Water is prepared from Water by ion-exchange, distillation, reverse osmosis or ultrafiltration, or by a combination of these processes.

It must be used immediately after preparation. However, it may be stored temporarily, if adequate countermeasures for preventing microbial proliferation are taken.

Description Purified Water is a clear and colorless liquid, having no odor.

Purity Total organic carbon <2.59>—Not more than 0.50 mg/L.

Conductivity <2.51> When the test is performed according to the following method, the conductivity (25°C) is not more than 2.1 μS·cm⁻¹.

Transfer a suitable amount of Purified Water to a beaker, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than 0.1 μS·cm⁻¹ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Purified Water in Containers

精製水(容器入り)

Purified Water in Containers is prepared from Purified Water by introducing it in a tight container.

It is allowable to describe it as “Purified Water” on the label.

Description Purified Water in Containers is a clear and colorless liquid, having no odor.

Purity Potassium permanganate-reducing substances—To 100 mL of Purified Water in Containers add 10 mL of dilute...
Sterile Water for Injection in Containers

Sterile Water for Injection in Containers is prepared from Water for Injection by introducing it into a hermetic container, sealing up the container, then sterilizing the product, or by making it sterile using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

It is allowable to describe it as “Water for Injection” on the label.

For Sterile Water for Injection in Containers prepared from Water for Injection obtained by distillation, an alternative name of “Distilled Water for Injection” may be used.

Description Sterile Water for Injection in Containers is a clear and colorless liquid, having no odor.

Purity Potassium permanganate-reducing substances—To 100 mL of Sterile Water for Injection in Containers add 10 mL of dilute sulfuric acid, boil, then add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color of the solution does not disappear.

Conductivity  The test is performed according to the following method, the conductivity (25°C) is not more than 25 μS·cm⁻¹ for containers with a nominal volume of 10 mL or less, and not more than 5 μS·cm⁻¹ for containers with a nominal volume greater than 10 mL.

Transfer a suitable amount of Sterile Water for Injection in Containers to a beaker, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than 0.1 μS·cm⁻¹ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Microbial limit The acceptance criteria of TAMC is 10⁶ CFU/mL. Perform the test using soybean-casein digest agar medium.

Containers and storage Containers—Hermetic containers.

Sterile Purified Water in Containers

Sterile Purified Water in Containers is prepared from Purified Water by introducing it into a hermetic container, sealing up the container, then sterilizing the product, or by making it sterile using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

Description Sterile Purified Water in Containers is a clear and colorless liquid, having no odor.

Purity Potassium permanganate-reducing substances—To 100 mL of Sterile Purified Water in Containers add 10 mL of dilute sulfuric acid, boil, then add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color of the solution does not disappear.

Conductivity When the test is performed according to the following method, the conductivity (25°C) is not more than 25 μS·cm⁻¹ for containers with a nominal volume of 10 mL or less, and not more than 5 μS·cm⁻¹ for containers with a nominal volume greater than 10 mL.

Transfer a suitable amount of Sterile Purified Water in Containers to a beaker, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than 0.1 μS·cm⁻¹ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Sterility It meets the requirements.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections can be used in place of hermetic containers.
Weil's Disease and Akiyami Combined Vaccine

Weil's Disease and Akiyami Combined Vaccine is a liquid for injection containing inactivated Weil's disease leptospira, Akiyami A leptospira, Akiyami B leptospira and Akiyami C leptospira. The product lacking more than a kind of Akiyami leptospira may be prepared, if necessary.

It conforms to the requirements of Weil's Disease and Akiyami Combined Vaccine in the Minimum Requirements for Biological Products.

Description Weil's Disease and Akiyami Combined Vaccine is a white-turbid liquid.

Wheat Starch

Amylum Tritici

このドキュメントは、欧州薬局方と米国薬局方とハーモニズムがあります。このドキュメントの一部は、ハーモニズムされていません(コムギデンプン)。

Wheat Starch consists of the starch granules obtained from caryopsis of wheat, Triticum aestivum Linné (Gramineae).

Description Wheat Starch occurs as white masses or powder.

It is practically insoluble in water and in ethanol (99.5).

Identification (1) Examine under a microscope <5.01> using a mixture of water and glycerinol (1:1), Wheat Starch presents large and small granules, and, very rarely, intermediate sizes. The large granules, usually 10–60 µm in diameter, are discoid or, more rarely, reniform when seen face-on. The central hilum and striations are invisible or barely visible and the granules sometimes show cracks on the edges. Seen in profile, the granules are elliptical and fusiform and the hilum appears as a slit along the main axis. The small granules, rounded or polyhedral, are 2–10 µm in diameter. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Wheat Starch add 50 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate <2.50> with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm, calculated as hydrogen peroxide).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.

A: Boiling flask (500 mL)
B: Funnel (100 mL)
C: Condenser
D: Test-tube
E: Tap

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Wheat Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the
tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

\[
\text{Amount (ppm) of sulfur dioxide} = \frac{V}{M} \times 1000 \times 3.203
\]

\(M\): Amount (g) of the sample

\(V\): Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

\(* (4)\) Foreign matter. Under a microscope \(<5.0\>\), Wheat Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any of fragments of the tissue of the original plant.

\(* (4)\) Loss on drying \(<2.4\>\) Not more than 15.0% (1 g, 130°C, 90 minutes).

\(* (4)\) Residue on ignition \(<2.4\>\) Not more than 0.6% (1 g).

\(* (4)\) Containers and storage Containers—Well-closed containers.

### White Ointment

白色軟膏

**Method of preparation**

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Beeswax</td>
<td>50 g</td>
</tr>
<tr>
<td>Sorbitan Sesquioleate</td>
<td>20 g</td>
</tr>
<tr>
<td>White Petrolatum</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Ointments, with the above materials.

**Description**

White Ointment is white in color. It has a slight, characteristic odor.

**Containers and storage**

Containers—Well-closed containers.

### Whole Human Blood

人全血液

Whole Human Blood is a liquid for injection which is prepared by mixing human blood cells and an anticoagulant solution for storage. It conforms to the requirements of Whole Human Blood in the Minimum Requirements for Biological Products.

**Description**

Whole Human Blood is a deep red liquid from which the erythrocytes settle upon standing, leaving a yellow supernatant layer. A gray layer which mainly consists of leukocytes may appear on the surface of the settled erythrocyte layer. The supernatant layer may become turbid in the presence of fat, or may show the faint color of hemoglobin.

### Wine

ブドウ酒

Wine is an alcoholic liquid obtained by fermenting the juice of the fruits of *Vitis vinifera* Linné (*Vitaceae*) or allied plants.

It contains not less than 11 vol% and not more than 14 vol% of ethanol (C₂H₆O: 46.07) (by specific gravity), and not less than 0.10 w/v% and not more than 0.40 w/v% of l-tartaric acid (C₄H₆O₆: 150.09).

It contains no artificial sweetener and no artificial coloring agent.

**Description**

Wine is a light yellow or reddish purple to red-purple liquid. It has a characteristic and aromatic odor. It has a slightly astringent and faintly irritating taste.

**Specific gravity** \(<2.5\>\) \(d_\text{20}^\circ\): 0.990 – 1.010

**Optical rotation** \(<2.4\>\) Boil 160 mL of Wine, neutralize with potassium hydroxide TS, and concentrate to 80 mL on a water bath. Cool, dilute with water to 160 mL, add 16 mL of lead subacetate TS, shake well, and filter. To 100 mL of the filtrate add 10 mL of a saturated solution of sodium sulfate decahydrate, shake well, filter, and use the filtrate as the sample solution. Allow 20 mL of the sample solution to stand for 24 hours, add 0.5 g of activated charcoal, shake, stop, and allow to stand for 10 minutes. Filter, and observe the optical rotation of the filtrate in a 200-mm cell. Multiply the optical rotation observed by 1.21, and designate as the optical rotation of Wine: it is between \(-0.3^\circ\) and \(+0.3^\circ\).

**Purity**

1. Total acid [as l-tartaric acid (C₄H₆O₆)]—To exactly 10 mL of Wine add 250 mL of freshly boiled and cooled water, and titrate \(<2.5\>\) with 0.1 mol/L sodium hydroxide VS (indicator: 1 mL of phenolphthalein TS).

   \[
   \text{Each mL of 0.1 mol/L sodium hydroxide VS} = \frac{7.504 \text{ mg of C}_4\text{H}_6\text{O}_6}{20} = 0.375 \text{ mg of C}_4\text{H}_6\text{O}_6
   \]

   Total acid is not less than 0.40 w/v% and not more than 0.80 w/v%.

2. Volatile acid [as acetic acid (C₂H₄O₂: 60.05)]—Transfer 100 mL of Wine to a beaker, add 1 mL of 1 mol/L sodium hydroxide VS and the same volume of 1 mol/L sodium hydroxide VS titrated in (1) to make the solution alkaline, and concentrate to 50 mL on a water bath. Cool, add water to make 100 mL, transfer to a 1000-mL distillation flask, containing previously added 100 g of sodium chloride. Wash the beaker with 100 mL of water, and combine the washings in the distillation flask. Add 5 mL of a solution of l-tartaric acid (3 in 20), and distil with steam cautiously to maintain the volume of the solution in the flask until 450 mL of the distillate is obtained for 45 minutes. Dilute the distillate to exactly 500 mL with water, and use this solution as the sample solution. Titrate \(<2.5\>\) a 250-mL portion of the sample solution with
0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 6.005 mg of C₂H₄O₂

The volatile acid is not more than 0.15 w/v%.

(3) Sulfur dioxide—Stopper a 750-mL round-bottomed flask with a stopper having two holes. Through one hole, insert a glass tube A extending nearly to the bottom of the flask. Through the other hole, insert a glass tube B ending to the neck of the flask. Connect the tube B to a Liebig’s condenser, and the end of the condenser to a joint of which inner diameter is 5 mm at the lower end. Connect the other end of the joint with a holed rubber stopper to a U tube having three bulbs as shown in the Figure. Pass carbon dioxide washed with a solution of potassium permanganate (3 in 100) through the tube A. Displace the air in the apparatus by carbon dioxide, and place 50 mL of a freshly prepared and diluted starch TS (1 in 5) and 1 g of potassium iodide in the U tube. From the other end of the U tube, add 1 to 2 drops of 0.01 mol/L iodine VS from a burette. While passing carbon dioxide, remove the stopper of the flask a little, add exactly 25 mL of Wine, 180 mL of freshly boiled and cooled water, 0.2 g of tannic acid, and 30 mL of phosphoric acid, and stopper again. Pass carbon dioxide for further 15 minutes, heat the distillation flask with caution so that 40 to 60 minutes have passed after the beginning of distillation. In this case, however, the coloration of the starch TS in the U tube is discharged, add 0.01 mL of the distillate may be obtained in 1 minute. When exactly 60 minutes have passed, heat the distillation flask with caution so that constant stirring and pushing down any material adhering to the wall of the dish until the contents of the dish become soft masses. Cool, add 5 mL of ethanol (99.5), and grind to a grue-like substance. Heat on a water bath, add 10 to 20 mL of ethanol (99.5) while agitating, boil, and transfer to a 100-mL volumetric flask. Wash the dish with seven 10-mL portions of hot ethanol (99.5), combine the washings with the contents of the flask, cool, and add ethanol (99.5) to make exactly 100 mL. Filter through a dry filter paper, evaporate 90 mL of the filtrate on a water bath, taking care not to boil the solution during the evaporation. Dissolve the residue in a small amount of ethanol (99.5), transfer to a 50-mL glass-stoppered volumetric cylinder, wash with several portions of ethanol (99.5), and add the washings to the solution in the cylinder to make 15 mL. Add three 7.5-mL portions of dehydrated diethyl ether, shake vigorously each time, and allow to stand. When the solution becomes quite clear, transfer to a tared, flat weighing bottle. Wash the volumetric cylinder with 5 mL of a mixture of dehydrated diethyl ether and ethanol (99.5) (3:2). Transfer the washings to the weighing bottle, and evaporate carefully on a water bath. When the liquid becomes sticky, dry at 105°C for 1 hour, and cool in a desiccator (silica gel), and weigh: the mass of the residue is not less than 0.45 g and not more than 0.90 g.

Reducing sugars—To a 25-mL portion of the sample solution obtained in the Optical rotation add 50 mL of boiling Fehling’s TS, and heat for exactly 2 minutes. Filter the separated precipitates by a tared glass filter by suction, wash successively with hot water, with ethanol (95) and with diethyl ether, and continue to dry the precipitates by suction. Heat the filter gently at first, and then strongly until the precipitates become completely black. Cool the precipitates in a desiccator (silica gel), and weigh as copper (II) oxide: the mass of cupric oxide does not exceed 0.325 g.

Sucrose—Transfer a 50-mL portion of the sample solution obtained in the Optical rotation to add 50 mL of boiling Fehling’s TS, and heat for exactly 2 minutes. Filter the separated precipitates by a tared glass filter by suction, wash successively with hot water, with ethanol (95) and with diethyl ether, and continue to dry the precipitates by suction. Heat the filter gently at first, and then strongly until the precipitates become completely black. Cool the precipitates in a desiccator (silica gel), and weigh as copper (II) oxide: the mass of cupric oxide does not exceed 0.325 g.

Benzaldehyde, cinnamic acid, and salicylic acid—Transfer exactly 50 mL of the sample solution obtained in (2) to a separator, add 10 g of sodium chloride and 2 mL of dilute hydrochloric acid, and extract with three 10-mL portions of diethyl ether. Combine the diethyl ether extracts, wash with
two 5-mL portions of water, and extract with three 10-mL portions of 0.1 mol/L sodium hydroxide VS. Combine the alkaline extracts, evaporate the diethyl ether by warming on a water bath, cool, neutralize with 1 mol/L hydrochloric acid VS, and add 5 mL of potassium chloride-hydrochloric acid buffer solution and water to make exactly 50 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry <2.2A> with this solution, using a solution prepared in the same manner instead of the sample solution as the blank: the absorbance does not exceed 0.15 at a wavelength between 220 nm and 340 nm.

Boric acid—Transfer 50 mL of Wine to a porcelain dish, add 5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite: a half portion of the residue does not respond to Qualitative Tests <1.09> (1) for borate. Dissolve another half portion of the residue in 5 mL of hydrochloric acid: it does not respond to Qualitative Tests <1.09> (2) for borate.

Methanol—Wine meets the requirements of the Methanol Test <1.12>, when proceeding with exactly 1 mL of ethanol layer obtained by Method 1 of the Alcohol Number Determination <1.01> and distilling without adding water after shaking with 0.5 g of calcium carbonate.

Formaldehyde—To 25 mL of Wine add 5 g of sodium chloride and 0.2 g of L-tartaric acid, distil, and obtain 15 mL of the distillate. To 5 mL of the distillate add 5 mL of acetyl acetone TS, mix, and heat on a water bath for 10 minutes: the solution has no more color than that of the following control solution.

Control solution: Using 5 mL of water instead of the distillate, perform the test in the same manner.

**Extract content** 1.9 – 3.5 w/v% Pipet 25 mL of Wine to a 200-mL tared beaker containing 10 g of sea sand (No. 1), previously dried at 105°C for 2.5 hours, and evaporate to dryness on a water bath. Dry the residue at 105°C for 2 hours, cool in a desiccator (silica gel), and weigh.

**Total ash** 0.13 – 0.40 w/v% Pipet 50 mL of Wine to a tared porcelain dish, and evaporate to dryness on a water bath. Ignite the residue to the constant mass, cool, and weigh.

**Assay (1)** Ethanol—Pipet Wine into a 100-mL volumetric flask at 15°C, transfer to a 300- to 500-mL flask, and wash this volumetric flask with two 15-mL portions of water. Add the washings to the sample in the flask, connect the flask to a distillation tube having a trap, and distil using the volumetric flask as a receiver. When about 80 mL of the distillate is obtained (it takes about 20 minutes), stop the distillation, allow to stand in water at 15°C for 30 minutes, and add water to make exactly 100 mL. Shake well, and determine the specific gravity at 15°C according to Method 3 under Specific Gravity <2.50>:

\[ d_{15}^3 = \frac{30.02 \text{ mg of } C_4H_6O_6}{30} \]

of powdered potassium chloride in 120 mL of diluted ethanol (1 in 6), and repeat the washings five times. Transfer the crystals together with the filter paper to a beaker, wash the filter with 50 mL of hot water, combine the washings in the beaker, and dissolve the crystals by heating. Titrate <2.50> the solution with 0.2 mol/L sodium hydroxide VS immediately (indicator: 1 mL of phenolphthalein TS). The number obtained by adding 0.75 to the amount (mL) of 0.2 mol/L sodium hydroxide VS consumed represents the amount (mL) of 0.2 mol/L sodium hydroxide VS consumed.

Each mL of 0.2 mol/L sodium hydroxide VS = 30.02 mg of C6H12O6

**Containers and storage** Containers—Tight containers.

### Wood Creosote

**ホクレオゾート**

Wood Creosote is a mixture of phenols obtained from by using wood tar derived from dry distillation of stems and branches of various plants of genus *Pinus* (*Pinaceae*), genus *Cryptomeria* (*Taxodiaceae*), genus *Fagus* (*Fagaceae*), genus *Afzelia* (genus *Intisia*); (*Leguminosae*), genus *Shorea* (*Dipterocarpaceae*) or genus *Tectona* (*Verbenaceae*), followed by distillation and collection at 180 to 230°C, then further purification and then re-distillation.

Wood Creosote contains not less than 23.0% and not more than 35% of guaiacol (C9H12O2: 124.14).

**Description** Wood Creosote is a colorless or pale yellow, clear liquid. It has a characteristic odor.

It is slightly soluble in water.

It is miscible with methanol and with ethanol (99.5).

Its saturated solution is acidic.

It is highly refractive.

It gradually changes in color by light or by air.

**Identification** Use the sample solution obtained in the Assay as the sample solution. Separately, dissolve 0.1 g of phenol, p-cresol, guaiacol, and 2-methoxy-4-methylphenol in methanol respectively, to make 100 mL. To 10 mL of each solution add methanol to make 50 mL, and use these solutions as standard solution (1), standard solution (2), standard solution (3) and standard solution (4). Perform the test with 10 μL each of the sample solution, standard solution (1), (2), (3) and (4) as directed under Liquid Chromatography <2.01> according to the following conditions: the main peaks obtained with the sample solution show the same retention times with those obtained with the standard solutions (1), (2), (3) and (4).

**Operating conditions**—Proceed as directed in the operating conditions in the Assay.

**Specific gravity** <2.50> **d_{20}^3:** not less than 1.076.

**Purity (1)** Coal Creosote—Accurately measure 10 mL of Wood Creosote, add methanol to make exactly 20 mL, and use this solution as the sample solution. Separately to 1 mg each of benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene add a small quantity of ethyl acetate,
if necessary, and add methanol to make 100 mL. To 1 mL of this solution add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography 2.02 according to the following conditions: No peaks are detected with the sample solution for the retention times corresponding to benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene of the standard solution. Change these conditions if any peak is detected for retention times that correspond to benzo[a]pyrene, benz[a]anthracene or dibenz[a,h]anthracene, to verify that such a peak does not belong to benzo[a]pyrene, benz[a]anthracene or dibenz[a,h]anthracene.

Operating conditions—
Detector: A mass spectrometer (EI).
Monitored ions:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular ion m/z</th>
<th>Fragment ion m/z</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[a]anthracene</td>
<td>228, 114</td>
<td></td>
<td>About 14 to 20 minutes</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>252, 125</td>
<td></td>
<td>About 20 to 25 minutes</td>
</tr>
<tr>
<td>Dibenz[a,h]anthracene</td>
<td>278, 139</td>
<td></td>
<td>About 25 to 30 minutes</td>
</tr>
</tbody>
</table>

Column: A quartz tube 0.25 mm in inside diameter and 30 m in length, with internal coating 0.25 – 0.5 μm in thickness made of 5% diphenyl-95% dimethyl polysiloxane for gas chromatography.

Column temperature: Inject sample at a constant temperature in vicinity of 45°C, then raise temperature to 240°C at the rate of 40°C per minute, maintain the temperature at 240°C for 5 minutes, then raise temperature to 300°C at the rate of 4°C per minute until reaching 300°C, then maintain temperature at 30°C for 3 minutes.

Injection port temperature: A constant temperature in vicinity of 250°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of benzo[a]pyrene is about 22 minutes.

Split ratio: Splitless.

System suitability—
Test for required detectability: Accurately measure 1 mL of the standard solution, add methanol to make exactly 10 mL, and use this solution as the solution for system suitability test. When the test is performed with conditions described above with 1 μL of the solution for system suitability test, the SN ratio of each substance is not less than 3.

System performance: When the procedure is run with conditions described above with 1 μL of the solution for system suitability test, the elution takes place in order of benzo[a]anthracene, benzo[a]pyrene and then dibenz[a,h]anthracene.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene is respectively not more than 10%.

(2) Acenaphthene—To 0.12 g of Wood Creosote add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of acenaphthene in methanol to make 50 mL. To 5 mL of this solution add methanol to make 20 mL. To 2 mL of this solution add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography 2.02 according to the following conditions: No peaks are detected with sample solution for the retention time corresponding to acenaphthene of the standard solution. Change these conditions if any peak is detected for the retention time corresponding to acenaphthene, to verify that such a peak does not belong to acenaphthene.

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A fused silica tube 0.25 mm inside diameter and 60 m in length, with internal coating 0.25. 0.5 μm in thickness made of polymethylsiloxane for gas chromatography.

Column temperature: Perform injection at a constant temperature in vicinity of 45°C, then raise the temperature by 11.5°C per minute until reaching 160°C, then raise the temperature by 4°C per minute until reaching 180°C, then raise the temperature by 8°C until reaching 270°C, then maintain temperature at 270°C for 3 minutes.

Injection port temperature: 250°C.
Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of acenaphthene is about 18 minutes.

Split ratio: Splitless.

System suitability—
Test for required detectability: Accurately measure 1 mL of the standard solution, add methanol to make exactly 10 mL, and use this solution as the solution for system suitability test. When the procedure is run with conditions described above with 1 μL of solution for system suitability test, the SN ratio of acenaphthene is not less than 3.

System performance: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of acenaphthene is not more than 6.0%.

(3) Other impurities
Add 2 mL of petroleum benzin to 1.0 mL of Wood Creosote, then add 2 mL of barium hydroxide test solution, agitate to mix and allow to stand. No blue or muddy brown color develops in the upper layer of the mixture. Furthermore, no red color develops in the lower layer.

Distilling range 2.57  200 – 220°C, not less than 85 vol%.

Assay To about 0.1 g of Wood Creosote, accurately weighed, add methanol to make exactly 50 mL. Pipet 10 mL of this solution add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, add methanol to about 30 mg of accurately measured guaiacol for assay to make exactly 50 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution under Liquid Chromatography 2.02 according to the following conditions, and determine the peak areas, \( A_F \) and \( A_S \), of guaiacol for each solution.
Amount (mg) of guaiacol (C₇H₈O₂)

\[ M_s = \frac{A_r}{A_s} \]

\( M_s \): Amount of guaiacol for assay (mg)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: Fill a stainless steel tube with internal diameter of 4.6 mm and length of 15 cm with 5 \( \mu \)m of octadecylsilanized silica gel for gas chromatography.

Column temperature: A constant temperature in vicinity of 40°C.

Mobile phase: Mixture of water and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of guaiacol is about 9 minutes.

System suitability—

System performance: Dissolve 2 mg each of guaiacol and phenol in methanol to make 10 mL. The procedure is run with 10 \( \mu \)L of this solution, the elution takes place in order of phenol then guaiacol, with the degree of separation of not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of guaiacol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Xylitol

キシリトール

\[
\text{C}_5\text{H}_{12}\text{O}_5 : 152.15
\]

meso-Xylitol

[87-99-0]

Xylitol, when dried, contains not less than 98.0% of C₅H₁₂O₅.

Description Xylitol occurs as white crystals or powder. It is odorless and has a sweet taste.

It is very soluble in water, slightly soluble in ethanol (95). It is hygroscopic.

Identification (1) To 1 mL of a solution of Xylitol (1 in 2) add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): blue-green color is produced without turbidity.

(2) Determine the infrared absorption spectrum of Xylitol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25s>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 5.0 g of Xylitol in 10 mL of freshly boiled and cooled water: the pH of this solution is between 5.0 and 7.0.

Melting point <2.60> 93.0 – 95.0°C

Purity (1) Clarity and color of solution—Dissolve 5 g of Xylitol in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 2.0 g of Xylitol. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%)

(3) Sulfate <1.14>—Perform the test with 4.0 g of Xylitol. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Heavy metals <1.07>—Proceed with 4.0 g of Xylitol according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 0.5 ppm).

(5) Nickel—Dissolve 0.5 g of Xylitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color is produced.

(6) Arsenic <1.17>—Prepare the test solution with 1.5 g of Xylitol according to Method 1, and perform the test (not more than 1.3 ppm).

(7) Sugars—Dissolve 5.0 g of Xylitol in 15 mL of water, add 4.0 mL of dilute hydrochloric acid, and heat in a water bath for 3 hours under a reflux condenser. After cooling, neutralize with sodium hydroxide TS (indicator: 2 drops of methyl orange TS). Then add water to make 50 mL, transfer 10 mL of this solution to a flask, add 10 mL of water and 40 mL of Fehling’s TS, boil gently for 3 minutes, and allow to stand to precipitate copper (I) oxide. Remove the supernatant liquid through a glass filter (G4), and wash the precipitate with warm water until the last washing does not show alkalinity. Filter these washings through the glass filter mentioned above. Dissolve the precipitate in the flask in 20 mL of iron (III) sulfate TS, filter the solution through the glass filter mentioned above, wash with water, combine the washings with the filtrate, heat at 80°C, and titrate <2.50> with 0.02 mol/L potassium permanganate VS; not more than 1.0 mL of 0.02 mol/L potassium permanganate VS is consumed.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Xylitol, previously dried, dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into an iodine flask, add 50 mL of potassium periodate TS exactly, and heat in a water bath for 15 minutes. After cooling, add 2.5 g of potassium iodide, stopper, shake well, allow to stand for 5 minutes in a dark place, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS = 1.902 mg of C₅H₁₂O₅

Containers and storage Containers—Tight containers.
Xylitol Injection

キシリトール注射液

Xylitol Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of xylitol (C₅H₁₂O₅: 152.15).

Method of preparation
Prepare as directed under Injections, with Xylitol.

No preservative may be added.

Description
Xylitol Injection is a clear, colorless liquid. It has a sweet taste.

Identification
Measure a volume of Xylitol Injection, equivalent to 0.1 g of Xylitol according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.1 g of xylitol in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 2 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95), ammonia solution (28) and water (25:4:3) to show a blackish brown color and the same shape, and 6 to 12 µm in length.

Total ash <2.54> Not more than 8.0% (1 g, 100°C, 8 hours).

Starch—Add iodine TS to Dried Yeast, and examine microscopically <5.01> no or only a few granules are tinted blackish purple.

Foreign insoluble matter <6.05> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay
Measure exactly a volume of Xylitol Injection, equivalent to about 15 mg of xylitol (C₅H₁₂O₅) according to the labeled amount, add water to make exactly 250 mL. Measure exactly 10 mL of this solution, and add water to make exactly 100 mL. Then, pipet 10 mL of this solution into an iodine flask, and proceed as directed in the Assay under Xylitol.

Each mL of 0.1 mol/L sodium thiosulfate VS = 1.902 mg of C₅H₁₂O₅

Containers and storage
Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Dried Yeast

乾燥酵母

Dried Yeast is dried and powdered cells of yeast belonging to Saccharomyces.

It contains not less than 400 mg of protein and not less than 100 µg of thiamine compounds [as thiamine hydrochloride (C₁₂H₁₇ClN₄OS.HCl: 337.27)] in each 1 g.

Description
Dried Yeast occurs as a light yellowish white to brown powder. It has a characteristic odor and taste.

Identification
Dried Yeast, when examined under a microscope <5.01>, shows isolated cells, spheroidal or oval in shape, and 6 to 12 µm in length.

Purity
(1) Rancidity—Dried Yeast is free from any unpleasant or rancid odor or taste.
(2) Starch—Add iodine TS to Dried Yeast, and examine microscopically <5.01>: no or only a few granules are tinted blackish purple.

Loss on drying <2.41> Not more than 9.0% (1 g).

Total ash <5.01> Not more than 9.0% (1 g).

Assay
(1) Protein—Weigh accurately about 50 mg of Dried Yeast and perform the test as directed under Nitrogen Determination <1.08>.

\[
\text{Amount (mg) of protein in 1 g of Dried Yeast} = \frac{N \times 6.25}{M}
\]

N: Amount (mg) of nitrogen (N)
M: Amount (g) of sample

(2) Thiamine—Weigh accurately about 1 g of Dried Yeast, add 1 mL of dilute hydrochloric acid and 80 mL of water, and heat in a water bath at 80°C to 85°C for 30 minutes with occasional shaking. After cooling, add water to make exactly 100 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add exactly 5 mL of acetic acid-sodium acetate TS and exactly 1 mL of enzyme TS, and allow to stand at 45°C to 50°C for 3 hours. Place exactly 2 mL of this solution onto a chromatographic column prepared by pouring 2.5 mL of a weakly acidic CM-bridged cellulose cation exchanger (H type) (40 to 110 µm in particle diameter) into a chromatographic tube about 1 cm in inside diameter and about 17 cm in length, and elute at the flow rate of about 0.5 mL per minute. Wash the upper part of the column with a small amount of water, and wash the column with two 10-mL portions of water at the flow rate of about 1 mL per minute. Elute the column with two 2.5-mL portions of diluted phosphoric acid (1 in 50) at the flow rate of about 0.5 mL per minute, and combine the eluate. To the eluate add exactly 1 mL of the internal standard solution and 0.01 g of sodium 1-octanesulfonate, and after dissolving, use this solution as the sample solution. Separately, weigh accurately about 15 mg of Thiamine Chloride Hydrochloride RS (previously determine the water <2.48> in the same manner as Thiamine Chloride Hydrochloride), dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 100
mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution and 3 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_2 \) and \( Q_3 \), of the peak area of thiamine to that of the internal standard.

\[
\text{Amount (μg) of thiamine in 1 g of Dried Yeast} = \frac{M_S}{M_T} \times \frac{Q_2}{Q_3} \times 12.5
\]

\( M_S \): Amount (mg) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis

\( M_T \): Amount (g) of the sample

**Internal standard solution**—Dissolve 0.01 g of phenacetin in acetonitrile to make 100 mL, and to 1 mL of this solution add diluted acetonitrile (1 in 5) to make 100 mL.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm)
Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 2.7 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). Dissolve 1.6 g of sodium 1-oxanesulfonate in 800 mL of this solution, and add 200 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of thiamine is about 8 minutes.
Selection of column: Proceed with 200 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of thiamine and the internal standard in this order with the resolution between these peaks being not less than 8.

**Containers and storage**—Containers—Tight containers.

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**Zaltoprofen**

サルトプロフェン

![Zaltoprofen structure](image)

\( \text{C}_{13}\text{H}_{14}\text{O}_{5}\text{S} \): 298.36
(2RS)-2-(10-Oxo-10,11-dihydrodibenzo[b,f]thiepin-2-yl)propanoic acid
[74711-43-6]

Zaltoprofen, when dried, contains not less than 99.0% and not more than 101.0% of \( \text{C}_{13}\text{H}_{14}\text{O}_{5}\text{S} \).

**Description**—Zaltoprofen occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in acetone, soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is gradually decomposed by light.

A solution of Zaltoprofen in acetonitrile (1 in 10) shows no optical rotation.

**Identification**—(1) To 0.2 g of Zaltoprofen add 0.5 g of sodium hydroxide, heat gradually to melt, and then carbonize. After cooling, add 5 mL of diluted hydrochloric acid (1 in 2); the gas evolved darkens moisten lead (II) acetate paper.

(2) Determine the absorption spectrum of a solution of Zaltoprofen in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Zaltoprofen as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point**—\( \angle \text{2.60°} \) 135 – 139°C

**Purity**—(1) Heavy metals <1.07>—Proceed with 2.0 g of Zaltoprofen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Zaltoprofen according to Method 3, using 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (2 in 25), and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Zaltoprofen in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than zaltoprofen and the peak having the relative retention time of about 0.7 with respect to zaltoprofen from the sample solution is not larger than the peak area of zaltoprofen from the standard solution.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (300:200:1).
Flow rate: Adjust the flow rate so that the retention time of zaltoprofen is about 4 minutes.
Time span of measurement: About 15 times as long as the retention time of zaltoprofen beginning after the solvent peak.

**System suitability**—
Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of zaltoprofen obtained with 20 μL of this solution is equivalent to 8 to 12% of that...
with 20 μL of the standard solution.

System performance: Dissolve 25 mg of zaltoprofen and 50 mg of isopropyl benzoate in 100 mL of ethanol (99.5). Pipet 1 mL of this solution, and add the mobile phase to make exactly 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, zaltoprofen and isopropyl benzoate are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of zaltoprofen is not more than 2.0%.

**Loss on drying**<sup>2.41</sup> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition**<sup>2.44</sup> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Zaltoprofen, dissolve in 50 mL of methanol, and titrate<sup>2.56</sup> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make the calculation as directed in the Assay.

**Containers**—Tight containers.

**Zaltoprofen Tablets**

ザルトプロフェン錠

Zaltoprofen Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of zaltoprofen (C<sub>17</sub>H<sub>14</sub>O<sub>3</sub>S: 298.36).

**Method of preparation** Prepare as directed under Tablets, with Zaltoprofen.

**Identification** Powder a suitable amount of Zaltoprofen Tablets. To a portion of the powder, equivalent to 80 mg of Zaltoprofen, add 30 mL of ethanol (99.5), shake well, and centrifuge. To 1 mL of the supernatant liquid add ethanol (99.5) to make 20 mL. To 2 mL of this solution add ethanol (99.5) to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry<sup>2.24</sup>: it exhibits maxima between 227 nm and 231 nm and between 329 nm and 333 nm, and a shoulder between 241 nm and 245 nm.

**Uniformity of dosage units**<sup>6.02</sup> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Zaltoprofen Tablets add 4 mL of water, shake to disintegrate, then add a suitable amount of ethanol (95), shake, add ethanol (95) to make exactly 200 mL, and centrifuge. Pipet an amount of the supernatant liquid, equivalent to about 22 mg of zaltoprofen for assay, previously dried at 105°C, to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of zaltoprofen for assay, previously dried at 105°C for 4 hours, dissolve in 20 mL of ethanol (99.5), and add the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, <i>A</i><sub>T</sub> and <i>A</i><sub>S</sub>, of the sample solution and standard solution at 340 nm as directed under Ultraviolet-visible Spectrophotometry<sup>2.24</sup>, using the dissolution medium as the control.

Dissolution rate (% with respect to the labeled amount of zaltoprofen (C<sub>17</sub>H<sub>14</sub>O<sub>3</sub>S))

\[
\text{Dissolution rate} = \frac{A_T / A_S 	imes V/V 	imes 1/C 	imes 180}{M_S}
\]

**Assay** To 10 tablets of Zaltoprofen Tablets add 40 mL of water, shake to disintegrate, then add a suitable amount of ethanol (95), shake, add ethanol (95) to make exactly 200 mL, and centrifuge. Pipet an amount of the supernatant liquid, equivalent to about 8 mg of zaltoprofen (C<sub>17</sub>H<sub>14</sub>O<sub>3</sub>S), add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of zaltoprofen for assay, previously dried at 105°C for 4 hours, add 4 mL of water and ethanol (95) to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography<sup>2.01</sup> according to the following conditions, and calculate the ratios, <i>Q</i><sub>T</sub> and <i>Q</i><sub>S</sub>, of the peak area of zaltoprofen to that of the internal standard.

Amount (mg) of zaltoprofen for assay

\[
\text{Amount (mg) of zaltoprofen} = M_S \times Q_T / Q_S \times V/20
\]

**Dissolution**<sup>6.10</sup> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Zaltoprofen Tablets is not less than 75%.

Start the test with 1 tablet of Zaltoprofen Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet <i>V</i> mL of the subsequent filtrate, add the dissolution medium to make exactly <i>V</i> mL so that each mL contains about 44 μg of zaltoprofen (C<sub>17</sub>H<sub>14</sub>O<sub>3</sub>S) according to the labeled amount, and use this solution as the sample solution. System performance: Dissolve 25 mg of zaltoprofen and 50 mg of isopropyl benzoate are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of zaltoprofen is not more than 2.0%.

**Internal standard solution**—A solution of benzyl benzoate in acetonitrile (1 in 1000).

**Internal standard solution**—A solution of benzyl benzoate in acetonitrile (1 in 1000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diamet-
ter and 15 cm in length, packed with octadeccsilanized silica gel for liquid chromatography (5 μm in particle diameter).

**Column temperature:** A constant temperature of about 25°C.

**Mobile phase:** A mixture of acetonitrile, water and acetic acid (100) (300:200:1).

**Flow rate:** Adjust the flow rate so that the retention time of zaltoprofen is about 4 minutes.

**System suitability—**

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, zaltoprofen and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of zaltoprofen to that of the internal standard is not more than 1.0%.

**Containers and storage** — Containers—Tight containers.

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**Zidovudine**

ジドブジン

\[
\text{C}_{10}\text{H}_{13}\text{N}_{5}\text{O}_{4}: \text{267.24} \\
3'-\text{Azido-3'-deoxythymidine} \\
\text{[30516-87-1]}
\]

Zidovudine contains not less than 97.0% and not more than 102.0% of \(\text{C}_{10}\text{H}_{13}\text{N}_{5}\text{O}_{4}\), calculated on the anhydrous basis.

**Description**  Zidovudine occurs as a white to pale yellowish white powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and sparingly soluble in water.

It gradually turns yellow-brown on exposure to light.

Melting point: about 124°C.

**Identification**  Determine the infrared absorption spectrum of Zidovudine as directed in the potassium bromide disc method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Zidovudine RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample and the RS separately in a small amount of water and dry them in a desiccator (in vacuum, phosphorus (V) oxide), and perform the test with the residues.

**Optical rotation** <2.49> \(\left[\alpha\right]_{D}^{20}: +60.5 - +63.0° (0.5 \text{ g calculated on the anhydrous basis, ethanol (99.5), 50 mL, 100 mm})\).
Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of zidovudine, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of zidovudine obtained from 10 mL of this solution is equivalent to 3.5 to 6.5% of that from 10 μL of the solution for system suitability test.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Water <2.48> Not more than 1.0% (0.25 g, coulometric titration).

Residue on ignition <2.44> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 50 mg of Zidovudine and Zidovudine RS (separately determine the water <2.48> using the same manner as Zidovudine), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of each solution, add the mobile phase to make them exactly 50 mL, and use these solutions as the sample and the standard solutions, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0D> according to the following conditions. Determine the peak areas of zidovudine, \( A_I \) and \( A_S \) of both solutions.

\[
M_S: \text{Amount (mg) of thymine for liquid chromatography} \\
M_I: \text{Amount (mg) of Zidovudine}
\]

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above conditions, the relative standard deviation of the peak area of zidovudine is not more than 2.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Zinc Chloride
塩化亜鉛

\( \text{ZnCl}_2: 136.29 \)

Zinc Chloride contains not less than 97.0% of \( \text{ZnCl}_2 \).

Description Zinc Chloride occurs as white, crystalline powder, rods, or masses. It is odorless.

It is very soluble in water, and freely soluble in ethanol (95), and its solution may sometimes be slightly turbid. The solution becomes clear on addition of a small amount of hydrochloric acid.

The pH of an aqueous solution of Zinc Chloride (1 in 2) is between 3.3 and 5.3.

It is deliquescent.

Identification An aqueous solution of Zinc Chloride (1 in 30) responds to the Qualitative Tests <1.09> for zinc salt and chloride.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Zinc Chloride in 10 mL of water and 2 drops of hydrochloric acid: the solution has no color, and is clear.

(2) Sulfate <1.14>—Perform the test with 2.0 g of Zinc Chloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Ammonium—Dissolve 0.5 g of Zinc Chloride in 5 mL of water, and warm with 10 mL of a solution of sodium hydroxide (1 in 6): the evolving gas does not change moistened red litmus paper to blue.

(4) Heavy metals—Dissolve 0.5 g of Zinc Chloride in 5 mL of water in a Nessler tube, shake thoroughly with 15 mL of potassium cyanide TS, add 1 drop of sodium sulfide TS, allow to stand for 5 minutes, and immediately observe from the top downward against a white background: the solution has no more color than the following control solution.

Control solution: To 2.5 mL of Standard Lead Solution add 3 mL of water and 15 mL of potassium cyanide TS, shake thoroughly, and add 1 drop of sodium sulfide TS (not more than 50 ppm).

(5) Alkali earth metals and alkali metals—Dissolve 2.0 g of Zinc Chloride in 120 mL of water, add ammonium sulfide TS to complete precipitation, add water to make 200 mL, shake thoroughly, and filter through dry filter paper. Discard the first 20 mL of the filtrate, take the following 100 mL of the filtrate, evaporate with 3 drops of sulfuric acid to dryness, and heat the residue strongly at 600°C to constant mass: the mass is not more than 10.0 mg.

(6) Arsenic <1.15>—Prepare the test solution with 0.40 g of Zinc Chloride according to Method 1, and perform the test (not more than 5 ppm).

(7) Oxychloride—Shake gently 0.25 g of Zinc Chloride
with 5 mL of water and 5 mL of ethanol (95), and add 0.3 mL of 1 mol/L hydrochloric acid VS: the solution is clear.

**Assay** Weigh accurately about 0.3 g of Zinc Chloride, add 0.4 mL of dilute hydrochloric acid and water to make exactly 200 mL. Measure exactly 20 mL of the solution, add 80 mL of water, 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.363 mg of ZnCl₂

**Containers and storage** Containers—Tight containers.

**Zinc Oxide**

酸化亜鉛

ZnO: 81.38

Zinc Oxide, when ignited, contains not less than 99.0% of ZnO.

**Description** Zinc Oxide occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95), in acetic acid (100) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

It gradually absorbs carbon dioxide from air.

**Identification** (1) Heat Zinc Oxide strongly: a yellow color develops on strong heating, and disappears on cooling.

(2) A solution of Zinc Oxide in dilute hydrochloric acid (1 in 10) responds to the Qualitative Tests <1.06> for zinc salt.

**Purity** (1) Carbonate, and clarity and color of solution—Mix 2.0 g of Zinc Oxide with 10 mL of water, add 30 mL of dilute sulfuric acid, and heat on a water bath with stirring: no effervescence occurs, and the solution obtained is clear and colorless.

(2) Alkalinity—To 1.0 g of Zinc Oxide add 10 mL of water, and boil for 2 minutes. Cool, filter through a glass filter (G3), and to the filtrate add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L hydrochloric acid VS: no color develops.

(3) Sulfate <1.14>—Shake 0.5 g of Zinc Oxide with 40 mL of water, and filter. Take 20 mL of the filtrate, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.096%).

(4) Iron—Dissolve 1.0 g of Zinc Oxide in 50 mL of dilute hydrochloric acid (1 in 2), dissolve 0.1 g of ammonium peroxodisulfate in this solution, and extract with 20 mL of 4-methyl-2-pentanone. Add 30 mL of acetic acid-sodium acetate buffer solution for Iron Limit Test, pH 4.5, to the 4-methyl-2-pentanone layer, extract again, and use the layer of the buffer solution as the test solution. Separately, perform the test in the same manner with 1.0 mL of Standard Iron Solution, and use the layer so obtained as the control solution. Add 2 mL each of l-ascorbic acid solution for Iron Limit Test (1 in 100) to the test solution and the control solution, respectively, mix, allow to stand for 30 minutes, add 5 mL of an ethanol (95) solution of α,α’-dipyridyl (1 in 200) and water to make 50 mL. After allowing to stand for 30 minutes, compare the color of the both liquids against a white back: the color of the liquid from the test solution is not stronger than that from the control solution (not more than 10 ppm).

(5) Lead—To 2.0 g of Zinc Oxide add 20 mL of water, then add 5 mL of acetic acid (100) with stirring, and heat on a water bath until solution is complete. Cool, and add 5 drops of potassium chromate TS: no turbidity is produced.

(6) Arsenic <1.11>—Dissolve 0.5 g of Zinc Oxide in 5 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 4 ppm).

**Loss on ignition** ≤2.43 Not more than 1.0% (1 g, 850°C, 1 hour).

**Assay** Weigh accurately about 0.8 g of Zinc Oxide, previously ignited at 850°C for 1 hour, dissolve in 2 mL of water and 3 mL of hydrochloric acid, and add water to exactly 100 mL. Pipet 10 mL of this solution, add 80 mL of water, then add a solution of sodium hydroxide (1 in 50) until a slight precipitate is produced. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 4.069 mg of ZnO

**Containers and storage** Containers—Tight containers.

**Zinc Oxide Oil**

チンク油

Zinc Oxide Oil contains not less than 45.0% and not more than 55.0% of zinc oxide (ZnO: 81.38).

**Method of preparation**

| Zinc Oxide | 500 g |
| Fixed oil | a sufficient quantity |
| To make | 1000 g |

Mix the above ingredients. An appropriate quantity of Castor Oil or polysorbate 20 may be used partially in place of fixed oil.

**Description** Zinc Oxide Oil is a white to whitish, slimy substance, separating a part of its ingredients when stored for a prolonged period.

**Identification** Mix thoroughly, and place 0.5 g of Zinc Oxide Oil in a crucible, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite it strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add
Zinc Oxide Ointment

Zinc Oxide Ointment contains not less than 18.5% and not more than 21.5% of zinc oxide (ZnO: 81.38).

**Method of preparation**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc Oxide</td>
<td>200 g</td>
</tr>
<tr>
<td>Liquid Paraffin</td>
<td>30 g</td>
</tr>
<tr>
<td>White Ointment</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Ointments, with the above ingredients. White Beeswax, Sorbitan Sesquioleate or White Petrolatum may be used instead of White Ointment.

**Description**  
Zinc Oxide Ointment is white in color.

**Identification**

Place 1 g of Zinc Oxide Ointment in a crucible, melt by warming, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite strongly: a yellow color develops, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

**Purity**

Calcium, magnesium and other foreign inorganic matters—Place 2.0 g of Zinc Oxide Ointment in a crucible, melt by warming, and heat gradually raising the temperature, until the mass is thoroughly charred. Ignite the mass strongly until the residue becomes uniformly yellow, and cool. Add 6 mL of dilute hydrochloric acid, and heat on a water bath for 5 to 10 minutes: the solution is colorless and clear. Filter the solution, add 10 mL of water to the filtrate, and add ammonia TS until the precipitate first formed redissolves. Add 2 mL each of ammonium oxalate TS and disodium hydrogen phosphate TS to this solution: the solution remains unchanged or becomes very slightly turbid within 5 minutes.

**Assay**  
Weigh accurately about 0.8 g of Zinc Oxide Oil, mixed well, place in a crucible, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite until the residue becomes uniformly yellow, and cool. Dissolve the residue in 1 mL of water and 1.5 mL of hydrochloric acid, and add water to make exactly 100 mL. Add 80 mL of water to exactly 20 mL of this solution, and add a solution of sodium hydroxide (1 in 50) until a small amount of precipitates begins to form in the solution. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 4.069 mg of ZnO

Containers and storage  
Containers—Tight containers.
ethanol (99.5). It effloresces in dry air.

**Identification**

1. A solution of Zinc Sulfate Hydrate (1 in 20) responds to the Qualitative Tests $<1.09$ for zinc salt.
2. A solution of Zinc Sulfate Hydrate (1 in 20) responds to the Qualitative Tests $<1.09$ for sulfite.

**pH**

$<2.54>$ Dissolve 1.0 g of Zinc Sulfate Hydrate in 20 mL of water: the pH of the solution is between 4.4 and 6.0.

**Purity**

1. Clarity and color of solution—Dissolve 0.25 g of Zinc Sulfate Hydrate in 5 mL of water: the solution is clear and colorless.
2. Heavy metals $<1.07>$—Dissolve 1.0 g of Zinc Sulfate Hydrate in 10 mL of water contained in a Nessler tube. Add 20 mL of potassium cyanide TS, and mix well. Add 2 drops of sodium sulfide TS, and allow the mixture to stand for 5 minutes. Observe vertically against a white background, the color of the solution is not more intense than the following control solution.

   **Control solution:** To 1.0 mL of Standard Lead Solution add 10 mL of water and 20 mL of potassium cyanide TS, and mix well. Add 2 drops of sodium sulfide TS (not more than 10 ppm).

3. Alkali earth metals and alkali metals—Dissolve 2.0 g of Zinc Sulfate Hydrate in 150 mL of water, add a suitable amount of ammonium sulfide TS to complete the precipitation, and add water to make exactly 200 mL. Shake well, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, take exactly 100 mL of the subsequent filtrate, evaporate to dryness, and ignite as directed under Residue on Ignition $<2.44>$: the mass of the residue is not more than 5.0 mg.

4. Arsenic $<1.11>$—Prepare the test solution with 1.0 g of Zinc Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

**Loss on drying**

$<2.41>$ Not less than 35.5% and not more than 38.5% (1 g, 105°C, 3 hours).

**Assay**

Weigh accurately about 0.3 g of Zinc Sulfate Hydrate, and dissolve in water to make exactly 100 mL. Measure exactly 25 mL of this solution, add 100 mL of water and 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate $<2.50>$ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

$= 2.876 \text{ mg of } \text{ZnSO}_4\cdot7\text{H}_2\text{O}$

**Containers and storage**

Containers—Tight containers.

---

**Zinc Sulfate Ophthalmic Solution**

硫酸亜鉛点眼液

Zinc Sulfate Ophthalmic Solution contains not less than 0.27 w/v% and not more than 0.33 w/v% of zinc sulfate hydrate (ZnSO₄·7H₂O: 287.55).

**Method of preparation**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc Sulfate Hydrate</td>
<td>3 g</td>
</tr>
<tr>
<td>Boric Acid</td>
<td>20 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>Fennel Oil</td>
<td>2 mL</td>
</tr>
<tr>
<td>Purified Water or Purified Water</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Ophthalmic Solution, with the above ingredients.

**Description**

Zinc Sulfate Ophthalmic Solution is a clear, colorless liquid.

**Identification**

1. Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests $<1.09>$ for zinc salt.
2. Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests $<1.09>$ for borate.
3. Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests $<1.09>$ for chloride.

**Assay**

Pipet accurately 25 mL of Zinc Sulfate Ophthalmic Solution, add 100 mL of water and 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate $<2.50>$ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

$= 2.876 \text{ mg of } \text{ZnSO}_4\cdot7\text{H}_2\text{O}$

**Containers and storage**

Containers—Tight containers.
Zinostatin Stimalamer

ジノスタチン スチマラマー

It is freely soluble in water, and practically insoluble in ethanol (95%) and in diethyl ether.

**Identification** (1) Dissolve 10 mg of Zinostatin Stimalamer in 1 mL of sodium hydroxide TS, and add a drop of copper (II) sulfate TS: a purple color develops.

(2) Dissolve 1 mg of Zinostatin Stimalamer in 1 mL of 0.05 mol/L phosphate buffer solution, pH 7.0, add 0.5 mL of a solution of trichloroacetic acid (1 in 5), and shake: a white precipitate is formed.

(3) Determine the absorption spectra of solutions of Zinostatin Stimalamer and Zinostatin Stimalamer RS in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectra of Zinostatin Stimalamer and Zinostatin Stimalamer RS as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Absorbance** <2.24> $E_{1\%}^{1cm}$ (268 nm): 15.5 – 18.5 (4 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 10 mL).

**Optical rotation** <2.49> $[\alpha]_{D}^{20}$: –30.0 – –38.0° (20 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 5 mL, 100 mm).

**pH** <2.54> Dissolve 10 mg of Zinostatin Stimalamer in 1 mL of water: the pH of the solution is between 4.5 and 5.5.

**Purity** (1) Clarity and color of solution—Dissolve 20 mg of Zinostatin Stimalamer in 2 mL of 0.05 mol/L phosphate buffer solution, pH 5.0: the solution is clear, and the absorbance at 400 nm of this solution after addition of 3 mL of 0.05 mol/L phosphate buffer solution, determined as directed under Ultraviolet-visible Spectrophotometry <2.24>, is not more than 0.25.

(2) Heavy metals <1.07>—Weigh accurately 40 mg of Zinostatin Stimalamer, place in a crucible, carbonize and incinerate according to Method 2, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. After cooling, weigh the residue $M_{T}$ g. Then, moisten the residue with 0.1 mL of diluted hydrochloric acid (1 in 5), add 1 mL of water, 85 $\mu$L of diluted ammonia TS (1 in 2) and 0.1 mL of dilute acetic acid, and add water so that the mass is $M_{T} + 2.0$ g. Adjust the pH of this solution to 3.2 to 3.4 with diluted ammonia TS (1 in 20) or diluted hydrochloric acid (1 in 50), add water so that the mass is $M_{T} + 2.5$ g, and use this solution as the test solution. Separately, prepare the blank solution in the same manner without the sample. Separately, take 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid, and evaporate to dryness according to Method 2. After cooling, weigh the residue $M_{S}$ g. Then, moisten the residue with 0.1 mL of diluted hydrochloric acid (1 in 5), and proceed in the same manner as directed in the preparation of the test solution. After adjusting the pH of the solution so obtained to 3.2 to 3.4, add 80 $\mu$L of Standard Lead Solution, and add water so that the mass is $M_{S} + 2.5$ g, and use this solution as the control solution. Add 10 $\mu$L each of diluted sodium sulfide TS (1 in 6) to the test solution, the blank solution and the control solution, mix,
and allow to stand for 5 minutes. Determine the absorbances, \( A_T \), \( A_0 \) and \( A_S \) of the test solution, the blank solution and the control solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.42\): \( A_T - A_0 \) is not larger than \( A_S - A_0 \) (not more than 20 ppm).

(3) Styrene-maleic acid alternating copolymer partial butyl ester and neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)—

(i) Test solutions

Solution A: Dissolve 36.6 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.23 mL of \( N,N,N',N' \)-tetramethylethylenediamine and water to make 100 mL.

Solution B: Dissolve 33.3 g of acrylamide and 0.89 g of \( N,N' \)-methylenbisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution C: Dissolve 5.98 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.46 mL of \( N,N,N',N' \)-tetramethylethylenediamine and water to make 100 mL.

Solution D: Dissolve 10.0 g of acrylamide and 2.5 g of \( N,N' \)-methylenbisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution E: Dissolve 4 mg of riboflavin in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution F: Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.4 g of glycerin in water to make 500 mL.

Buffer solution for sample: To 50 mL of Solution C add 20 mL of water and 10 mL of glycerin solution (3 in 5).

(ii) Gels

Resolving gel: Mix 2.5 mL of Solution A and 7.5 mL of Solution B. Mix the mixture with 10 mL of freshly prepared ammonium peroxydisulfate solution (7 in 5000) after degassing under reduced pressure. Pour this mixture into a glass tube, 5 mm in inside diameter and 10 cm in length, to make 7 cm height, put water gently on the upper surface of the mixture, and allow to polymerize for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

Stacking gel: Mix 1 mL of Solution C, 2 mL of Solution D, 1 mL of Solution E and 4 mL of water, pour 0.2 mL of the mixture on the resolving gel, put water gently on the upper surface of the mixture, and allow to polymerize under a fluorescent light for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

(iii) Standard solution

Weigh accurately about 6 mg of styrene-maleic acid alternating copolymer partial butyl ester, calculated on the anhydrous basis, and dissolve in the buffer solution for sample to make exactly 20 mL. Separately, weigh accurately about 6 mg of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3), and dissolve in the buffer solution for sample to make exactly 20 mL. Pipet 1 mL each of these solutions, add the buffer solution for sample to make exactly 20 mL, and use this solution as the standard solution.

(iv) Sample solution

Weigh accurately about 5 mg of Zinostatin Stimalamer, calculated on the anhydrous basis, dissolve in the buffer solution for sample to make exactly 10 mL.

(v) Procedure

Mount the gel in the electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the top reservoir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100 \( \mu L \) each of the sample solution and standard solution onto the surface of separate gels, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as the bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached at 5 cm from the upper end of the gel.

(vi) Staining and decolorization Dissolve 0.1 g of Coomassie brilliant blue G-250 in 100 mL of trichloroacetic acid solution (1 in 2), and mix before using 1 volume of this solution and 2 volumes of water. Immerse the gels for 15 hours in this mixture, and transfer into about 20 mL of acetic acid (100) solution (7 in 100) to remove the excess of dye. Replace the acetic acid (100) solution until the back ground of the gel becomes colorless.

(vii) Determination

Determine the peak areas, \( T_1 \), \( T_2 \), \( A_1 \), \( A_2 \), \( A_3 \) and \( A_4 \) of styrene-maleic acid alternating copolymer partial butyl ester and neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) obtained from the sample solution and the standard solution, based on the absorbance at 600 nm of the gels determined by using a densitometer. Calculate the amounts of styrene-maleic acid alternating copolymer partial butyl ester and neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) by the following formula:

\[
\text{Amount} (\% \text{ of styrene-maleic acid alternating copolymer partial butyl ester}) = \frac{M_S}{M_T} \times \frac{T_1}{A_1} \times \frac{A_3}{A_5} \times \frac{5/2}{5/2}
\]

\[
\text{Amount} (\% \text{ of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)}) = \frac{M_S}{M_T} \times \frac{(P_S/100)/M_T}{A_1/2} \times \frac{5/2}{5/2}
\]

\( M_S \): Amount (mg) of styrene-maleic acid alternating copolymer partial butyl ester, calculated on the anhydrous basis

\( M_T \): Amount (mg) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3), calculated on the anhydrous basis

\( M_1 \): Amount (mg) of sample, calculated on the anhydrous basis

\( P_S \): Purity (\% of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)

(4) Neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1)—Weigh accurately about 10 mg of Zinostatin Stimalamer, calculated on the anhydrous basis, dissolve in the mobile phase to make exactly 1 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 10 mg of neocarzinostatin (separately determine the water \(<2.48\) in the same manner as Zinostatin Stimalamer), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 0.2 mL each of the sample stock solution and the standard stock solution, add to them exactly 1.5 mL each of a solution, prepared by dissolving 38.1 g of sodium tetraborate decahydrate in dilute sodium hydroxide TS to make 1000 mL, add exactly 1.2 mL of a solution of sodium 2,4,6-trinitrobenzenesulfonate (1 in 20), allow to stand for 10 minutes at room temperature, then add
exactly 6 mL of sodium sulfite-sodium dihydrogen phosphate TS, shake thoroughly, and use these solutions as the sample solution and the standard solution, respectively. Separately, pipet 0.2 mL of the sample stock solution, add 1.5 mL of a solution, prepared by dissolving 38.1 g of sodium tetraborate decahydrate in dilute sodium hydroxide TS to make 1000 mL, add exactly 1.2 mL of water, allow to stand for 10 minutes at room temperature, then add exactly 6 mL of sodium sulfite-sodium dihydrogen phosphate TS, shake thoroughly, and use this solution as the blank solution. Perform the test with exactly 0.25 mL each of the sample solution, the standard solution and the blank solution as directed under Liquid Chromatography (2.07) under the following conditions, and determine the peak area, A*1, of trinitrobenzenesulfonic acid derivative of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) obtained from the sample solution, the peak area, A*6, of trinitrobenzenesulfonic acid derivative of neocarzinostatin obtained from the standard solution, which retention time is almost the same as that of trinitrobenzenesulfonic acid derivative of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) obtained from the sample solution, and the peak area, A*0, obtained from the blank solution. Calculate the amount of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) by the following formula: not more than 5.0%.

\[
\text{Amount} (\%) \text{ of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1)} = \frac{M/S}{M/T} \times \frac{(A*1 - A*0)}{A*1} \times 2 \times 2.280
\]

\[M/S: \text{Amount (mg) of neocarzinostatin calculated on the anhydrous basis}
\]
\[M/T: \text{Amount (mg) of sample, calculated on the anhydrous basis}
\]

Operating conditions—

Detector: A visible absorption photometer (wavelength: 436 nm).

Column: Pre-column is a stainless steel column 7.5 mm in inside diameter and 7.5 cm in length, packed with silica gel for liquid chromatography (10 μm in particle size). Separation column is a stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with silica gel for liquid chromatography (10 μm in particle size), which is coupled to the pre-column.

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.78 g of potassium dihydrogen phosphate and 5.52 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of trinitrobenzenesulfonic acid derivative of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) is about 21 minutes.

System suitability—

System performance: When the procedure is run with 0.25 mL of the standard stock solution under the above operating conditions except at 254 nm, the number of theoretical plates and the symmetry factor of the peak of neocarzinostatin are not less than 2000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 3 times with 0.25 mL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trinitrobenzenesulfonic acid derivative of neocarzinostatin is not more than 10%.

(5) Manufacturing process origin inorganic salts—Being specified separately.

Water ≤2.48 Getting not more than 12.0% (10 mg, coulometric titration).

Assay—Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics (4.02) according to the following conditions. Perform the procedures of (iii), (iv) and (v) without exposure to direct or indirect sunlight.

(i) Test organism—Micrococcus luteus ATCC 9341

(ii) Culture medium—Use the medium 1 in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Zinostatin Stimalamer RS equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 50 mL, and use this solution as the high concentration standard solution. Pipet 5 mL of the high concentration standard solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 20 mL, and use this solution as the low concentration standard solution.

(iv) Sample solutions—Weigh accurately an amount of Zinostatin Stimalamer equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 50 mL, and use this solution as the high concentration sample solution. Pipet 5 mL of the high concentration sample solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 20 mL, and use this solution as the low concentration sample solution.

(v) Procedure—Allow to stand at 3 to 5°C for 2 hours before incubation.

Containers and storage—Containers—Tight containers. Storage—Light-resistant, and not exceeding –20°C.

Zolpidem Tartrate

ゾルピデム酒石酸塩

\[
\text{(C}_{10}\text{H}_{12}\text{N}_{2}\text{O}_{2})_2\text{C}_{6}\text{H}_{12}\text{O}_{6}: \text{764.87}
\]

N,N,6-Trimethyl-2-(4-methylphenyl)imidazo[1,2-a]pyridine-3-acetamide hemi-(2R,3R)-tartrate [99294-93-6]

Zolpidem Tartrate contains not less than 98.5% and not more than 101.0% of [(C_{10}H_{12}N_{2}O_{2})_{2} C_{6}H_{12}O_{6}], calculated on the anhydrous basis.

Description—Zolpidem Tartrate occurs as a white, crystal-
line powder.

It is freely soluble in acetic acid (100), soluble in \(N,N\)dimethylformamide and in methanol, sparingly soluble in water, and slightly soluble in ethanol (99.5) and in acetic anhydride. It dissolves in 0.1 mol/L hydrochloric acid TS.

It gradually changes to yellow in color on exposure to light.

Optical rotation \([\alpha]D\) about +1.8° (1 g, \(N,N\)-dimethylformamide, 20 mL, 100 mm).

Identification (1) Dissolve 50 mg of Zolpidem Tartrate in 5 mL of acetic acid (100) and add 3 drops of Dragendorff’s TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of a solution of Zolpidem Tartrate in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Zolpidem Tartrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Zolpidem Tartrate in methanol (1 in 10) responds to the Qualitative Tests <1.06> (3) for tartrate.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Zolpidem Tartrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 10 mg of Zolpidem Tartrate in 20 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each area of the peak other than the peak of zolpidem from the sample solution is not larger than the peak area of zolpidem from the standard solution.

Operating conditions—
Detector: A ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel tube 4.6 mm in inside diameter and 7.5 cm in length, packed with octadecysilanized silica gel for liquid chromatography (5 \(\mu\)m particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: To 4.9 g of phosphoric acid add 1000 mL of water, and adjust the pH to 5.5 with triethylamine. To 11 volumes of this solution add 5 volumes of methanol and 4 volumes of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of zolpidem is about 5 minutes.
Time span of measurement: About 5 times as long as the retention time of zolpidem.

System suitability—
System performance: Dissolve 10 mg each of Zolpidem Tartrate and benzyl parahydroxybenzoate in 100 mL of methanol. When the procedure is run with 5 \(\mu\)L of this solution under the above operating conditions, zolpidem and benzyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of zolpidem is not more than 5.0%.

(3) Residual solvent Being specified separately.

Water <2.47> Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Zolpidem Tartrate, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) and titrate <2.5D> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 38.24 mg of \((C_{19}H_{21}N_{3}O)_{2}C_{4}H_{6}O_{6}\)

Containers and storage Containers—Tight containers.
Storage conditions—Light-resistant.

Zolpidem Tartrate Tablets

ゾルピデム酒石酸塩錠

Zolpidem Tartrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of zolpidem tartrate \([(C_{19}H_{21}N_{3}O)_{2}C_{4}H_{6}O_{6}]: 764.87]\).

Method of preparation Prepare as directed under Tablets, with Zolpidem Tartrate.

Identification To 1 tablet of Zolpidem Tartrate Tablets add 100 mL of 0.1 mol/L hydrochloric acid TS, shake for 30 minutes, and filter. Discard the first 20 mL of the filtrate, to a volume of the subsequent filtrate, equivalent to 1 mg of Zolpidem Tartrate according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 235 nm and 239 nm and between 292 nm and 296 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Zolpidem Tartrate Tablets add V/10 mL of 0.1 mol/L hydrochloric acid TS, and disintegrate the tablet by shaking for 15 minutes. Add 2V/5 mL of methanol, then add exactly V/10 mL of the internal standard solution, shake for 15 minutes, and add methanol to make V mL so that each mL contains about 0.1 mg of zolpidem tartrate \([(C_{19}H_{21}N_{3}O)_{2}C_{4}H_{6}O_{6}].\) Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of zolpidem tartrate for assay (separately determine the water <2.48>) in the same manner as
Zolpidem Tartrate Tablets, and dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add exactly 25 mL of the internal standard solution, then add methanol to make 250 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of zolpidem tartrate [(C\textsubscript{19}H\textsubscript{21}N\textsubscript{3}O\textsubscript{2}.C\textsubscript{4}H\textsubscript{6}O\textsubscript{6}] = M\textsubscript{s} × Q\textsubscript{1} / Q\textsubscript{a} × V / 250

M\textsubscript{s}: Amount (mg) of zolpidem tartrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of benzyl parahydroxybenzoate in methanol (1 in 1000).

**Dissolution** 6.10 When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Zolpidem Tartrate Tablets is not less than 80%.

Start the test with 1 tablet of Zolpidem Tartrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the 2nd fluid for dissolution test to make exactly V mL so that each mL contains about 2.8 μg of zolpidem tartrate [(C\textsubscript{19}H\textsubscript{21}N\textsubscript{3}O\textsubscript{2}.C\textsubscript{4}H\textsubscript{6}O\textsubscript{6}] according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of zolpidem tartrate for assay (separately determine the water < 2.48% in the same manner as Zolpidem Tartrate), and dissolve in water to make exactly 100 mL. Pipet 25 mL of this solution, add the 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A\textsubscript{s} and A\textsubscript{a}, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using diluted the 2nd fluid for dissolution test (1 in 2) as the blank.

Dissolution rate (%) with respect to the labeled amount of zolpidem tartrate [(C\textsubscript{19}H\textsubscript{21}N\textsubscript{3}O\textsubscript{2}.C\textsubscript{4}H\textsubscript{6}O\textsubscript{6}] = M\textsubscript{s} × A\textsubscript{a} / A\textsubscript{s} × V / V′ × 1 / C × 45 / 4

M\textsubscript{s}: Amount (mg) of zolpidem tartrate for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of zolpidem tartrate [(C\textsubscript{19}H\textsubscript{21}N\textsubscript{3}O\textsubscript{2}.C\textsubscript{4}H\textsubscript{6}O\textsubscript{6}] in 1 tablet

**Assay** To 20 Zolpidem Tartrate Tablets add V/10 mL of 0.1 mol/L hydrochloric acid TS, and disintegrate the tablet by shaking for 15 minutes. Add 2V/5 mL of methanol, then add exactly V/10 mL of the internal standard solution, shake for 15 minutes, and add methanol to make V mL so that each mL contains about 1 mg of zolpidem tartrate [(C\textsubscript{19}H\textsubscript{21}N\textsubscript{3}O\textsubscript{2}.C\textsubscript{4}H\textsubscript{6}O\textsubscript{6}] in 1 tablet of Zolpidem Tartrate Tablets. Centrifuge this solution, add to 1 mL of the supernatant liquid add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of zolpidem tartrate for assay (separately determine the water < 2.48% in the same manner as Zolpidem Tartrate), and dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add exactly 2.5 mL of the internal standard solution, then add methanol to make 250 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and calculate the ratio, Q\textsubscript{1} and Q\textsubscript{2}, of the peak area of zolpidem to that of the internal standard.

Amount (mg) of zolpidem tartrate [(C\textsubscript{19}H\textsubscript{21}N\textsubscript{3}O\textsubscript{2}.C\textsubscript{4}H\textsubscript{6}O\textsubscript{6}] in 1 tablet of Zolpidem Tartrate Tablets

= M\textsubscript{s} × Q\textsubscript{1} / Q\textsubscript{a} × V / 500

M\textsubscript{s}: Amount (mg) of zolpidem tartrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of benzyl parahydroxybenzoate in methanol (1 in 100).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 4.9 g of phosphoric acid add 1000 mL of water, and adjust to pH 5.5 with triethylamine. To 550 mL of this solution add 250 mL of methanol and 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of zolpidem is about 5 minutes.

**System suitability**—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, zolpidem and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of zolpidem to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.
Crude Drugs

Acacia

*Gummi Arabicum*

アラビアゴム

Acacia is the secretions obtained from the stems and branches of *Acacia senegal* Willdenow or other species of the same genus (*Leguminosae*).

**Description** Colorless or light yellow-brown, translucent or somewhat opaque spheroidal tears, or angular fragments with numerous fissures on the surface; very brittle; the fractured surface glassy and occasionally iridescent.

Odorless; tasteless, but produces a mucilaginous sensation on the tongue.

Pulverized Acacia (1.0 g) dissolves almost completely in 2.0 mL of water, and the solution is acid.

It is practically insoluble in ethanol (95%).

**Identification** To 1 g of powdered Acacia add 25 mL of water and 1 mL of sulfuric acid, and heat under a reflux condenser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of D-galactose in 1 mL water, add methanol to make 10 mL, and use this solution as the standard solution (1). Proceed with L-arabinose and with L-rhamnose monohydrate in the same manner as for the preparation of the standard solution (1), and use so obtained solutions as the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and water (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: the three spots from the sample solution are denser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of D-galactose in 1 mL water, add methanol to make 10 mL, and use this solution as the standard solution (1). Proceed with L-arabinose and with L-rhamnose monohydrate in the same manner as for the preparation of the standard solution (1), and use so obtained solutions as the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and water (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: the three spots from the sample solution are the same with the spots of D-galactose, L-arabinose and L-rhamnose from the standard solution in the color tone and the RF value, respectively.

**Purity** (1) Insoluble residue—To 5.0 g of pulverized Acacia add 100 mL of water and 10 mL of dilute hydrochloric acid, and dissolve by gentle boiling for 15 minutes with swirling. Filter the warm mixture through a tared glass filter (G3), wash the residue thoroughly with hot water, and dry at 105°C for 5 hours: the mass of the residue does not exceed 10.0 mg.

(2) Tannin-bearing gums—To 10 mL of a solution of Acacia (1 in 50) add 3 drops of iron (III) chloride TS: no dark green color is produced.

(3) Glucose—Dissolve 10 mg of glucose in 1 mL of water, add methanol to make 10 mL, and use this solution as the standard solution. Proceed with the sample solution obtained in the Identification and the standard solution obtained here as directed in the Identification: any spot at the RF value corresponding to glucose from the standard solution does not appear from the sample solution.

**Loss on drying** <5.0> Not more than 17.0% (6 hours).

**Total ash** <5.0> Not more than 4.0%.

**Acid-insoluble ash** <5.0> Not more than 0.5%.

**Containers and storage** Containers—Well-closed containers.

**Powdered Acacia**

*Gummi Arabicum Pulveratum*

アラビアゴム末

Powdered Acacia is the powder of Acacia.

**Description** Powdered Acacia occurs as a white to yellowish white powder. It is odorless, tasteless, but produces a mucilaginous sensation on the tongue.

Under a microscope <5.0>, Powdered Acacia, immersed in olive oil or liquid paraffin, reveals colorless, angular fragments or nearly globular grains. Usually starch grains or vegetable tissues are not observed or very trace, if any.

Powdered Acacia (1.0 g) dissolves almost completely in 2.0 mL of water, and the solution is acid.

It is practically insoluble in ethanol (95%).

**Identification** To 1 g of Powdered Acacia add 25 mL of water and 1 mL of sulfuric acid, and heat under a reflux condenser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of D-galactose in 1 mL water, add methanol to make 10 mL, and use this solution as the standard solution (1). Proceed with L-arabinose and with L-rhamnose monohydrate in the same manner as for the preparation of the standard solution (1), and use so obtained solutions as the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and water (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 5 hours: the mass of the residue does not exceed 5.01 mg. Powdered Acacia, immersed in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of D-galactose in 1 mL water, add methanol to make 10 mL, and use this solution as the standard solution (1). Proceed with L-arabinose and with L-rhamnose monohydrate in the same manner as for the preparation of the standard solution (1), and use so obtained solutions as the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and water (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: the three spots from the sample solution are the same with the spots of D-galactose, L-arabinose and L-rhamnose from the standard solution in the color tone and the RF value, respectively.

**Purity** (1) Insoluble residue—To 5.0 g of Powdered Acacia add 100 mL of water and 10 mL of dilute hydrochloric acid, and dissolve by gentle boiling for 15 minutes with swirling. Filter the warm mixture through a tared glass filter (G3), wash the residue thoroughly with hot water, and dry at 105°C for 5 hours: the mass of the residue does not exceed 10.0 mg.

(2) Tannin-bearing gums—To 10 mL of a solution of Acacia (1 in 50) add 3 drops of iron (III) chloride TS: no dark green color is produced.

(3) Glucose—Dissolve 10 mg of glucose in 1 mL of water, add methanol to make 10 mL, and use this solution as the standard solution. Proceed with the sample solution obtained in the Identification and the standard solution obtained here as directed in the Identification: any spot at the RF value corresponding to glucose from the standard solution does not appear from the sample solution.

**Loss on drying** <5.0> Not more than 17.0% (6 hours).

**Total ash** <5.0> Not more than 4.0%.

**Acid-insoluble ash** <5.0> Not more than 0.5%.

**Containers** Containers—Well-closed containers.
Acid-insoluble ash

Containers—Tight containers.

Purity (1)

Stem—When perform the test of foreign material other than stems contained in Achyranthes Root does not exceed 1.0%.

Loss on drying <5.01% Not more than 17.0% (6 hours).

Total ash <5.01% Not more than 10.0%.

Acid-insoluble ash <5.01% Not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Achyranthes Root

Achyranthis Radix

ゴシツ

Achyranthes Root is the root of Achyranthes fauriei Leveillé et Vaniot or Achyranthes bidentata Blume (Amaranthaceae).

Description Main root or main root with some lateral roots, with or without short remains of rhizome at the crown; main root, long cylindrical and sometimes somewhat tortuous, 15 – 90 cm in length, 0.3 – 0.7 cm in diameter; externally grayish yellow to yellow-brown, with numerous longitudinal wrinkles, and with scattering scars of lateral roots. Fractured surface is flat; grayish white to light brown on the circumference, and with yellowish white xylem in the center. Hard and brittle, or flexible.

Odor, slight; taste, slightly sweet, and mucilaginous.

Under a microscope <5.01%, a transverse section reveals a rather distinct cambium separating the cortex from the xylem; small protoxylem located at the center of the xylem, and surrounded by numerous vascular bundles arranged on several concentric circles; parenchyma cells containing sand crystals of calcium oxalate; starch grains absent.

Identification Shake vigorously 0.5 g of pulverized Achyranthes Root with 10 mL of water: a lasting fine foam is produced.

Purity (1) Stem—When perform the test of foreign matter <5.01%, the amount of stems contained in Achyranthes Root does not exceed 5.0%.

(2) Heavy metals <1.07%—Proceed with 3.0 g of pulverized Achyranthes Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.1%—Prepare the test solution with 0.40 g of pulverized Achyranthes Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <5.01%—The amount of foreign matter other than stems contained in Achyranthes Root does not exceed 1.0%.

Loss on drying <5.01% Not more than 17.0% (6 hours).

Total ash <5.01% Not more than 10.0%.

Acid-insoluble ash <5.01% Not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Agar

Agar

カンテン

Agar is the solid residue obtained by freezing dehydration of a mucilage derived from Gelidium elegans Kuetzing, other species of the same genus (Gelidiaceae), or other red algae (Rhodophyta).

Description White, translucent rectangular column, string or flakes. Rectangular column about 26 cm in length, 4 cm square in cross section; a string of about 35 cm in length and about 3 mm in width; flakes about 3 mm in length; externally, with wrinkles and somewhat lustrous, light and pliable.

Odorless; tasteless and mucilagenous.

It is practically insoluble in organic solvents.

A boiling solution of Agar (1 in 100) is neutral.

Identification (1) To a fragment of Agar add dropwise iodine TS: a dark blue to reddish purple color develops.

(2) Dissolve 1 g of Agar in 65 mL of water by boiling for 10 minutes with constant stirring, and add a sufficient amount of hot water to make up the water lost by evaporation: the solution is clear. Cool the solution between 30°C and 39°C: the solution forms a firm, resilient gel, which does not melt below 85°C.

Purity (1) Sulfuric acid—Dissolve 1.0 g of Agar in 100 mL of water by boiling: the solution is not acidic.

(2) Sulfurous acid and starch—To 5 mL of the solution obtained in (1) add 2 drops of iodine TS: the solution is not decolorized immediately, and does not show a blue color.

(3) Insoluble matter—To 7.5 g of Agar add 500 mL of water, boil for 15 minutes, and add water to make exactly 500 mL. Measure exactly 100 mL of the solution, add 100 mL of hot water, heat to boiling, filter while hot through a tared glass filter (G3), wash the residue with a small amount of hot water, and dry the residue at 105°C for 3 hours: the mass of the residue is not more than 15.0 mg. the amount of foreign material other than stems contained in Achyranthes Root does not exceed 1.0%.

(4) Water absorption—To 5.0 g of Agar add water to make 100 mL, shake well, allow to stand at 25°C for 24 hours, and filter through moistened glass wool in a 100-mL graduated cylinder: the volume of the filtrate is not more than 75 mL.

Loss on drying <5.01% Not more than 22.0% (6 hours).

Total ash <5.01% Not more than 4.5%.

Acid-insoluble ash <5.01% Not more than 0.5%.
Containers and storage  Containers—Well-closed containers.

**Powdered Agar**

*Agar Pulveratum*

Powdered Agar is the powder of Agar.

**Description**  Powdered Agar appears as a white powder, is odorless, and is tasteless and mucilagenous.

Under a microscope <5.01>, Powdered Agar, immersed in olive oil or liquid paraffin, reveals angular granules with striations or nearly spheroidal granules 5 to 60 μm in diameter.

It becomes transparent in chloral hydrate TS. It is practically insoluble in organic solvents.

A boiling solution of Powdered Agar (1 in 100) is neutral.

**Identification**  (1) To a part of Powdered Agar add dropwise iodine TS: a dark blue to reddish purple color develops.

(2) Dissolve 1 g of Powdered Agar in 65 mL of water by boiling for 10 minutes with constant stirring, and add a sufficient amount of hot water to maintain the original volume lost by evaporation: the solution is clear. Cool the solution between 30°C and 39°C: the solution forms a firm, resilient gel, which does not melt below 85°C.

**Purity**  (1) Sulfuric acid—Dissolve 1.0 g of Powdered Agar in 100 mL of water by boiling: the solution is not acid.

(2) Sulfurous acid and starch—To 5 mL of the solution obtained in (1) add 2 drops of iodine TS: the solution is not decolorized immediately, and does not show a blue color.

(3) Insoluble matter—To 7.5 g of Powdered Agar add 500 mL of water, boil for 15 minutes, and add water to make exactly 500 mL. Take exactly 100 mL of the solution, add 100 mL of hot water, heat to boiling, filter while hot through a tared glass filter (G3), wash the residue with a small amount of hot water, and dry the residue at 105°C for 3 hours: the mass of the residue is not more than 15.0 mg.

(4) Water absorption—To 5.0 g of Powdered Agar add water to make 100 mL, shake well, allow to stand at 25°C for 24 hours, and filter through moistened glass wool in a 100-mL graduated cylinder: the volume of the filtrate is not more than 75 mL.

**Loss on drying**  <5.01>  Not more than 22.0% (6 hours).

**Total ash**  <5.01>  Not more than 4.5%.

**Acid-insoluble ash**  <5.01>  Not more than 0.5%.

Containers and storage  Containers—Tight containers.

**Akebia Stem**

*Akebiae Caulis*

モクツウ

Akebia Stem is the climbing stem of *Akebia quinata* Decaisne or *Akebia trifoliata* Koidzumi (Lardizabalaceae), usually cut transversely.

**Description**  Circular or ellipsoidal sections 0.2–0.3 cm in thickness, and 1–3 cm in diameter; phloem on both fractured surfaces is dark grayish brown; xylem reveals light brown vessel portions and grayish white medullary rays lined alternately and radially; pith light grayish yellow, and distinct; flank grayish brown, and with circular or transversely elongated elliptical lenticels.

Almost odorless; slightly acrid taste.

Under a microscope <5.01>, a transverse section reveals ring layers mainly consisting of fiber bundles with crystal cells and stone cell groups and surrounding the outside of the phloem in arc shape. Medullary rays of the phloem consisting of sclerenchymatous cells containing solitary crystals; portion near cambium is distinct; cells around the pith remarkably thick-walled; xylem medullary rays and parenchymatous cells around the pith contain solitary crystals of calcium oxalate and starch grains less than 8 μm in diameter.

**Identification**  To 0.5 g of pulverized Akebia Stem add 10 mL of water, boil, allow to cool, and shake vigorously: lasting fine foams are produced.

**Total ash**  <5.01>  Not more than 10.0%.

Containers and storage  Containers—Well-closed containers.

**Alisma Rhizome**

*Alismatis Rhizoma*

タクシャ

Alisma Rhizome is the tuber of *Alisma orientale* Juzepczuk (Alismataceae), from which periderm has been usually removed.

**Description**  Spherical or conical tubers, 3–8 cm in length, 3–5 cm in diameter, sometimes a 2- to 4-branched irregular tuber; externally light grayish brown to light yellow-brown, and slightly annulate; many remains of root appearing as small warty protrusions; fractured surface nearly dense, the outer portion grayish brown, and the inner part white to light yellow-brown in color; rather light in texture and difficult to break.

Slight odor and slightly bitter taste.

**Purity**  (1) Heavy metals <1.07>—Proceed with 1.0 g of pulverized Alisma Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Alisma Rhizome according to Method 4, and perform the test (not more than 5 ppm).

**Total ash**  <5.01>  Not more than 5.0%.

**Acid-insoluble ash**  <5.01>  Not more than 0.5%.

Containers and storage  Containers—Well-closed containers.
Powdered Alisma Rhizome

Alismatis Rhizoma Pulveratum

タクシャ末

Powdered Alisma Rhizome is the powder of Alisma Rhizome.

Description Powdered Alisma Rhizome occurs as a light grayish brown powder, and has a slight odor and a slightly bitter taste.

Under a microscope, Powdered Alisma Rhizome reveals mainly starch grains, fragments of parenchyma containing them, parenchyma cells containing yellow contents, and fragments of vascular bundles. Starch grains, spheroidal to ellipsoidal simple grains, 3 – 15 μm in diameter.

Purity (1) Heavy metals ≤ 0.02%. Perform the test as directed under Thin-layer Chromatography (2.01). Add 5 mL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100:20:5:2.2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among several spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same Rf value.

Purity (2) Resin—Warm 0.5 g of pulverized Aloe with 10 mL of diethyl ether on a water bath, and filter. Wash the residue and the filter paper with 3 mL of diethyl ether. Combine the filtrate and the washing, and evaporate the diethyl ether solution: the mass of the residue is not more than 5.0 mg.

(2) To 0.2 g of pulverized Aloe add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of barbaloin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.01). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (20:5:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among several spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same Rf value.

Acid-insoluble ash ≤ 0.5%. Containers—Well-closed containers.

Aloe

アロエ

Aloe is the dried juice of the leaves mainly of Aloe ferox Miller, or of hybrids of the species with Aloe africana Miller or Aloe spicata Baker (Liliaceae).

It contains not less than 4.0% of barbaloin, calculated on the basis of dried material.

Description Aloe occurs as blackish brown to dark brown, irregular masses; sometimes the external surface covered with a yellow powder; the fractured surface smooth and glassy.

Odor, characteristic; taste, extremely bitter.

Identification (1) Dissolve 0.5 g of pulverized Aloe in 50 mL of water by warming. After cooling, add 0.5 g of siliceous earth, and filter. Perform the following tests using the filtrate as the sample solution.

(i) Dissolve 0.2 g of sodium tetraborate decahydrate in 5 mL of the sample solution by warming in a water bath. Add a few drops of this solution into 30 mL of water, and shake: a green fluoresence is produced.

(ii) Shake 2 mL of the sample solution with 2 mL of nitric acid: a yellow-brown color which changes gradually to green is produced. Then warm this colored solution in a water bath: the color of the solution changes to red-brown.

(ii) Shake 2 mL of the sample solution with 2 mL of nitric acid: a yellow-brown color which changes gradually to green is produced. Then warm this colored solution in a water bath: the color of the solution changes to red-brown.
Containers—Well-closed containers.

**Assay**

Weigh accurately about 0.1 g of Powdered Aloe, and heat under a reflux condenser on a water bath for 30 minutes. After cooling, filter, and add methanol to the filtrate to make exactly 10 mL. Pipet 5 mL of the solution, add methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Separately, weigh accurately about 10 mg of barbaloin for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, add 40 mg of oxalic acid dihydrate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of barbaloin, $A_I$ and $A_S$, of both solutions.

$$M_S = \frac{M_b \times A_I}{A_S} \times \frac{1}{2}$$

**Liquid Chromatography**

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

**Operating conditions—**

System performance: To about 10 mg of barbaloin for assay add 40 mg of oxalic acid dihydrate, and dissolve in methanol to make 100 mL. To 5 mL of the solution add 5 mL of a solution of ethenzamide in methanol (1 in 2000) and methanol to make 10 mL. When the procedure is run with 5 μL of this solution under the above operating conditions except the wavelength of 300 nm, barbaloin and ethenzamide are eluted in this order with the resolution between these peaks being not less than 2.0.
System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of barbaloïn is not more than 1.5%.

Containers and storage  Containers—Tight containers.

Alpinia Officinarum Rhizome

Alpiniae Officinarum Rhizoma

Alpinia Officinarum Rhizome is the rhizome of Alpinia officinarum Hance (Zingiberaceae).

Description  Alpinia Officinarum Rhizome is a slightly curved and cylindrical rhizome, sometimes branched; 2 – 8 cm in length, 6 – 15 mm in diameter; externally red-brown to dark brown with fine striped lines, grayish white nodes and several traces of rootlet; hard to break; fracture surface, light brown in color and thickness of cortex is approximately the same as that of stele.

Odor, characteristic; taste, extremely pungent.

Under a microscope <5.01>, transverse section reveals epidermal cells often containing resin-like substances; cortex, endodermis and stele present beneath the epidermis; cortex and stele divided by endodermis; vascular bundles surrounded by fibers, scattered throughout the cortex and stele, cortex and stele composed of parenchyma interspersed with oil cells; parenchymatous cells containing solitary crystals of calcium oxalate and starch grains, starch grains generally simple (sometimes 2- to 8-compound), ovate, oblong or narrowly ovate, 10 – 40 µm in diameter and with an eccentric navel.

Identification  To 0.5 g of pulverized Alpinia Officinarum Rhizome add 5 mL of acetone, shake for 5 minutes, and filter. Perform the test with the filtrate as directed under Thin-layer Chromatography <2.03>. Spot 5 µL of the filtrate on a plate of silica gel for thin-layer chromatography, dehydrate with Silicon Dioxide add 3 mL of diluted sulfuric acid (1 in 3), heat until white vapors evolve, then after cooling add 20 mL of water, and filter. The filtrate neutralized to pH 7.0 by adding 50 mL of water, and filter. The filtrate neutralized to pH 7.0 by adding the filtrate to the combined liquid until a slight precipitate forms, then add, while shaking vigorously, dilute hydrochloric acid dropwise to the combined liquid until a slight precipitate forms, then add, while shaking vigorously, dilute hydrochloric acid dropwise to dissolve the precipitate. Add 0.45 g of hydroxyaluminium chloride to this solution, heat, then after cooling add 0.45 g of sodium acetate trihydrate and 6 mL of dilute acetic acid, and add water to make 150 mL. Perform the test with 50 mL of this solution as the test solution. Prepare the control solution by adding to 2.0 mL of Standard Lead Solution, 0.15 g of hydroxyaluminium chloride, 0.15 g of sodium acetate trihydrate and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 40 ppm).

Purity (1)  Heavy metals <1.07>—To 1.5 g of Aluminum Silicate Hydrate with Silicon Dioxide add 50 mL of water and 5 mL of hydrochloric acid, and boil gently for 20 minutes while thorough shaking. After cooling, centrifuge, and separate the supernatant liquid. Wash the precipitate twice with 10 mL portions of water, centrifuging each time, and combine the supernatant liquids. Add ammonia solution (28) dropwise to the combined liquid until a slight precipitate forms, then add, while shaking vigorously, dilute hydrochloric acid dropwise to dissolve the precipitate. Add 0.45 g of hydroxyaluminium chloride to this solution, heat, then after cooling add 0.45 g of sodium acetate trihydrate and 6 mL of dilute acetic acid, and add water to make 150 mL. Perform the test with 50 mL of this solution as the test solution. Prepare the control solution by adding to 2.0 mL of Standard Lead Solution, 0.15 g of hydroxyaluminium chloride, 0.15 g of sodium acetate trihydrate and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 40 ppm).

(2) Arsenic <1.11>—To 1.0 g of Aluminum Silicate Hydrate with Silicon Dioxide add 5 mL of dilute hydrochloric acid, heat gently until boiling begins then cool quickly, and centrifuge. To the precipitate add 5 mL of dilute hydrochloric acid, shake thoroughly, and centrifuge. Repeat this operation with 10 mL of water, combine all extracts, and concentrate the extract to make 5 mL by heating on a water bath. Perform the test using this solution as the test solution (not more than 2 ppm).

Containers and storage  Containers—Well-closed contain-

Aluminum Silicate Hydrate with Silicon Dioxide

Kaseki

Aluminum Silicate Hydrate with Silicon Dioxide is a mineral substance, mainly composed of aluminum silicate hydrate and silicon dioxide.

It is not the same substance with the mineralogical talc.

Description  Aluminum Silicate Hydrate with Silicon Dioxide occurs as white to light red powdered crystalline masses, which becomes easily fine powder on crushing. The powder is roughish and easily adheres to skin, and becomes slightly darken and obtains plasticity when moisten with water.

It has a characteristic odor and almost tasteless. It feels like as sand of fine grains by chewing.

Under a microscope <5.01>, the powder of Aluminum Silicate Hydrate with Silicon Dioxide, thoroughly grained between a slide glass and a cover glass together with mounting medium, shows numbers of round to polygonal crystals not smaller than 10 µm in diameter.

Identification  To 0.5 g of powdered Aluminum Silicate Hydrate with Silicon Dioxide add 3 mL of diluted sulfuric acid (1 in 3), heat until white vapors evolve, then after cooling add 20 mL of water, and filter. The filtrate neutralized to be a weak acidity with ammonia TS responds to the Qualitative Tests <1.09> (1), (2) and (4) for aluminum salt.

Purity (1)  Heavy metals <1.07>—To 1.0 g of Aluminum Silicate Hydrate with Silicon Dioxide add 50 mL of water and 5 mL of hydrochloric acid, and boil gently for 20 minutes while thorough shaking. After cooling, centrifuge, and separate the supernatant liquid. Wash the precipitate twice with 10 mL portions of water, centrifuging each time, and combine the supernatant liquids. Add ammonia solution (28) dropwise to the combined liquid until a slight precipitate forms, then add, while shaking vigorously, dilute hydrochloric acid dropwise to dissolve the precipitate. Add 0.45 g of hydroxyaluminium chloride to this solution, heat, then after cooling add 0.45 g of sodium acetate trihydrate and 6 mL of dilute acetic acid, and add water to make 150 mL. Perform the test with 50 mL of this solution as the test solution. Prepare the control solution by adding to 2.0 mL of Standard Lead Solution, 0.15 g of hydroxyaluminium chloride, 0.15 g of sodium acetate trihydrate and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 40 ppm).

(2) Arsenic <1.11>—To 1.0 g of Aluminum Silicate Hydrate with Silicon Dioxide add 5 mL of dilute hydrochloric acid, heat gently until boiling begins then cool quickly, and centrifuge. To the precipitate add 5 mL of dilute hydrochloric acid, shake thoroughly, and centrifuge. Repeat this operation with 10 mL of water, combine all extracts, and concentrate the extract to make 5 mL by heating on a water bath. Perform the test using this solution as the test solution (not more than 2 ppm).

Containers and storage  Containers—Well-closed contain-
Amomum Seed

Amomi Semen

シュクシャ

Amomum Seed is the seed mass of *Amomum xanthioides* Wallich (*Zingiberaceae*).

**Description**
Approximately spherical or ellipsoidal mass, 1 – 1.5 cm in length, 0.8 – 1 cm in diameter; externally grayish brown to dark brown, and with white powder in those dried by spreading lime over the seeds; the seed mass is divided into three loculi by thin membranes, and each loculus contains 10 to 20 seeds joining by aril; each seed is polygonal and spherical, 0.3 – 0.5 cm in length, about 0.3 cm in diameter, externally dark brown, with numerous, fine protrusions; hard tissue; under a magnifying glass, a longitudinal section along the raphe reveals oblong section, with deeply indented hilum and with slightly indented chalaza; white perisperm covering light yellow endosperm and long embryo.

Characteristic aroma when cracked, and taste acrid.

**Total ash** < 5.01%  Not more than 9.0%.

**Acid-insoluble ash** < 5.01%  Not more than 3.0%.

**Essential oil content** < 5.01%  Perform the test with 30.0 g of pulverized Amomum Seed: the volume of essential oil is not less than 0.6 mL.

**Containers and storage**  Containers—Well-closed containers.

Powdered Amomum Seed

Amomi Semen Pulveratum

シュクシャ末

Powdered Amomum Seed is the powder of Amomum Seed.

**Description**  Powdered Amomum seed occurs as a grayish brown powder, and has a characteristic aroma and an acrid taste.

Under a microscope < 5.01%, Powdered Amomum Seed reveals fragments of wavy perisperm cells filled with starch grains and containing in each cell a calcium oxalate crystal; yellow and long epidermal cells of seed coat and fragments of thin-walled tissue perpendicular to them; fragments of groups of brown, thick-walled polygonal stone cells.

**Total ash** < 5.01%  Not more than 9.0%.

**Acid-insoluble ash** < 5.01%  Not more than 3.0%.

**Essential oil content** < 5.01%  Perform the test with 30.0 g of Powdered Amomum Seed: the volume of essential oil is not less than 0.4 mL.

**Containers and storage**  Containers—Tight containers.

Anemarrhena Rhizome

Anemarrhenae Rhizoma

チモ

Anemarrhena Rhizome is the rhizome of *Anemarrhena asphodeloides* Bunge (*Liliaceae*).

**Description**  Rather flat and cord-like rhizome, 3 – 15 cm in length, 0.5 – 1.5 cm in diameter, slightly bent and branched; externally yellow-brown to brown; on the upper surface, a longitudinal furrow and hair-like remains or scars of leaf sheath forming fine ring-nodes; on the lower surface, scars of root appearing as numerous round spot-like hollows; light and easily broken. Under a magnifying glass, a light yellow-brown transverse section reveals an extremely narrow cortex; stele porous, with many irregularly scattered vascular bundles.

Odor, slight; taste, slightly sweet and mucous, followed by bitterness.

**Identification** (1)  Shake vigorously 0.5 g of pulverized Anemarrhena Rhizome with 10 mL of water in a test tube: a lasting fine foam is produced. Filter the mixture, and to 2 mL of the filtrate add 1 drop of iron (III) chloride TS: a dark green precipitate is produced.

(2)  Warm 0.5 g of pulverized Anemarrhena Rhizome with 2 mL of acetic anhydride on a water bath for 2 minutes while shaking, then filter, and to the filtrate add carefully 1 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact.

**Purity** (1)  Heavy metals < 1.07%—Proceed with 3.0 g of pulverized Anemarrhena Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2)  Arsenic < 1.11%—Prepare the test solution with 0.40 g of pulverized Anemarrhena Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3)  Foreign matter < 5.01%—The amount of fiber, originating from the dead leaves, and other foreign matters contained in Anemarrhena Rhizome is not more than 3.0%.

**Total ash** < 5.01%  Not more than 7.0%.

**Acid-insoluble ash** < 5.01%  Not more than 2.5%.

**Containers and storage**  Containers—Well-closed containers.

Angelica Dahurica Root

Angelicae Dahuricae Radix

ビャクシ

Angelica Dahurica Root is the root of *Angelica dahurica* Bentham et Hooker filius ex Franchet et Savatier (*Umbelliferae*).

**Description**  Main root from which many long roots are branched out and nearly fusiform and conical in whole
shape, 10 - 25 cm in length; externally grayish brown to dark brown, with longitudinal wrinkles, and with numerous scars of rootlets laterally elongated and protruded. A few remains of leaf sheath at the crown and ring-nodes closely protruded near the crown. In a transverse section, the outer region is grayish white in color, and the central region is sometimes dark brown in color.

Odor, characteristic; taste, slightly bitter.

Identification  To 0.2 g of pulverized Angelica Dahurica Root add 5 mL of ethanol (95%), shake for 5 minutes, and filter. Examine the filtrate under ultraviolet light (main wavelength: 365 nm): a blue to blue-purple fluorescence develops.

Purity (1)  Leaf sheath—When perform the test of foreign matter <5.01>, the amount of leaf sheath contained in Angelica Dahurica Root does not exceed 3.0%.

(2)  Heavy metals <1.07>—Proceed with 3.0 g of pulverized Angelica Dahurica Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3)  Arsenic <1.17>—Prepare the test solution with 0.40 g of pulverized Angelica Dahurica Root according to Method 4, and perform the test (not more than 5 ppm).

(4)  Foreign matter <5.02>—The amount of foreign matter other than leaf sheath contained in Angelica Dahurica Root is not more than 1.0%.

Total ash <5.01>  Not more than 7.0%.
Acid-insoluble ash <5.01>  Not more than 2.0%.
Extract content <5.01>  Dilute ethanol-soluble extract: not less than 25.0%.

Containers and storage  Containers—Well-closed containers.

Apricot Kernel

Arménicae Semen

キョウニン

Apricot Kernel is the seed of Prunus armeniaca Linné, Prunus armeniaca Linné var. ansu Maximowicz or Prunus sibirica Linné (Rosaceae).

It contains not less than 2.0% of amygdalin, calculated on the basis of dried material.

Description  Flattened, somewhat asymmetric ovoid seed, 1.1 - 1.8 cm in length, 0.8 - 1.3 cm in width, 0.4 - 0.7 cm in thickness; sharp at one end and rounded at the other end where chalaza situated; seed coat brown and its surface being powdery with rubbing easily detachable stone cells of epidermis; numerous vascular bundles running from chalaza throughout the seed coat, appearing as thin vertical furrows; seed coat and thin semitransparent white albumen easily separate from cotyledon when soaked in boiling water; cotyledon, white in color. Almost odorless; taste, bitter and oily.

Under a microscope <5.01>, surface of epidermis reveals stone cells on veins protruded by vascular bundles, forming angular circle to ellipse and approximately uniform in shape, with uniformly thickened walls, and 60 - 90 μm in diameter; in lateral view, stone cell appearing obtusely triangular and its wall extremely thickened at the apex.

Identification  To 1.0 g of ground Apricot Kernel add 10 mL of methanol, immediately heat under a reflux condenser on a water bath for 10 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a spot with a bluish white fluorescence appears at around RI value 0.7. Spray evenly thymol-sulfuric acid-methanol TS for spraying upon the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and RI value with the red-brown spot from the standard solution.

Purity (1)  Rancidity—Grind Apricot Kernel with hot water: no unpleasant odor of rancid oil is perceptible.

(2)  Foreign matter <5.04>—Apricot Kernel does not contain fragments of endocarp and other foreign matter.

Loss on drying <5.01>  Not more than 7.0% (6 hours).

Assay  Weigh accurately 0.5 g of ground Apricot Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser on a water bath for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, AT and A5, of amygdalin.

\[
\text{Amount (mg) of amygdalin} = M_5 \times \frac{A_T}{A_5} \times 2
\]

M5: Amount (mg) of amygdalin for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylazanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with
μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

**Apricot Kernel Water**

キョウニン水

Apricot Kernel Water contains not less than 0.09 w/v% and not more than 0.11 w/v% of hydrogen cyanide (HCN: 27.03).

Method of preparation Prepare by one of the following methods.

(1) To Apricot Kernels, previously crushed and pressed to remove fixed oils as much as possible, add a suitable amount of Water, Purified Water or Purified Water in Containers, and carry out steam distillation. Determine the amount of hydrogen cyanide in the distillate by the method as directed in the Assay, and carry on the distillation until the content of hydrogen cyanide in the distillate is about 0.14 w/v%. To the distillate add Ethanol in about 1/3 of the volume of the distillate, and dilute with a mixture of Purified Water or Purified Water in Containers and Ethanol (3:1) until the content of hydrogen cyanide meets the specification.

(2) Dissolve 7.5 mL of freshly prepared mandelonitrile in 1000 mL of a mixture of Purified Water or Purified Water in Containers and Ethanol (3:1), mix well, and filter. Determine the amount of hydrogen cyanide in the solution as directed in the Assay, and, if the amount is more than that specified above, dilute the solution to the specified concentration by the addition of the mixture of Purified Water or Purified Water in Containers and Ethanol (3:1) until the content of hydrogen cyanide meets the specification.

**Aralia Rhizome**

**Araliae Cordatae Rhizoma**

ドケカツ

Aralia Rhizome is usually the rhizome of *Aralia cordata* Thunberg (*Araliaceae)*.

Description Aralia Rhizome is curved, irregular cylindrical to masses occasionally with remains of short roots. 4 – 12 cm in length, 2.5 – 7 cm in diameter, often cut crosswise or lengthwise. 1 to several, enlarged dents by remains of stems on the upper part or rarely 1.5 – 2.5 cm in diameter, remains of short stem. The outer surface is dark brown to yellow-brown, with longitudinally wrinkles, bases or dents of root. The transverse section of rhizome reveals dark brown to yellow-brown, scattered brownish small spots with oil canals, and with numerous splits.

Odor, characteristic; taste, slightly bitter.

Under a microscope 5.012, a transverse section of rhizome reveals the outermost layer to be cork layer, rarely composed of cork stone cells, followed these appeared several layers of collenchyma. Vascular bundle and medullary rays is distinct, pith broad. Phloem fibre bundles are sometimes observed at the outer portion of phloem. Oil canals composed of schizogenous intercellular space in cortex and pith. Cortex composed of vessels, xylem fibres, and occasionally thick-wall xylem parenchyma. Vascular bundles scattered on the pith. And, parenchymatous cells observed rosette aggregates of calcium oxalate. Starch grains composed of simple grains, 2- to 6- compound grains.

Identification To 1 g of pulverized Aralia Rhizome add 10 mL of methanol, shake for 5 minutes, filter, and use the fil-
Arecaceae 

*Areca catechu* Linné (Palmae).

**Description** Rounded-conical or flattened nearly spherical seed 1.5 - 3.5 cm high and 1.5 - 3 cm in diameter; hilum at the center of its base and usually forming a dent; externally grayish red-brown to grayish yellow-brown, with a network of pale lines; hard in texture; cross section dense in texture, exhibiting a marbled appearance of grayish brown seed coat alternating with white albumen; center of the seed often hollow.

Odor, slight; taste, astringent and slightly bitter.

**Identification** Weigh 3 g of pulverized Areca in a glass-stoppered centrifuge tube, and add 30 mL of diethyl ether and 5 mL of sodium hydroxide TS, stopper tightly, shake for 5 minutes, centrifuge, and separate the diethyl ether layer. Evaporate the diethyl ether on a water bath, dissolve the residue in 1.5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of arecoline hydrobromide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and water (10:6:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iodine of acetone, water and acetic acid (100) (10:6:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (30:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes; a purple spot appears at an Rf value of about 0.6.

Loss on drying <5.01> Not more than 12.0%.

**Total ash** <5.01> Not more than 9.0%.

**Acid-insoluble ash** <5.01> Not more than 1.5%.

**Extract content** <5.01> Dilute ethanol-soluble extract: not less than 15.0%.

**Containers and storage** Containers—Well-closed containers.

*Areca* 

*Arecae Semen*

ビンロウジ

Areca is the seed of *Areca catechu* Linné (Palmae).

**Description** Rounded-conical or flattened nearly spherical seed 1.5 - 3.5 cm high and 1.5 - 3 cm in diameter; hilum at the center of its base and usually forming a dent; externally grayish red-brown to grayish yellow-brown, with a network of pale lines; hard in texture; cross section dense in texture, exhibiting a marbled appearance of grayish brown seed coat alternating with white albumen; center of the seed often hollow.

Odor, slight; taste, astringent and slightly bitter.

**Identification** Weigh 3 g of pulverized Areca in a glass-stoppered centrifuge tube, and add 30 mL of diethyl ether and 5 mL of sodium hydroxide TS, stopper tightly, shake for 5 minutes, centrifuge, and separate the diethyl ether layer. Evaporate the diethyl ether on a water bath, dissolve the residue in 1.5 mL of methanol, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and water (10:6:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iodine of acetone, water and acetic acid (100) (10:6:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes; a purple spot appears at an Rf value of about 0.6.

Loss on drying <5.01> Not more than 12.0%.

**Total ash** <5.01> Not more than 9.0%.

**Acid-insoluble ash** <5.01> Not more than 1.5%.

**Extract content** <5.01> Dilute ethanol-soluble extract: not less than 15.0%.

**Containers and storage** Containers—Well-closed containers.

**Artemisia Capillaris Flower**

*Artemisiae Capillaris Flos*

インチンコウ

Artemisia Capillaris Flower is the capitulum of *Artemisia capillaris* Thunberg (Compositae).

**Description** Capitulum of ovoid to spherical, capitula, about 1.5 - 2 mm in length, about 2 mm in diameter, with linear leaves, peduncles, and thin stem. Outer surface of capitulum, light green to light yellow-brown in color; peduncle, green-brown to dark brown in color. Under a magnifying glasses, the capitulum; involucral scale, in 3 - 4 succorous rows, outer scale of ovate with obtuse, inner scale of elliptical, 1.5 mm in length, longer than outer one, with keel midrib and thin membranous margin. Floret; tubular, marginal flower of female, disk flower of hermaphrodite. Achene of obovoid, 0.8 mm in length. Light in texture. Odor, characteristic, slight; taste, slightly acrid, which gives slightly numbing sensation to the tongue.

**Identification** To 0.5 g of pulverized Artemisia Capillaris Flower add 10 mL of methanol, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and n-hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a principal spot with a blue fluorescence appears at an Rf value of about 0.5.

**Purity** Stem—When perform the test of foreign matter <5.01>, Artemisia Capillaris Flower does not contain any stem more than 2 mm in diameter.

Loss on drying <5.01> Not more than 12.0% (6 hours).

**Total ash** <5.01> Not more than 9.0%.

**Acid-insoluble ash** <5.01> Not more than 2.0%.

**Extract content** <5.01> Dilute ethanol-soluble extract: not less than 15.0%.

**Containers and storage** Containers—Well-closed containers.

**Asiasarum Root**

*Asiasari Radix*

サイシン

Asiasarum Root is the root with rhizome of *Asiasarum sieboldii* F. Maekawa or *Asiasarum*...
heterotropoides F. Maekawa var. mandshuricum F. Maekawa (Aristolochiaceae).

**Description**
Asiasarum Root is a nearly cylindrical rhizome with numerous thin and long roots, externally light brown to dark brown. The root, about 15 cm in length, about 0.1 cm in diameter, with shallow longitudinal wrinkles on the surface, and brittle. The rhizome, 2–4 cm in length, 0.2–0.3 cm in diameter, often branched, with longitudinal wrinkles on the surface; internode short; each node has several scars of petiole and peduncle, and several thin and long roots.

Odor, characteristic; taste, acrid, with some sensation of numbness on the tongue.

**Purity (1)**
Terrestrial part—When perform the test of foreign matter <5.01>, any terrestrial parts are not found.

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Asiasarum Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter <5.01>—The amount of foreign matter other than terrestrial part contained in Asiasarum Root is not more than 1.0%.

(4) Aristolochic acid I—To exactly 2.0 g of pulverized Asiasarum Root add exactly 50 mL of diluted methanol (3 in 4), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve exactly 1.0 mg of aristolochic acid I for crude drugs purity test in diluted methanol (3 in 4) to make exactly 100 mL. Pipet 1 mL of this solution, add diluted methanol (3 in 4) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, according to the following conditions: the sample solution shows no peak at the retention time corresponding to aristolochic acid I from the standard solution. If the sample solution shows such a peak, repeat the test under different conditions to confirm that the peak in question is not aristolochic acid I.

**Operating conditions**—
Detector: An ultraviolet or visible absorption photometer (wavelength: 400 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate and 2 mL of phosphoric acid in water to make 1000 mL and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of aristolochic acid I is about 15 minutes.

**System suitability**—
Test for required detectability: Measure exactly 1 mL of the standard solution, and add diluted methanol (3 in 4) to make exactly 10 mL. Confirm that the ratio, S/N, of the signal (S) and noise (N) of aristolochic acid I obtained from 20 μL of this solution is not less than 3. In this case, S means the peak height on the chromatogram not including noise obtained by drawing an average line of the detector output, and N is 1/2 of the difference between the maximum and minimum output signals of the baseline around the peak in the range of 20 times the width at half-height of the peak.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aristolochic acid I is not more than 5.0%.

(5) Total BHC’s and total DDT’s <5.01>—Not more than 0.2 ppm, respectively.

**Total ash <5.01**—Not more than 10.0%.

Acid-insoluble ash <5.01>—Not more than 3.0%.

**Essential oil content <5.01>**—Perform the test with 30.0 g of pulverized Asiasarum Root: the volume of essential oil is not less than 0.6 mL.

**Containers and storage**
Containers—Well-closed containers.

### Asparagus Tuber

**Asparagi Tuber**

Asparagus Tuber is the tuber of *Asparagus cochinichinensis* Merrill (*Liliaceae*), from which most of the cork layer is removed, usually, after being steamed.

**Description**
Asparagus Tuber is a fusiform to cylindrical tuber, 5 – 15 cm in length, 5 – 20 mm in diameter; externally light yellow-brown to light brown, translucent and often with longitudinal wrinkles; flexible, or hard and easily broken in texture; fractured surface, grayish yellow, glossy and horny.

Odor, characteristic; taste, sweet at first, followed by a slightly bitter aftertaste.

Under a microscope <5.01>, a transverse section of Asparagus Tuber reveals stone cells and bundles of them on outer layer of cortex; mucilaginous cells containing raphides of calcium oxalate in the parenchyma cells of cortex and stele; no starch grains.

**Identification**
To 1 g of coarsely cut Asparagus Tuber add 5 mL of a mixture of 1-butanol and water (40:7), shake for 30 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.01>, according to the following conditions: the sample solution shows no spot on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 2 minutes: the spot of a red-brown at first then changes to brown color appears at an Rf value of about 0.4.

**Purity (1)**
Heavy metals <1.07>—Proceed with 3.0 g of pulverized Asparagus Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Asparagus Tuber according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying <5.01>**—Not more than 18.0% (6 hours).

**Total ash <5.01>**—Not more than 3.0%.
Astragalus Root

Astragali Radix

オウギ

Astragalus Root is the root of *Astragalus membranaceus* Bunge or *Astragalus mongholicus* Bunge (Leguminosae).

**Description** Nearly cylindrical root, 30 – 100 cm in length, 0.7 – 2 cm in diameter, with small bases of lateral root dispersed on the surface, twisted near the crown; externally light grayish yellow to light yellow-brown, and covered with irregular, dispersed longitudinal wrinkles and horizontal lenticel-like patterns; difficult to break; fractured surface fibrous. Under a magnifying glass, a transverse section reveals an outer layer composed of periderm; cortex light yellowish white, xylem light yellow, and zone near the cambium somewhat brown in color; thickness of cortex from about one-third to one-half of the diameter of xylem; xylem medullary ray from xylem to cortex in thin root, but often appearing as radiating cracks in thick root; usually pith unobservable.

Odor, slight; taste, sweet.

**Identification** Put 1 g of pulverized Astragalus Root in a glass-stoppered centrifuge tube, add 5 mL of potassium hydroxide TS and 5 mL acetonitrile, and stop the vial tightly. After shaking this for 10 minutes, centrifuge, and use the upper layer as the sample solution. Separately, dissolve 1 mg of astragalin IV for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of solution and standard solution on a plate of silica gel for thin-layer chromatography. Put 1 g of pulverized Astragalus Root in a glass-stoppered centrifuge tube, add 5 mL of potassium hydroxide TS and 5 mL acetonitrile, and stop the vial tightly. After shaking this for 10 minutes, centrifuge, and use the upper layer as the sample solution. Separately, dissolve 1 mg of astragalin IV for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of solution and standard solution on a plate of silica gel for thin-layer chromatography. Under a microscope, a vertical section of Astragalus Root reveals an outer layer composed of periderm; cortex light yellowish white, xylem light yellow, and zone near the cambium somewhat brown in color; thickness of cortex from about one-third to one-half of the diameter of xylem; xylem medullary ray from xylem to cortex in thin root, but often appearing as radiating cracks in thick root; usually pith unobservable.

Odor, slight; taste, sweet.

**Purity** (1) Root of *Hedysarum* species and others—Under a microscope <5.07>, a vertical section of Astragalus Root reveals no crystal fiber containing solitary crystals of calcium oxalate outside the fiber bundle.

(2) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Astragalus Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.17>—Prepare the test solution with 0.40 g of pulverized Astragalus Root and 0.2 mL of 0.1N sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spots among the several spots from the sample solution has the same color tone and Rf value with the brownish yellow fluorescent spot from the standard solution.

**Essential oil content** <5.01> Perform the test with 50.0 g of pulverized Astragalus Root: the volume of essential oil is not less than 0.7 mL.

**Total ash** <5.01> Not more than 7.0%.

**Acid-insoluble ash** <5.01> Not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.
Powdered Atractylodes Lancea Rhizome

Atractylodis Lanceae Rhizoma Pulveratum

Powdered Atractylodes Lancea Rhizome is the powder of Atractylodes Lancea Rhizome.

**Description** Powdered Atractylodes Lancea Rhizome occurs as a yellow-brown powder. It has a characteristic odor, and a slightly bitter taste.

Under a microscope <5.01>, Powdered Atractylodes Lancea Rhizome reveals mainly parenchyma cells, spherocrystals of inulin, fragments of parenchyma cells containing fine needle crystals of calcium oxalate as their contents; and further fragments of light yellow thick-walled fibers, stone cells and cork cells; a few fragments of reticulate and scalariform vessels, and small yellow-brown secreted masses or oil drops; starch grains absent.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of Powdered Atractylodes Lancea Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Atractylodes Lancea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Powdered atractylodes rhizome—To 0.5 g of Powdered Atractylodes Lancea Rhizome add 5 mL of ethanol (95), macerate by warming in a water bath for 2 minutes, and filter. To 2 mL of the filtrate add 0.5 mL of vanillin-hydrochloric acid TS, and shake immediately: no red to red-purple color develops within 1 minute.

**Total ash** <5.01> Not more than 7.0%.

**Acid-insoluble ash** <5.01> Not more than 1.5%.

**Essential oil content** <5.01> Perform the test with 50.0 g of Powdered Atractylodes Lancea Rhizome: the volume of essential oil is not less than 0.5 mL.

**Containers and storage** Containers—Tight containers.

Atractylodes Rhizome

Atractylodis Rhizoma

Atractylodes Rhizome is the rhizome of *Atractylodes japonica* Koidzumi ex Kitamura (Wa-byakujutsu), or is the rhizome of *Atractylodes macrocephala* Koidzumi (Atractylodes ovata De Candolle) (Kara-byakujutsu) (Compositae).

**Description (1)** Wa-byakujutsu—Periderm-removed rhizome is irregular masses or irregularly curved cylinder, 3 – 8 cm in length, 2 – 3 cm in diameter; externally light grayish yellow to light yellowish white, with scattered grayish brown parts. The rhizome covered with periderm is externally grayish brown, often with node-like protuberances and coarse wrinkles. Difficult to break, and the fractured surface is fibrous. A transverse section, with fine dots of light yellow-brown to brown secrete.

Odor, characteristic; taste, somewhat bitter.

Under a microscope <5.01>, a transverse section reveals periderm with stone cell layers; fiber bundles in the parenchyma of the cortex, often adjoined to the outside of the phloem; oil sacs containing light brown to brown substances, situated at the outer end of medullary rays; in the xylem, radially lined vessels, surrounding large pith, and distinct fiber bundle surrounding the vessels; in pith and in medullary rays, oil sacs similar to those in cortex, and in parenchyma, crystals of inulin and small needle crystals of calcium oxalate.

(2) Kara-byakujutsu—Irregularly enlarged mass, 4 – 8 cm in length, 2 – 5 cm in diameter; externally grayish yellow to dark brown, having sporadic, knob-like small protrusions. Difficult to break; fractured surface has a light brown to dark brown xylem remarkably fibrous.

Odor, characteristic; taste, somewhat sweet, but followed by slight bitterness.

Under a microscope <5.01>, a transverse section usually reveals periderm with stone cells, absence of fibers in the cortex; oil sacs containing yellow-brown contents in phloem ray and also at the outer end of it; xylem with radially lined vessels surrounding large pith, and distinct fiber bundle surrounding the vessels; pith and medullary ray exhibit oil sacs as in cortex; parenchyma contains crystals of inulin and small needle crystals of calcium oxalate.

**Identification** Macerate 0.5 g of pulverized Atractylodes Rhizome with 5 mL of ethanol (95) by warming in a water bath for 2 minutes, and filter. To 2 mL of the filtrate add 0.5 mL of vanillin-hydrochloric acid TS, and shake immediately: a red to red-purple color develops and persists.

**Purity (1)** Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Atractylodes Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(2) Atractylodes lancea rhizome—To 2.0 g of pulverized Atractylodes Rhizome add exactly 5 mL of hexane, shake for 5 minutes, filter, and use this filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Sprayer evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 100°C for 5 minutes: no green to grayish green spot appears at the Rf value of between 0.3 and 0.6.

**Total ash** <5.01> Not more than 7.0%.

**Acid-insoluble ash** <5.01> Not more than 1.0%.

**Essential oil content** <5.01> Perform the test with 50.0 g of pulverized Atractylodes Rhizome: the volume of essential oil is not less than 0.5 mL.

**Containers and storage** Containers—Well-closed containers.
Powdered Atractylodes Rhizome

*Atractylodis Rhizoma Pulveratum*

Powdered Atractylodes Rhizome is the powder of Atractylodes Rhizome.

**Description** Powdered Atractylodes Rhizome occurs as a light brown to yellow-brown powder, and has a characteristic odor and a slightly bitter or slightly sweet taste, followed by a slightly bitter aftertaste.

Under a microscope, Powdered Atractylodes Rhizome reveals mainly parenchyma cells, crystals of inulin and fragments of parenchyma cells containing small needle crystals of calcium oxalate; fragments of light yellow thick-walled fibers, stone cells and cork cells; a few fragments of reticulate and scalariform vessels; small yellow-brown secretory masses or oil droplets; starch grains absent.

**Identification** Macerate 0.5 g of Powdered Atractylodes Rhizome with 5 mL of ethanol (95) by warming in a water bath for 2 minutes, and filter. To 2 mL of the filtrate add 0.5 mL of vanillin-hydrochloric acid TS, and shake immediately: a red to red-purple color develops and persists.

**Purity**

(1) Arsenic *<1.1>—Prepare the test solution with 0.40 g of Powdered Atractylodes Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(2) *Atractylodes lancea rhizome*—To 2.0 g of Powdered Atractylodes Rhizome add exactly 5 mL of hexane, shake for 5 minutes, filter, and use this filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography *<2.0>.* Spot 10 μL of the solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 100°C for 5 minutes: no green to grayish green spot appears at the RF value of between 0.3 and 0.6.

**Total ash** *<5.0>—Not more than 7.0%.

**Acid-insoluble ash** *<5.0>—Not more than 1.0%.

**Essential oil content** *<5.0>—Perform the test with 50.0 g of Powdered Atractylodes Rhizome: the volume of essential oil is not less than 0.4 mL.

**Containers and storage** Containers—Tight containers.

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**Bakumondoto Extract**

麦門冬湯エキス

Bakumondoto Extract contains not less than 1.2 mg of ginesenoside Rb₁ (C₅₄H₉₂O₂₃: 1109.29), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid (C₁₈H₂₈O₇: 822.93), per extract prepared with the amount specified in the Method of preparation.

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ophiopogon Tuber</td>
<td>10 g</td>
</tr>
<tr>
<td>Pinellia Tuber</td>
<td>5 g</td>
</tr>
<tr>
<td>Brown Rice</td>
<td>5 g</td>
</tr>
<tr>
<td>Jujube</td>
<td>3 g</td>
</tr>
<tr>
<td>Ginseng</td>
<td>2 g</td>
</tr>
<tr>
<td>Glycyrrhiza</td>
<td>2 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1), using the crude drugs shown above.

**Description** Bakumondoto Extract occurs as a light yellow to blackish brown powder or viscous extract. It has a slight odor, and a sweet taste.

**Identification**

(1) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of 1-butanol, shake, centrifuge, and use the water layer as the sample solution. Separately, to 3.0 g of ophiopogon tuber add 50 mL of water, and heat under a reflux condenser for 1 hour. After cooling, take 20 mL of the extract, add 5 mL of 1-butanol, shake, centrifuge, and use the water layer as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.0>.* Spot 2 μL of the sample solution and 5 μL of the standard solution as bands on the original line of a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), water and acetic acid (100) (120:80:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and RF value with the dark blue-green spot (RF value: about 0.3) from the standard solution (Ophiopogon Tuber).

(2) Shake 5.0 g of dry extract (or 15 g of the viscous extract) with 15 mL of water, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of cycloartenyl ferulate for thin-layer chromatography in 1 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.0>.* Spot 30 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, acetone and acetic acid (100) (50:20:1) to a distance of about 10 cm, and air-dry the plate. When examine the plate under ultraviolet light (main wavelength: 365 nm), one of the spot among the several spots obtained from the sample solution has the same color tone and RF value with the bluish white fluorescent spot from the standard solution. Or when examine the plate under ultraviolet light (main wavelength: 365 nm) after spraying evenly a mixture of sulfuric acid and ethanol (99.5) (1:1) and heating at 105°C for 5 minutes, one of the spot among the several spots obtained from the sample solution has the same color tone and RF value with the yellow fluorescent spot from the standard solution (Brown Rice).

(3) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 5 mL
of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb1 RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and RT value with the purple spot from the standard solution (Ginseng).

(4) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and RT value with the yellow-brown spot from the standard solution (Glycyrrhiza).

**Purity (1)** Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed in Extracts (4), and perform the test (not more than 30 ppm).

**Arsenic (2)** Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

**Loss on drying (2.41)** The dry extract: Not more than 7.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

**Total ash (5.01)** Not more than 10.0%, calculated on the dried basis.

**Assay (1)** Ginsenoside Rb1—Weigh accurately about 2 g of the dry extract (or an amount of the viscous extract, equivalent to 2 g of dried substance), add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 15 mL of diluted methanol (3 in 5), and repeat the same procedure. Combine all of the supernatant liquid, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of sodium hydroxide TS, allow to stand for 30 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 20 mL. Apply exactly 5 mL of this solution to a column about 10 mm in inside diameter, packed with 0.36 g of octadecylsilanized silica gel for pre-treatment (55 – 105 μm in particle size), and washed just before using with methanol and then diluted methanol (3 in 10), and wash the column in sequence with 2 mL of diluted methanol (3 in 10), 1 mL of sodium carbonate TS and 10 mL of diluted methanol (3 in 10). Finally, elute with methanol to collect exactly 5 mL, and use this as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb1 RS (separately determine the water), and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rb1 in each solution.

Amount (mg) of ginsenoside Rb1 (C_{42}H_{62}O_{16})

\[ M_S = \frac{S}{T/A_S} \times \frac{1}{5} \]

M_S: Amount (mg) of Ginsenoside Rb1 RS, calculated on the anhydrous basis

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with carbamoyl group bound silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: A mixture of acetonitrile and water (4:1).

Flow rate: 1.0 mL per minute (the retention time of ginsenoside Rb1 is about 16 minutes).

**System suitability—**

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ginsenoside Rb1 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb1, is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

Amount (mg) of glycyrrhizic acid (C_{42}H_{62}O_{16})

\[ M_S = \frac{S}{T/A_S} \times \frac{1}{2} \]

M_S: Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica
Containers and storage
Containers—Tight containers.

Bear Bile
*Fel Ursi*

**Bear Bile** is the dried bile of *Ursus arctos* Linné or allied animals (*Ursidae*).

**Description** Indefinite small masses; externally yellow-brown to dark yellow-brown; easily broken; fractured surface has a glassy luster, and is not wet.

Usually in a gall sac, occasionally taken out, the gall sac consists of a fibrous and strong membrane, 9–15 cm in length and 7–9 cm in width; externally dark brown and translucent.

Odor, slight and characteristic; taste, extremely bitter.

**Identification** To 0.1 g of pulverized Bear Bile, add 5 mL of methanol, warm in a water bath for 10 minutes. After cooling, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of sodium tauroursodeoxycholate for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetic acid (100), toluene and water (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution has the transverse section reveals thick cuticle; parenchyma cells of palisade tissue and sponge tissue being similar in form; in the vascular bundle, medullary ray consisting of 2 to 7 rows of one-cell line, appearing as bones of Japanese fan; polygonal solitary crystals and clustered crystals of calcium oxalate present sparsely in cells on both outer and inner sides of the vascular bundle, but no crystals in mesophyll.

**Identification** (1) Macerate 0.5 g of pulverized Bearberry Leaf with 10 mL of boiling water, shake the mixture for a few minutes, allow to cool, and filter. Place 1 drop of the filtrate on filter paper, and add 1 drop of iron (III) chloride TS: a dark purple color appears.

(2) To 0.2 g of pulverized Bearberry Leaf add 10 mL of a mixture of ethanol (95) and water (7:3), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of arbutin for thin-layer chromatography in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl formate, water and formic acid (8:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) upon the plate, and heat at 105°C for 10 minutes: one spot among several spots from the sample solution and that from the standard solution show a yellow-brown to blackish brown color and the same Rf value.

**Purity** (1) Twig—When perform the test of foreign matter <5.01>, the amount of twigs contained in Bearberry Leaf does not exceed 4.5%.

(2) Foreign matter <5.01>—The amount of foreign matter other than twigs contained in Bearberry Leaf does not exceed 2.0%.

**Total ash** <5.01> Not more than 4.0%.
Acid-insoluble ash <5.0% Not more than 1.5%.

Assay Weigh accurately about 0.5 g of pulverized Bearberry Leaf in a glass-stoppered centrifuge tube, add 40 mL of water, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 40 mL of water, and proceed in the same manner. To the combined extracts add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of arbutin for assay, previously dried for 12 hours (in vacuum, silica gel), dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine the peak areas, A_T and A_S, of arbutin.

\[
\text{Amount (mg) of arbutin} = M_S \times \frac{A_T}{A_S}
\]

M_S: Amount (mg) of arbutin for assay

Operating conditions—
Detectors: An ultraviolet spectrophotometer (wavelength: 280 nm).
Column: A stainless steel column 4 – 6 mm in inside diameter and 15 – 25 cm in length, packed with octadecylsilanized silica gel (5 – 10 μm in particle diameter).
Column temperature: A constant temperature of about 20°C.
Mobile phase: A mixture of water, methanol and 0.1 mol/L hydrochloric acid TS (94:5:1).
Flow rate: Adjust the flow rate so that the retention time of arbutin is about 6 minutes.
Selection of column: Dissolve 0.05 g each of arbutin for assay, hydroquinone and gallic acid in water to make 100 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of arbutin, hydroquinone and gallic acid in this order, and clearly dividing each peak.
System repeatability: Repeat the test five times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of arbutin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Uva Ursi Fluidextract
ウワウルシ流エキス

Uva Ursi Fluidextract contains not less than 3.0 w/v% of arbutin.

Method of preparation Prepare an infusion from Bearberry Leaf, in coarse powder, as directed under Fluidextracts, using hot Purified Water or hot Purified Water in Containers. Remove a part of the accompanying tannin, evaporate the mixture under reduced pressure, if necessary, and add Purified Water or Purified Water in Containers to adjust the percentage. It may contain an appropriate quantity of Ethanol.

Description Uva Ursi Fluidextract is a yellow-brown to dark red-brown liquid, and has a bitter and astringent taste.

It is miscible with water and with ethanol (95).

Identification To 1 mL of Uva Ursi Fluidextract add 30 mL of a mixture of ethanol (95) and water (7:3), shake, filter, and use the filtrate as the sample solution. Proceed as directed in the Identification (2) under Bearberry Leaf.

Purity Heavy metals <1.07>—Prepare the test solution with 1.0 g of Uva Ursi Fluidextract as direct in the Fluidextracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

Assay Pipet 1 mL of Uva Ursi Fluidextract, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay under Bearberry Leaf.

\[
\text{Amount (mg) of arbutin} = M_S \times \frac{A_T}{A_S}
\]

M_S: Amount (mg) of arbutin for assay

Containers and storage Containers—Tight containers.

Belladonna Root

Belladonnæ Radix
ベルドンナコン

Belladonna Root is the root of Atropa belladonna Linné (Solanaceae).

When dried, it contains not less than 0.4% of hyoscyamine (C_{17}H_{23}NO_{3}: 289.37).

Description Cylindrical root, usually 10 – 30 cm in length, 0.5 – 4 cm in diameter; often cut crosswise or lengthwise; externally grayish brown to grayish yellow-brown, with longitudinal wrinkles; periderm often removed; fractured surface is light yellow to light yellow-brown in color and is powdery.

Almost odorless; taste, bitter.

Identification Place 2.0 g of pulverized Belladonna Root in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate RS in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff’s TS for spraying on the plate: the principal spot from the sample solution is the same in color tone and Rf value with a yellow-red spot from the standard solution.

Purity (1) Stem and crown—When perform the test of
Belladonna Extract

Belladonna Extract contains not less than 0.85% and not more than 1.05% of hyoscyamine (C₁₇H₂₃NO₃: 289.37).

Method of preparation

To 1000 g of a coarse powder of Belladonna Root add 4000 mL of 35 vol% Ethanol, and digest for 3 days. Press the mixture, add 2000 mL of 35 vol% Ethanol to the residue, and digest again for 2 days. Combine all the extracts, and allow to stand for 2 days. Filter, and prepare the viscous extract as directed under Extracts. An appropriate quantity of Ethanol and Purified Water or Purified Water in Containers may be used in place of 35 vol% Ethanol.

Description

Belladonna Extract has a dark brown color, a characteristic odor and a bitter taste.

Identification

Mix 0.5 g of Belladonna Extract with 30 mL of ammonia TS in a flask, transfer the mixture to a separator, then add 40 mL of ethyl acetate, and shake the mixture. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Proceed as directed in the Identification under Belladonna Root.

Purity

Heavy metals <1.07>—Prepare the test solution with 1.0 g of Belladonna Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

Assay

Weigh accurately about 0.4 g of Belladonna Extract, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 25 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 25 mL each of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. Filter this solution through a filter of a porosity of not more than 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate RS (previously determine the loss on drying <2.4>) in the same conditions as Atropine Sulfate Hydrate, dissolve in the mobile phase to make exactly 25 mL, and use this solution as the sample stock solution. Pipet 5 mL of the standard stock solution, add exactly 3 mL of the internal standard solution, then add 25 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Calculate the ratios, Q₁ and Q₃, of the peak area of hyoscyamine (atropine), to that of the internal standard of each solution.

\[
\text{Amount (mg) of hyoscyamine (C₁₇H₂₃NO₃)} = M_S \times \frac{Q_1}{Q_3} \times \frac{1}{5} \times 0.8551
\]

\[
M_S: \text{Amount (mg) of Atropine Sulfate RS, calculated on the dried basis}
\]

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption spectrometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 900 mL of water, add 10 mL of triethylamine, adjust with phosphoric acid to pH 3.5, and add water to make 1000 mL, and mix this solution with acetonitrile (9:1).

Flow rate: Adjust the flow rate so that the retention time of atropine is about 14 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and determine the resolution. Use a column giving elution of atropine and the internal standard in this order with the resolution between these peaks being not less than 4.

Containers and storage

Containers—Well-closed containers.

Belladonna Extract / Crude Drugs
Benincasa Seed

Benincasae Semen

Benincasa seed is the seed of *Benincasa cerifera* Savi (1) or *Benincasa cerifera* Savi forma *emarginata* K. Kimura et Sugiyama (2) (*Cucurbitaceae*).

**Description** (1) Flattened, ovate to orbicular–ovate seed, 10–13 mm in length, 6–7 mm in width, about 2 mm in thickness; slightly acute at base; hilum and germ pore form two protrusions; externally light grayish yellow to light yellowish brown; prominent band along with marginal edge of seed; under a magnifying glass, surface of the seed is with fine wrinkles and minute hollows.

(2) Flattened, ovate to ellipsoidal seed, 9–12 mm in length, 5–6 mm in width, about 2 mm in thickness; hilum and germ pore form two protrusions as in (1); externally light grayish yellow, smooth, no prominent band along with marginal edge of seed.

Both (1) and (2) odorless; bland taste and slightly oily.

Under a microscope \(<5.01\>\), a transverse section of (1) reveals the outermost layer of seed coat composed of a single-layered and palisade like epidermis, the epidermis obvious at prominent band along with marginal edge of seed; a transverse section of (2) reveals the outermost layer composed of a single-layered epidermis coated with cuticle, often detached; hypodermis of (1) and (2) composed of slightly sclerified parenchyma beneath epidermis; inside of the parenchyma several layers of stone cells lie; the innermost layer of seed coat composed of parenchyma several cells thick; perispem coated with cuticle, composed of parenchyma several cells thick; endosperm composed of a row of compressed cells; cotyledon contains oil drops and aleurone grains, occasionally starch grains.

**Identification** To about 0.5 g of pulverized Benincasa Seed add 10 mL of a mixture of methanol and water (4:1), shake for 10 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography \(<2.02\>. Spot 20 \mu L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (8:6:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): two bluish white spots appear an RF value of about 0.4, and the spot having the smaller RF value shows more intense fluorescence.

**Purity** Foreign matter \(<5.01\>—It contains not more than 2.0%.

**Loss on drying** \(<5.01\> Not more than 11.0% (6 hours).

**Total ash** \(<5.01\> Not more than 5.0%.

**Acid-insoluble ash** \(<5.01\> Not more than 1.5%.

**Extract content** \(<5.01\> Dilute ethanol-soluble extract: not less than 3.0%.

**Containers and storage** Containers—Well-closed containers.

Benzoin

**Benzoinum**

Benzoin is the resin obtained from *Styrax benzoin* Dryander or other species of the same genus (*Styracaceae*).

**Description** Benzoin occurs as grayish brown to dark red-brown blocks varying in size; the fractured surface exhibiting whitish to light yellow-red grains in the matrix; hard and brittle at ordinary temperature but softened by heat.

Odor, characteristic and aromatic; taste, slightly pungent and acrid.

**Identification** (1) Heat a fragment of Benzoin in a test tube; it evolves an irritating vapor, and a crystalline sublimate is produced.

(2) Digest 0.5 g of Benzoin with 10 mL of diethyl ether, decant 1 mL of the diethyl ether into a porcelain dish, and add 2 to 3 drops of sulfuric acid: a deep red-brown to deep red-purple color develops.

**Purity** Ethanol-insoluble substances—Boil gently 1.0 g of Benzoin with 30 mL of ethanol (95) on a water bath for 15 minutes under a reflux condenser. After cooling, collect the insoluble substances through a tared glass filter (G3), and wash with three 5-mL portions of ethanol (95). Dry the residue at 105°C for 4 hours: the mass of the residue does not exceed 0.30 g.

**Total ash** \(<5.01\> Not more than 2.0%.

**Acid-insoluble ash** \(<5.01\> Not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

Bitter Cardamon

**Alpiniae Fructus**

**ヤクチ**

Bitter Cardamon is the fruit of *Alpinia oxyphylla* Miqoul (*Zingiberaceae*).

**Description** Spherical to fusiform fruit, with both ends somewhat pointed; 1 – 2 cm in length, 0.7 – 1 cm in width; externally brown to dark brown, with numerous longitudinal, knob-like protruding lines; pericarp 0.3 – 0.5 mm in thickness, closely adhering to the seed mass, and difficult to separate; inside divided vertically into three loculi by thin membranes, each loculus containing 5 to 8 seeds adhering by aril; seeds irregularly polygonal, about 3.5 mm in diameter, brown to dark brown in color, and hard in texture.

Odor, characteristic; taste, slightly bitter.

**Total ash** \(<5.01\> Not more than 10.0%.

**Acid-insoluble ash** \(<5.01\> Not more than 2.5%.

**Essential oil content** \(<5.01\> Perform the test with 50.0 g of
pulverized Bitter Cardamon: the volume of essential oil is not less than 0.4 mL.

Containers and storage  Containers—Well-closed containers.

Bitter Orange Peel

Aurantii Pericarpium

Bitter Orange Peel is the pericarp of the ripe fruit of Citrus aurantium Linné or Citrus aurantium Linné var. daidai Makino (Rutaceae).

Description  Usually quartered sections of a sphere, sometimes warped or flattened, 4 – 8 cm in length, 2.5 – 4.5 cm in width and 0.5 – 0.8 cm in thickness; the outer surface is dark red-brown to grayish yellow-brown, with numerous small dents associated with oil sacs; the inner surface is white to light grayish yellow-red, with irregular indented reticulation left by vascular bundles; light and brittle in texture.

Odor, characteristic aroma; taste, bitter, somewhat mucilaginous and slightly pungent.

Identification  To 1.0 g of Bitter Orange Peel add 10 mL of ethanol (95), allow to stand for 30 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of naringin for thin-layer chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in ammonia gas: a spot from the sample solution and a grayish green spot from the standard solution show the same color tone and the same RF value.

Loss on drying  Not more than 14.0% (6 hours).

Total ash  Not more than 5.5%.

Acid-insoluble ash  Not more than 0.5%.

Essential oil content  Perform the test with 50.0 g of pulverized Bitter Orange Peel provided that 1 mL of silicon resin is previously added to the test sample in the flask: the volume of essential oil is not less than 0.2 mL.

Containers and storage  Containers—Well-closed containers.

Orange Peel Syrup

Orange Peel Tincture

Method of preparation

<table>
<thead>
<tr>
<th>Orange Peel Tincture</th>
<th>Simple Syrup</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 mL</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Tinctures, with the above ingredients. An appropriate quantity of Sucrose and Purified Water or Purified Water in Containers may be used in place of Simple Syrup.

Description  Orange Peel Syrup is a brownish yellow to reddish brown liquid. It has a characteristic odor, a sweet taste and a bitter aftertaste.

Specific gravity  $d_{20}^\infty$; about 1.25

Identification  To 25 mL of Orange Peel Syrup add 50 mL of ethyl acetate, shake for 5 minutes, allow to stand until clear ethyl acetate layer separate, and take the ethyl acetate layer, and evaporate on a water bath to dryness. Dissolve the residue in 10 mL of ethanol (95), filter if necessary, and use this solution as the sample solution. Separately, dissolve 10 mg of naringin for thin-layer chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography in 10 mL of ethanol (95), filter if necessary, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Prepare the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in ammonia gas: a spot from the sample solution and a grayish green spot from the standard solution show the same color tone and the same RF value.

Containers and storage  Containers—Tight containers.

Orange Peel Syrup

Method of preparation

<table>
<thead>
<tr>
<th>Bitter Orange Peel, in coarse powder</th>
<th>Simple Syrup</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 g</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Tinctures, with the above ingredients. An appropriate quantity of Ethanol and Purified Water or Purified Water in Containers may be used in place of 70 vol% Ethanol.

Description  Orange Peel Tincture is a yellowish brown liquid. It has a characteristic odor, and a bitter taste.

Specific gravity  $d_{20}^\infty$; about 0.90

Identification  To 5.0 mL of Orange Peel Tincture add 5 mL of ethanol (95), filter if necessary, and use the filtrate as
the sample solution. Proceed as directed in the Identification under Bitter Orange Peel.

**Alcohol number** <1.01> Not less than 6.6 (Method 2).

**Containers and storage** Containers—Tight containers.

### Bitter Tincture

*Tinctura Amara*

苦味チンキ

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitter Orange Peel, in coarse powder</td>
<td>50 g</td>
</tr>
<tr>
<td>Swertia Herb, in coarse powder</td>
<td>5 g</td>
</tr>
<tr>
<td>Zanthoxylum Fruit, in coarse powder</td>
<td>5 g</td>
</tr>
<tr>
<td>70 vol% Ethanol</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Tinctures, with the above ingredients. An appropriate quantity of Ethanol and Purified Water or Purified Water in Containers may be used in place of 70 vol% Ethanol.

**Description** Bitter Tincture is a yellow-brown liquid. It has a characteristic aroma and a bitter taste.

**Identification**

1. To 1 mL of Bitter Tincture add 5 mL of methanol, then add 0.1 g of magnesium in ribbon form and 1 mL of hydrochloric acid, and allow to stand: the solution is red-purple in color.

2. Use Bitter Tincture as the sample solution. Separately, to 5.0 g of pulverized Bitter Orange Peel add 100 mL of diluted ethanol (7 in 10), stopper the vessel tightly, shake for 30 minutes, filter, and use the filtrate as the standard solution (1). Proceed with 0.5 g each of pulverized Swertia Herb and Zanthoxylum Fruit in the same manner, and use the solutions so obtained as the standard solution (2) and the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL of the sample solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (5:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a blue-purple fluorescent spot appears at around Rf value 0.3.

**Total ash** <0.01> Not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

### Brown Rice

*Oryzae Fructus*

コウヘイ

Brown Rice is the caryopsis of *Oryza sativa* Linné (*Gramineae*).

**Description** Brown Rice occurs as ellipsoidal, slightly flattened, 4 - 6 mm in length; externally translucent, light yellowish white to light brown. Slightly cave in and a white embryo at one end; a brown small dent of scar of style at the other end; few longitudinally striates on the surface.

Odor, slight; taste, slightly sweet.

Under a microscope <5.01>, a transverse section of the caryopsis reveals the outermost layer composed of pericarp; vascular bundles in the pericarp; seed coat adhering closely to the pericarp; in the interior, 1 or 2 aleuron layers; parenchymatous cells of endosperm contain simple or compound starch grains.

**Identification** (1) To 0.1 g of pulverized Brown Rice add 50 mL of water, and heat in a water bath for 5 minutes. After cooling, add 1 drops of iodine TS, and shake: a blue-purple color develops.

(2) To 1 g of pulverized Brown Rice add 5 mL of ethyl acetate, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer chromatography <2.03>. Spot 10 µL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (5:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a blue-purple fluorescent spot appears at around Rf value 0.3.

**Containers and storage** Containers—Well-closed containers.

### Bupleurum Root

*Bupleuri Radix*

サイコ

Bupleurum Root is the root of *Bupleurum falcatum* Linné (*Umbelliferae*). It contains not less than 0.35% of the total saponin (saikosaponin a and saikosaponin d), calculated on the basis of dried material.

**Description** Single or branched root of long cone or column shape, 10 - 20 cm in length, 0.5 - 1.5 cm in diameter; occasionally with remains of stem on the crown; externally light brown to brown and sometimes with deep wrinkles; easily broken, and fractured surface somewhat fibrous.

Odor, characteristic, and taste, slightly bitter.

Under a microscope <5.01>, a transverse section reveals the thickness of cortex reaching 1/3 ~ 1/2 of the radius, tangen-
Burdock Fruit

Arctii Fructus

**Description**

Burdock Fruit is slightly curved, long obovate achene, 5 – 7 mm in length, 2.0 – 3.2 mm in width, 0.8 to 1.5 mm in thickness; externally grayish brown to brown, with black spots; hollow about 1 mm in diameter at one broad end; flat, indistinct, longitudinal ridge at the other narrow end. 100 fruits weigh 1.0 – 1.5 g.
Practically odorless; taste, bitter and oily.
Under a microscope, transverse section reveals an exocarp of single-layered epidermal tissue, mesocarp of slightly sclerified parenchyma, and endocarp of a single layer of stone cells; seed coat composed of radially elongated, sclerified epidermis, and parenchyma several cells thick; parenchymatous cells of the mesocarp contain a brown substance; stone cells of endocarp contain solitary, discrete crystals of calcium oxalate; cotyledons with starch grains, oil drops, aleurone grains, and minute crystals of calcium oxalate.

**Identification** To 0.5 g of pulverized Burdock Fruit add 20 mL of methanol, shake for 10 minutes, filter, and use filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of acetone, ethyl acetate and water (15:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: a red-purple spot appears at an Rf value of about 0.4.

**Loss on drying** Not more than 12.0% (6 hours).

**Total ash** Not more than 7.0%.

**Acid-insoluble ash** Not more than 1.0%.

**Extract content** Dilute ethanol-extract: not less than 15.0%.

**Containers and storage** Well-closed containers.

**Calumba**

*Calumba Radix*

コロンボ

Calumba is the cross-sectioned root of *Jateorhiza columba* Miers (*Menispermaceae*).

**Description** Disk-like slices, 0.5 – 2 cm in thickness, 3 – 8 cm in diameter; mostly with concave center and slightly waved; side surface grayish brown in color, with irregular wrinkles; cut surface light yellow and powdery, with pale and dark radiating stripes; cortex rather yellowish; cambium and its neighborhood light grayish brown, warty protrusions in the center; hard in texture, but brittle.

Odor characteristic; taste, bitter.

**Identification** To 3 g of pulverized Calumba add 30 mL of water, allow to stand for 5 minutes with occasional shaking, and filter. To 2 mL of the filtrate add gently 1 mL of sulfuric acid, and after cooling, add carefully chloroform TS to make two layers: a light red to red color develops at the zone of contact.

**Purity (1)** Heavy metals —Proceed with 3.0 g of pulverized Calumba according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

**Powdered Calumba**

*Calumba Radix Pulverata*

コロンボ末

Powdered Calumba is the powder of Calumba.

**Description** Powdered Calumba occurs as a grayish yellow powder, and has a characteristic odor and a bitter taste.

Under a microscope, Powdered Calumba reveals numerous starch grains, fragments of parenchyma cells containing them; fragments of cork cells, stone cells, fibers, substitute fibers, vessels, tracheids, and also solitary crystals of calcium oxalate; starch grains consisting of solitary grains or 2- to 3-compound grains; hilum, unevenly scattered, usually 25 – 50 μm, but up to 90 μm in diameter.

**Identification** To 3 g of Powdered Calumba add 30 mL of water, allow to stand for 5 minutes with occasional shaking, and filter. To 2 mL of the filtrate add gently 1 mL of sulfuric acid, and after cooling, add carefully chloroform TS to make two layers: a light red to red color develops at the zone of contact.

**Purity (2)** Arsenic —Prepare the test solution with 0.40 g of Powdered Calumba according to Method 4, and perform the test (not more than 5 ppm).

**Containers and storage** Well-closed containers.

**Capsicum**

*Capsici Fructus*

トウガラシ

Capsicum is the fruit of *Capsicum annuum* Linné (*Solanaceae*).

It contains not less than 0.10% of total capsaicins (E)-capsaicin and dihydrocapsaicin), calculated on the basis of dried material.

**Description** Elongated conical to fusiform fruit, often bent, 3 – 10 cm in length, about 0.8 cm in width; outer surface lustrous and dark red to dark yellow-red; interior of pericarp hollow and usually divided into two loculi, containing numerous seeds nearly circular and compressed, light yellow-red, about 0.5 cm in diameter.

Usually it remains of calyx and peduncle.
Odor, slight and characteristic; taste, hot and acrid.

**Identification** To 2.0 g of pulverized Capsicum add 5 mL of ethanol (95), warm on a water bath for 5 minutes, cool, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (E)-capsaicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and methanol (19:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly 2,6-dibromo-N-chloro-1,4-benzoquinone monooimine TS on the plate, and allow to stand in ammonia gas: a spot from the sample solution and a blue spot from the standard solution show the same color tone and the same Rf value.

**Purity** Foreign matter <5.07>—The amount of foreign matter contained in Capsicum does not exceed 1.0%. Loss on drying <5.07> Not more than 14.0% (6 hours).

**Total ash** <5.07> Not more than 8.0%.

**Acid-insoluble ash** <5.07> Not more than 1.2%.

**Assay** Weigh accurately about 0.5 g of medium powder of Capsicum in a glass-stoppered centrifuge tube, add 30 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of methanol, shake for 5 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure again, combine the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of (E)-capsaicin for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, ATC and ATD, of (E)-capsaicin and dihydrocapsaicin (the relative retention time to (E)-capsaicin is about 1.3) in the sample solution, and the peak area, AS, of (E)-capsaicin in the standard solution.

\[
\text{Amount (mg) of total capsaicins} = M_s \times (A_{TC} + A_{TD}) / A_S \times 0.08
\]

Where

- \(M_s\) is the amount (mg) of (E)-capsaicin for assay

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 281 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of (E)-capsaicin is about 20 minutes.

**System suitability**—

System performance: Dissolve 1 mg each of (E)-capsaicin for assay and 4-hydroxy-3-methoxybenzyl nonyl acid amide in methanol to make 50 mL. When the procedure is run with 20 µL of this solution under the above operating conditions, 4-hydroxy-3-methoxybenzyl nonyl acid amide and (E)-capsaicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of (E)-capsaicin is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

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**Powdered Capsicum**

*Capsici Fructus Pulveratus*

Powdered Capsicum is the powder of Capsicum. It contains not less than 0.10% of total capsaicins ((E)-capsaicin and dihydrocapsaicin), calculated on the basis of dried material.

**Description** Powdered Capsicum occurs as a yellow-red powder. It has a slight, characteristic odor and a hot, acrid taste.

Under a microscope <5.07>, Powdered Capsicum reveals fragments of parenchyma containing oil droplets and yellow-red chromoplasts; fragments of outer pericarp with thick cuticle; fragments of stone cells from inner surface of pericarp, with wavy curved side walls; fragments of thin vessels; fragments of seed coat with thick wall, and fragments of parenchyma consisting of small cells of endosperm containing fixed oil and aleurone grains.

**Identification** To 2.0 g of Powdered Capsicum add 5 mL of ethanol (95), warm on a water bath for 5 minutes, cool, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (E)-capsaicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and methanol (19:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly 2,6-dibromo-N-chloro-1,4-benzoquinone monooimine TS on the plate, and allow to stand in ammonia gas: a spot from the sample solution and blue spot from the standard solution show the same in color tone and Rf value.

**Loss on drying** <5.07> Not more than 14.0% (6 hours).

**Total ash** <5.07> Not more than 8.0%.

**Acid-insoluble ash** <5.07> Not more than 1.2%.

**Assay** Weigh accurately about 0.5 g of medium powder of Powdered Capsicum in a glass-stoppered centrifuge tube, add 30 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of methanol, shake for 5 minutes, centrifuge, and sepa-
rate the supernatant liquid. Repeat this procedure again, combine the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of (E)-capsaicin for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{TC}$ and $A_{TD}$, of (E)-capsaicin and dihydrocapsaicin (the relative retention time to (E)-capsaicin is about 1.3) in the sample solution, and the peak area, $A_S$, of (E)-capsaicin in the standard solution.

\[
\text{Amount (mg) of total capsaicins} = M_S \times \frac{(A_{TC} + A_{TD})}{A_S} \times 0.08
\]

$M_S$: Amount (mg) of (E)-capsaicin for assay

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 281 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of (E)-capsaicin is about 20 minutes.

**System suitability—**

System performance: Dissolve 1 mg each of (E)-capsaicin for assay and 4-hydroxy-3-methoxybenzyl nonylic acid amide in methanol to make 50 mL. When the procedure is run with 20 µL of this solution under the above operating conditions, 4-hydroxy-3-methoxybenzyl nonylic acid amide and (E)-capsaicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of (E)-capsaicin is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

---

**Capsicum Tincture**

トウガラシチンキ

Capsicum Tincture contains not less than 0.010 w/v% of total capsaicins ((E)-capsaicin and dihydrocapsaicin).

**Method of preparation**

<table>
<thead>
<tr>
<th>Capsicum, in medium cutting</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td></td>
<td>To make 1000 mL</td>
</tr>
</tbody>
</table>

Prepare as directed under Tinctures, with the above ingredients.

**Description** Capsicum Tincture is a yellow-red liquid. It has a burning, pungent taste.

Specific gravity $d^3$: about 0.82

**Identification** Proceed as directed in the Identification under Capsicum, using Capsicum Tincture as the sample solution. Spot 20 µL each of the sample solution and the standard solution.

**Alcohol number <1.01>** Not less than 9.7 (Method 2).

**Assay** Pipet 2 mL of Capsicum Tincture, add methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of (E)-capsaicin for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{TC}$ and $A_{TD}$, of (E)-capsaicin and dihydrocapsaicin (the relative retention time to (E)-capsaicin is about 1.3) in the sample solution, and the peak area, $A_S$, of (E)-capsaicin in the standard solution.

\[
\text{Amount (mg) of total capsaicins} = M_S \times \frac{(A_{TC} + A_{TD})}{A_S} \times 0.032
\]

$M_S$: Amount (mg) of (E)-capsaicin for assay

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 281 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of (E)-capsaicin is about 20 minutes.

**System suitability—**

System performance: Dissolve 1 mg each of (E)-capsaicin for assay and 4-hydroxy-3-methoxybenzyl nonylic acid amide in methanol to make 50 mL. When the procedure is run with 20 µL of this solution under the above operating conditions, 4-hydroxy-3-methoxybenzyl nonylic acid amide and (E)-capsaicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of (E)-capsaicin is not more than 1.5%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.
Capsicum and Salicylic Acid Spirit

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsicum Tincture</td>
<td>40 mL</td>
</tr>
<tr>
<td>Salicylic Acid</td>
<td>50 g</td>
</tr>
<tr>
<td>Liquefied Phenol</td>
<td>20 mL</td>
</tr>
<tr>
<td>Castor Oil</td>
<td>100 mL</td>
</tr>
<tr>
<td>aromatic substance</td>
<td>a suitable quantity</td>
</tr>
<tr>
<td>Ethanol</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Spirits, with the above ingredients.

**Description** Capsicum and Salicylic Acid Spirit is a light brown-yellow liquid.

**Specific gravity** \( \frac{12}{10} \) about 0.84

**Identification**

1. Shake 10 mL of Capsicum and Salicylic Acid Spirit with 15 mL of sodium hydrogen carbonate TS and 10 mL of diethyl ether, and separate the water layer. To 1 mL of the solution add hydrochloric acid-potassium chloride buffer solution, \( \text{pH} 2.0 \), to make 200 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate ene hydrate (1 in 200): a red-purple color is produced (salicylic acid).

2. To 0.5 mL of Capsicum and Salicylic Acid Spirit add 20 mL of water and 5 mL of dilute hydrochloric acid, extract with 20 mL of diethyl ether, wash the diethyl ether extract with two 5-mL portions of sodium hydrogen carbonate TS, and then extract with 20 mL of dilute sodium hydroxide TS. To 1 mL of the extract add 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, shake, and allow to stand for 10 minutes. Add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

3. To 0.2 mL of Capsicum and Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chloroform, and use the extract as the sample solution. Dissolve 0.01 g of salicylic acid and 0.02 g of phenol in 5 mL and 25 mL of chloroform, respectively, and use both solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.01> \). Spot 5 \( \mu L \) of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and dry-air dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots from the sample solution exhibit the same \( R_f \) values as those from standard solution (1) and standard solution (2). Spray evenly iron (III) chloride TS upon the plate: the spot from standard solution (1) and the corresponding spot from the sample solution reveal a purple color.

**Alcohol number** \( <1.01> \) Not less than 8.1 (Method 2).

Prepare the sample solution as follows: Pipet 5 mL of Capsicum and Salicylic Acid Spirit at 15 ± 2°C into a glass-stoppered, conical flask containing exactly 45 mL of water while shaking vigorously, allow to stand, and filter the lower layer. Discard the first 15 mL of the filtrate. Pipet 25 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, and add water to make exactly 100 mL.

**Containers and storage** Containers—Tight containers.

Cardamon

**Cardamomi Fructus**

Cardamon is the fruit of *Elettaria cardamomum* Maton (*Zingiberaceae*). The capsules are removed from the seeds before use.

**Description** Nearly ellipsoidal, 1–2 cm in length, 0.5–1 cm in diameter; externally, light yellow with three blunt ridges and many longitudinal lines; 0.1–0.2 cm beak at one end; pericarp thin, light and fibrous; interior longitudinally divided into three loculi by thin membranes, each loculus containing 3 to 7 seeds joining by aril; seed irregularly angular ovoid, 0.3–0.4 cm in length, dark brown to blackish brown; the dorsal side convex, the ventral side longitudinally grooved; external surface coarsely tuberculated.

Seed has a characteristic aroma, and pungent, slightly bitter taste; pericarp, odorless and tasteless.

**Total ash** \( <5.01> \) Not more than 6.0% (seed).

**Acid-insoluble ash** \( <5.01> \) Not more than 4.0% (seed).

**Essential oil content** \( <5.01> \) Perform the test with 30.0 g of the pulverized seeds of Cardamon: the volume of essential oil is not less than 1.0 mL.

**Containers and storage** Containers—Well-closed containers.

Cassia Seed

**Cassiae Semen**

Cassia Seed is the seed of *Cassia obtusifolia* Linné or *Cassia tora* Linné (*Leguminosae*).

**Description** Short cylindrical seed, 3 – 6 mm in length, 2 – 3.5 mm in diameter; acuminate at one end and flat at the other; externally green-brown to brown and lustrous, with light yellow-brown longitudinal lines or bands on both sides; hard in texture; cross section round or obtuse polygonal; under a magnifying glass, albumen enclosing a bent, dark-colored cotyledon.

When ground, characteristic odor and taste.

**Identification** Place 0.1 g of pulverized Cassia Seed, previously dried in a desiccator (silica gel) for 48 hours, on a slide glass, put a glass ring 10 mm in both internal diameter and height on it, then cover with moistened filter paper, and heat gently the slide glass over a small flame. Take off the filter paper when a yellow color has developed on the upper surface of it, and place 1 drop of potassium hydroxide TS on
the surface of the filter paper where a sublimate is present: a red color appears.

**Purity**  Foreign matter <5.0%—The amount of foreign matter contained in Cassia Seed does not exceed 1.0%.

**Total ash** <5.0%  Not more than 5.0%.

**Containers and storage**  Containers—Well-closed containers.

### Catalpa Fruit

**Catalpalae Fructus**

キササゲ

Catalpa Fruit is the fruit of Catalpa ovata G. Don or Catalpa bungei C. A. Meyer (Bignoniaceae).

**Description**  Slender stick-like fruit, 30 – 40 cm in length and about 0.5 cm in diameter; externally, dark brown; inner part contains numerous seeds; seed compressed or semitubular, about 3 cm in length and about 0.3 cm in width, externally greyish brown; hairs, about 1 cm in length, attached to both ends of seed; pericarp, thin and brittle.

Odor, slight; taste, slightly astringent.

**Identification**  To 1.0 g of pulverized Catalpa Fruit add 20 mL of water, warm on a water bath for 5 minutes, and filter immediately. Transfer the filtrate to a separator, and extract with two 20-mL portions of 1-butanol. Combine the extracts, evaporate to dryness under reduced pressure on a water bath, dissolve the residue in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.0%L). Spot 5 μL of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (20:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultra-violet light (main wavelength: 254 nm): one spot among the several spots obtained from the sample solution has the same color tone and the same Rf value. Prescribe that the moving distance of the spot corresponding to parahydroxybenzoic acid from the sample solution is 1: a dark purple spot develops at the relative moving distance of about 0.3.

**Purity**  Peduncle—When perform the test of foreign matter <5.0%, the amount of peduncles contained in Catalpa Fruit does not exceed 5.0%.

**Total ash** <5.0%  Not more than 6.0%.

**Acid-insoluble ash** <5.0%  Not more than 0.5%.

**Extract content** <5.0%  Dilute ethanol-soluble extract: not less than 8.0%.

**Containers and storage**  Containers—Well-closed containers.

### Chotosan Extract

釣藤散エキス

Chotosan Extract contains not less than 24 mg and not more than 72 mg of hesperidin, not less than 8 mg and not more than 24 mg of glycyrrhizic acid (C12H20O16: 822.93), and not less than 0.3 mg of the total alkaloid (rhyncophylline and hirsutine), per extract prepared with the amount specified in the Method of preparation.

**Method of preparation**

<table>
<thead>
<tr>
<th></th>
<th>1)</th>
<th>2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncaria Hook</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Citrus Unshiu Peel</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Pinellia Tuber</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Ophiopogon Tuber</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Poria Sclerotium</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Ginseng</td>
<td>2 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Saposhnikovia Root and Rhizome</td>
<td>2 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Chrysanthemum Flower</td>
<td>2 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Glycyrrhiza</td>
<td>1 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Ginger</td>
<td>1 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Gypsum</td>
<td>5 g</td>
<td>3 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

**Description**  Chotosan Extract is a light brown to blackish brown, powder or viscous extract. It has a slight odor, and has a pungent and slightly sweet first, then bitter taste.

**Identification**  (1) Shake 2.0 g of a dry extract (6.0 g of a viscous extract) with 20 mL of water and 2 mL of ammonia TS, and then shake with 20 mL of diethyl ether, separate the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg each of rhyncophylline for thin-layer chromatography and hirsutine for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.0%L). Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with one of the two dark purple spots from the standard solution (Uncaria Hook).

(2) Shake 2.0 g of a dry extract (6.0 g of a viscous extract) with 10 mL of water, add 10 mL of 1-butanol, and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of hesperidin for thin-layer chromatography and hirsutine for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.

**Method of preparation**
Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6.3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-N-chloro-1,4-benzoquinone monoxide TS on the plate, allow to stand in an ammonia gas: one of the spots among the several spots obtained from the sample solution has the same color tone and Rf value with the blue spot from the standard solution.

(3) Shake 2.0 g of a dry extract (6.0 g of a viscous extract) with 10 mL of water, add 5 mL of 1-butanol and shake, centrifuge, remove the 1-butanol layer, and use the aqueous layer as the sample solution. Separately, heat 3.0 g of Ophiopogon Tuber in 50 mL of water under a reflux condenser for 1 hour. After cooling, shake 20 mL of the extract with 5 mL of 1-butanol, centrifuge, remove the 1-butanol layer, and use the aqueous layer as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 2 μL of the sample solution and 5 μL of the standard solution as bands on original line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of a ethanol (99.5), water and acetic acid (100) (120:80:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the dark blue-green spot (around Rf value 0.3) from the standard solution (Ophiopogon Tuber).

(4) Shake 2.0 g of a dry extract (6.0 g of a viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb1, RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the purple spot from the standard solution (Ginseng).

(5) Shake 2.0 g of a dry extract (6.0 g of a viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of 4'-O-glycosyl-5-O-methylvisammolin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the blue spot from the standard solution (Ginseng), and air-dry the plate. Spray evenly vanillin-sulfuric acid on the plate, heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Chrysanthemum Flower).

(6) Shake 2.0 g of a dry extract (6.0 g of a viscous extract) with 10 mL of water, add 20 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of lutocin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 μL of the sample solution and 3 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and formic acid (5:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(7) Shake 2.0 g of a dry extract (6.0 g of a viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(8) Shake 1.0 g of a dry extract (3.0 g of a viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6] gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the red-purple spot from the standard solution (Ginger).

(9) Shake 1.0 g of a dry extract (3.0 g of a viscous extract) with 30 mL of methanol, centrifuge, and separate the supernatant liquid. Shake the residue with 30 mL of water, centrifuge, and separate the supernatant liquid. Add ammonium oxalate TS to this solution: a white precipitate is formed, and it does not dissolve by addition of dilute acetic acid, but it dissolve by addition of dilute hydrochloric acid. (Gypsophila)

**Purity (1)**

Heavy metals <1.07>—Prepare the test solution
with 1.0 g of a dry extract (1.0 g of a viscous extract, calculated on the dried basis) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic less than 1.1 ppm—Prepare the test solution with 0.67 g of a dry extract (0.67 g of a viscous extract, calculated on the dried basis) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying less than 7.5% (1 g, 105°C, 5 hours).

A viscous extract—Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash not more than 15.0%, calculated on the dried basis.

Assay (1) Hesperidin—Weigh accurately about 0.1 g of a dry extract (or amount of viscous extract, equivalent to 0.1 g of dried substance), add exactly 50 mL of diluted tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add diluted tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the filtrate as the sample solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography less than 2.0 ppm according to the following conditions, and determine the peak areas, and not more than 1.5, respectively.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).
Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Amount (mg) of glycyrrhizic acid (C_{24}H_{24}O_{13})
\[ M_S = \frac{A_T \times A_S}{A_T} \times \frac{1}{20} \]

Assay (2) Glycyrrhizic acid—Weigh accurately about 0.5 g of a dry extract (or amount of viscous extract, equivalent to 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography less than 2.0 ppm according to the following conditions, and determine the peak areas, and not more than 1.5, respectively.

Amount (mg) of glycyrrhizic acid (C_{24}H_{24}O_{13})
\[ M_S = \frac{A_T \times A_S}{A_T} \times \frac{1}{20} \]

Amount (mg) of Glycyrrhizic Acid RS, calculated on the dried basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).
Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

(3) Total alkaloid (rhynophylline and hirsutine)—Weigh accurately about 1 g of a dry extract (or amount of viscous extract, equivalent to 1 g of dried substance), add 20 mL of diethyl ether, shake, add 3 mL of 1 mol/L hydrochloric acid TS and 7 mL of water, shake for 10 minutes, centrifuge, and separate the ether layer. To the aqueous layer add 20 mL of diethyl ether, and repeat the above process. To the aqueous layer add 10 mL of sodium hydroxide TS and 20 mL of diethyl ether, shake for 10 minutes, centrifuge, and separate the supernatant liquid. Repeat the above process twice more with the residue using 20 mL portion of diethyl ether. Combine all supernatant liquids, evaporate to dryness under reduced pressure at not more than 40°C, and dissolve the residue in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of rhynophylline for assay and about 5 mg of hirsutine for assay, and dissolve in a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography less than 2.0 ppm according to the following conditions, and determine the peak areas of rhynophylline and hirsutine, and not more than 1.5, respectively.

Amount (mg) of rhynophylline and hirsutine
\[ M_S = \frac{A_T \times A_S}{A_T} \times \frac{1}{20} \]
Chrysanthemum Flower / Crude Drugs

Chrysanthemum Flower is the capitulum of 1) Chrysanthemum morifolium Ramatulle or 2) Chrysanthemum indicum Linné (Compositae).

Description 1) Chrysanthemum Flower is capitulum, 15–40 mm in diameter; involucrare consisting of 3–4 rows of involucral scales; the outer involucral scale linear to lanceolate, inner involucral scale narrow ovate to ovate; ligulate flowers are numerous, white to yellow; tubular flowers in small number, light yellow-brown; tubular flowers occasionally degenerate; outer surface of involucre green-brown to brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

2) Chrysanthemum Flower is capitulum, 3–10 mm in diameter; involucrare consisting of 3–5 rows of involucral scales; the outer involucral scale linear to lanceolate, inner involucral scale narrow ovate to ovate; ligulate flower is single, yellow to light yellow-brown; tubular flowers in numerous, light yellow-brown; outer surface of involucre yellow-brown to brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

Identification To 1 g of pulverized Chrysanthemum Flower add 20 mL of methanol, shake for 10 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of methanol, and use this as the sample solution. Separately, dissolve 1 mg of luteolin for thin-layer chromatography in 1 mL of methanol, and use this as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (25:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of several spots obtained from the sample solution has the same color tone and the same Rf value with the dark green spot obtained from the standard solution.

Loss on drying <5.0%> Not more than 15.0% (6 hours).

Total ash <5.0%> Not more than 8.5%.

Acid-insoluble ash <5.0%> Not more than 1.0%.

Extract content <5.0%> Dilute ethanol-soluble extract: not less than 30.0%.

Containers and storage Containers—Well-closed containers.

Cimicifuga Rhizome

Cimicifugae Rhizoma

Cimicifuga Rhizome is the rhizome of Cimicifuga simplex Turczaninow, Cimicifuga dahurica Maximowicz, Cimicifuga foetida Linné or Cimicifuga heracleifolia Komarov (Ranunculaceae).

Description Knotted, irregularly shaped rhizome, 6–18 cm in length, 1–2.5 cm in diameter; externally dark brown to blackish brown, with many remains of roots, often with scars of terrestrial stems; the center of the scar dented, and the circumference being pale in color and showing a radial pattern; fractured surface fibrous; pith dark brown in color and often hollow; light and hard in texture.

Almost odorless; taste, bitter and slightly astringent.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Cimicifuga Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Cimicifuga Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Rhizome of Astilbe thunbergii Miq.—Under a microscope <5.01>, pulverized Cimicifuga Rhizome does not contain crystal druses in the parenchyma.

Total ash <5.0%> Not more than 9.0%.

Acid-insoluble ash <5.0%> Not more than 1.5%.

Extract content <5.0%> Dilute ethanol-soluble extract: not less than 18.0%.

Containers and storage Containers—Well-closed contain-
Cinnamon Bark

Cinnamomi Cortex

粉末桂皮

Cinnamon Bark is the bark of the trunk of *Cinnamomum cassia* Blume (*Lauraceae*), or such bark from which a part of the periderm has been removed.

**Description** Usually semi-tubular or tubularly rolled pieces of bark, 0.1–0.5 cm in thickness, 5–50 cm in length, 1.5–5 cm in diameter; the outer surface dark red-brown, and the inner surface red-brown and smooth; brittle; the fractured surface is slightly fibrous, red-brown, exhibiting a light brown, thin layer.

Characteristic aroma; taste, sweet and pungent at first, later rather mucilaginous and slightly astringent.

Under a microscope, a transverse section of Cinnamon Bark reveals a primary cortex and a secondary cortex divided by an almost continuous ring consisting of stone cells; nearly round bundles of fibers in the outer region of the ring; wall of each stone cell often thickened in a U-shape; secondary cortex lacking stone cells, and with a small number of sclerenchymatous fibers coarsely scattered; parenchyma scattered with oil cells, mucilage cells and cells containing starch grains; medullary rays with cells containing fine needles of calcium oxalate.

**Identification** To 2.0 g of pulverized Cinnamon Bark add 10 mL of diethyl ether, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography.<p>

To 2.0 g of powdered Cinnamon Bark add 1 mL of diethyl ether, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography.<p>

**Purity (1)** Petiole—Under a microscope, Powdered Cinnamon Bark does not reveal epidermal cells, hairs, cells containing chlorophyll granules, and fragments of vascular bundle.<p>

**Purity (2)** Total BHC's and total DDT's—Not more than 0.2 ppm, respectively.<p>

**Loss on drying**—Not more than 15.0% (6 hours).<p>

**Total ash**—Not more than 6.0%.<p>

**Essential oil content** Perform the test with 50.0 g of powdered Cinnamon Bark provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.5 mL.<p>

**Containers and storage** Containers—Well-closed containers.

Powdered Cinnamon Bark

*Cinnamomi Cortex Pulveratus*

粉末桂皮

Powdered Cinnamon Bark is the powder of Cinnamon Bark.

**Description** Powdered Cinnamon Bark is red-brown to brown in color. It has a characteristic aroma and a sweet, pungent taste with a slightly mucilaginous and astringent aftertaste.

Under a microscope, Powdered Cinnamon Bark reveals starch grains, fragments of parenchyma cells containing them; fragments of fibers, oil cells containing yellow-brown oil droplets, stone cells, cork stone cells, cork tissue, and fine crystals of calcium oxalate. Starch grains are simple and compound grains 6 to 20 μm in diameter.

**Identification** To 2.0 g of Powdered Cinnamon Bark add 10 mL of diethyl ether, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography.<p>

**Purity (1)** Petiole—Under a microscope, Powdered Cinnamon Bark does not reveal epidermal cells, hairs, cells containing chlorophyll granules, and fragments of vascular bundle.<p>

**Purity (2)** Total BHC's and total DDT's—Not more than 0.2 ppm, respectively.<p>

**Loss on drying**—Not more than 15.0% (6 hours).<p>

**Total ash**—Not more than 6.0%.<p>

**Essential oil content** Perform the test with 50.0 g of Powdered Cinnamon Bark provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.35 mL.<p>

**Containers and storage** Containers—Tight containers.

Cinnamon Oil

*Oleum Cinnamomi*

粉末桂皮油

Cinnamon Oil is the essential oil distilled with steam from the leaves and twigs or bark of *Cinnamomum cassia* Blume or from the bark of *Cinnamomum zeylanicum* Nees (*Lauraceae*). It contains not less than 60 vol% of the total aldehydes.
Cinnamon Oil is a yellow to brown liquid. It has a characteristic, aromatic odor and a sweet, pungent taste.

It is clearly miscible with ethanol (95) and with diethyl ether.

It is practically insoluble in water.

It is weakly acidic. Upon aging or long exposure to air, it darkens and becomes viscous.

Specific gravity at 20°C: 1.010–1.065

Identification

Shake 4 drops of Cinnamon Oil with 4 drops of nitric acid; the mixture forms white to light yellow crystals at a temperature below 5°C.

Purity

(1) Rosin—Mix 1.0 mL of Cinnamon Oil with 5 mL of ethanol (95), then add 3 mL of freshly prepared, saturated ethanol solution of lead (II) acetate trihydrate: no precipitate is produced.

(2) Heavy metals

Proceed with 1.0 mL of Cinnamon Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Assay

Pipet 5.0 mL of Cinnamon Oil into a cassia flask, add 70 mL of sodium hydrogensulfite TS, and heat the mixture in a water bath with frequent shaking to dissolve completely. To this solution add sodium hydrogensulfite TS to raise the lower level of the oily layer within the graduate portion of the neck. Allow to stand for 2 hours, and measure the volume (mL) of the separated oily layer.

Total aldehydes (vol%)

\[ \text{Area of aldehydes} = 5.0 - \text{(volume of separated oily layer)} \times 20 \]

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.

Citrus Unshiu Peel

Aurantii Nobilis Pericarpium

テンピ

Citrus Unshiu Peel is the pericarp of the ripe fruit of Citrus unshiu Marcowicz or Citrus reticulata Blanco (Rutaceae).

It contains not less than 4.0% of hesperidin, calculated on the basis of dried material.

Description

Irregular pieces of pericarp, about 2 mm in thickness; externally yellow-red to dark yellow-brown, with numerous small dents associated with oil sacs; internally white to light grayish yellow-brown; light and brittle in texture.

Odor, characteristic aroma; taste, bitter and slightly pungent.

Identification

To 0.5 g of pulverized Citrus Unshiu Peel add 10 mL of methanol, warm on a water bath for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbon-form and 1 mL of hydrochloric acid, and allow to stand: a red-purple color develops.

Purity

Total BHC’s and total DDT’s <5.0%—Not more than 0.2 ppm, respectively.

Loss on drying <5.0%—Not more than 13.0% (6 hours).

Total ash <5.0%—Not more than 4.0%.

Extract content <5.0%—Dilute ethanol-soluble extract: not less than 30.0%.

Essential oil content <5.0%—Perform the test with 50.0 g of pulverized Citrus Unshiu Peel provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.2 mL.

Assay

Weigh accurately about 0.1 g of powdered Citrus Unshiu Peel, add 30 mL of methanol, heat under a reflux condenser on a water bath for 15 minutes, centrifuge after cooling, and separate the supernatant liquid. To the residue add 20 mL of methanol, and proceed in the same manner. Combine the extracts, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of hespiridin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0% according to the following conditions, and determine the peak areas, A1 and A2, of hesperidin.

\[ \text{Amount (mg) of hesperidin} = M \times A_1 / A_2 \times 1/2 \]

M5: Amount (mg) of hesperidin for assay

Operating conditions


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (82:18:1).

Flow rate: 1.0 mL per minute (the retention time of hesperidin is about 15 minutes).

System suitability

System performance: Dissolve 1 mg each of hesperidin for assay and naringin for thin-layer chromatography in 10 mL of methanol, and add water to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, naringin and hesperidin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hesperidin is not more than 1.5%.

Containers and storage

Containers—Well-closed containers.

Storage—Light-resistant.
Clematis Root

Clematidis Radix

Description Clematis Root consists of short rhizome and numerous slender roots. The root, 10 – 20 cm in length, 1 – 2 mm in diameter, externally brown to blackish brown, with fine longitudinal wrinkles, brittle. The cortex easily separable from central cylinder; root, grayish white to light yellow-brown in the transverse section, light grayish yellow to yellow in the central cylinder; under a magnifying glass, central cylinder almost round, slight 2 – 4 sinuses on xylem. The rhizome, 2 – 4 cm in length, 5 – 20 mm in diameter, externally light grayish brown to grayish brown; cortex peeled off and fibrous, often with rising node; apex having the residue of lignified stem.

Odor, slight; practically tasteless.

Under a microscope, <5.01> transverse section of root reveals a uni-layered epidermis in the outermost layer; with exodermis lying just inside of the epidermis; cortex and stele divided by endodermis; cortex composed of parenchymatous tissue; xylem with 2 – 4 small concavities where phloem is present; parenchymatous cells contain both simple and 2- to 8-compound starch grains.

Identification (1) To 0.5 g of pulverized Clematis Root add 10 mL of water, and boil for 2 to 3 minutes. After cooling, shake vigorously: lasting fine foams appear.

(2) To 0.5 g of pulverized Clematis Root add 3 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To the filtrate add 1 mL of sulfuric acid gently: a brown color appears at the zone of contact.

Purity Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Clematis Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 8.5%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 15.0%.

Containers and storage Containers—Well-closed containers.

Clove

Caryophylli Flos

Description Dark brown to bright red buds, 1 – 1.8 cm in length, consisting of slightly compressed and four-sided receptacle, crowned by 4 thick sepals and 4 nearly spherical, membranous, imbricated petals, enclosing numerous stamens and a single style.

Odor, strong and characteristic; taste, pungent, followed by a slight numbness of the tongue.

Identification Mix 0.1 mL of the mixture of essential oil and xylene, obtained in the Essential oil content, with 2 mL of ethanol (95), and add 1 to 2 drops of iron (III) chloride TS: a green to blue color develops.

Purity (1) Stem—When perform the test of foreign matter <5.01>, the amount of the stem contained in Clove does not exceed 5.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than the stem contained in Clove does not exceed 1.0%.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Essential oil content <5.01> Perform the test with 10.0 g of pulverized Clove: the volume of essential oil is not less than 1.6 mL.

Containers and storage Containers—Well-closed containers.

Powdered Clove

Powdered Clove

Caryophylli Flos Pulveratus

Description Powdered Clove is the powder of Clove.

Identification Powdered Clove occurs as a dark brown powder. It has a strong, characteristic odor and a pungent taste, followed by slight numbness of the tongue.

Under a microscope <5.01>, Powdered Clove reveals epidermal tissue with stomata, collenchyma, parenchyma with oil sacs, and spongy parenchyma or its fragments; furthermore, a few fusiform thick-walled fibers, spiral vessels 6 – 10 μm in diameter, anther and pollen grains, and rosette aggregates of calcium oxalate 10 – 15 μm in diameter. Epidermis of anther shows characteristically reticulated walls; pollen grains tetrahedral 10 – 20 μm in diameter; rosette aggregates of calcium oxalate arranged in crystal cell rows, or contained in collenchyma cells and parenchyma cells.

Identification Mix 0.1 mL of a mixture of essential oil and xylene, obtained in the Essential oil content, with 2 mL of ethanol (95), and add 1 to 2 drops of iron (III) chloride TS: a green to blue color develops.

Purity Foreign matter <5.01>—Under a microscope, Powdered Clove does not contain stone cells or starch grains.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Essential oil content <5.01> Perform the test with 10.0 g of
Clove Oil

Oleum Caryophylli

Clove Oil is the volatile oil distilled with steam from the flower buds or leaves of Syzygium aromaticum Merrill et Perry (Eugenia caryophyllata Thunberg) (Myrtaceae).

- **It contains not less than 80.0 vol% of total eugenol.**

  **Description** Clove Oil is a colorless or light yellow-brown, clear liquid. It has a characteristic aroma and a burning taste.

- It is miscible with ethanol (95) and with diethyl ether.

- **It is slightly soluble in water.**

- **Identification (1)** To 5 drops of Clove Oil add 10 mL of calcium hydroxide TS, and shake vigorously: the oil forms a flocculent mass, and a white to light yellow color develops.

- **Identification (2)** Dissolve 2 drops of Clove Oil in 4 mL of ethanol (95), and add 1 to 2 drops of iron (III) chloride TS: a green color is produced.

- **Identification (3)** Dissolve 2 drops of Clove Oil in 4 mL of ethanol (95), and add 1 to 2 drops of iron (III) chloride TS: a green color is produced.

  **Refractive index** \( \eta^2_20 = 1.527 - 1.537 \)

  **Optical rotation** \( [\alpha]_20^B = 0 - 1.5^\circ (100 \text{ mm}) \)

  **Specific gravity** \( d_20^B = 1.040 - 1.068 \)

**Purity (1)** Clarity of solution

1. **Dissolve 1.0 mL of Clove Oil in 2.0 mL of diluted ethanol (7 in 10): the solution is clear.**

2. **Water-soluble phenols—To 1.0 mL of Clove Oil add 20 mL of boiling water, shake vigorously, filter the aqueous layer after cooling, and add 1 to 2 drops of iron (III) chloride TS: a yellow-green, but no blue or violet, color develops.**

3. **Heavy metals—Proceed with 1.0 mL of Clove Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).**

**Assay** Take 10.0 mL of Clove Oil in a Cassia flask, add 70 mL of sodium hydroxide TS, shake for 5 minutes and warm for 10 minutes in a water bath with occasional shaking, add sodium hydroxide TS to the volume after cooling, and allow to stand for 18 hours. Measure the volume (mL) of the separated oily layer.

Total eugenol (vol%)

\[ = \left[ 10 - \text{(volume of separated oily layer)} \right] \times 10 \]

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

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**Cnidium Monnieri Fruit**

**Cnidii Monnieri Fructus**

ジャショウシ

Cnidium Monnieri Fruit is the fruit of Cnidium monnieri Cusson (Umbelliferae).

**Description** Elliptical cremocarp, often each mericarp separated; 2 - 3 mm in length, 1 - 2 mm in width; externally light brown to brown, each mericarp usually with five winged longitudinal ridges; inner surface of mericarp almost flat.

Odor, characteristic; it gives characteristic aroma, later a slight sensation of numbness on chewing.

Under a microscope \( \times \), a transverse section reveals one oil canal between longitudinal ridges, usually two oil canals in the inner part of mericarp facing to gynophore; longitudinal ridges composed of slightly lignified parenchymatous cells, with vascular bundles in the base; epidermal cells and parenchymatous cells of longitudinal ridges contain solitary crystals of calcium oxalate; parenchymatous cells of albumen contain oil drops and aleurone grains, and occasionally starch grains.

**Identification** To 1 g of pulverized Cnidium Monnieri Fruit add 10 mL of ethyl acetate, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of osthole for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \times \). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and the \( R_f \) value with the bluish white fluorescent spot from the standard solution.

**Loss on drying** \( \times \) Not more than 12.0% (6 hours).

**Total ash** \( \times \) Not more than 17.0%.

**Acid-insoluble ash** \( \times \) Not more than 6.0%.

**Extract content** \( \times \) Dilute ethanol-soluble extract: not less than 8.0%.

**Containers and storage** Containers—Well-closed containers.

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**Cnidium Rhizome**

**Cnidii Rhizoma**

センキュウ

Cnidium Rhizome is the rhizome of Cnidium officinale Makino (Umbelliferae), usually passed through hot water.
Description  Irregular massive rhizome, occasionally cut lengthwise; 5 – 10 cm in length, and 3 – 5 cm in diameter; externally grayish brown to dark brown, with gathered nodes, and with knobbed protrusions on the node; margin of the vertical section irregularly branched; internally grayish white to grayish brown, translucent and occasionally with hollows; dense and hard in texture.

Odor, characteristic; taste, slightly bitter.

Under a microscope <5.01>, a transverse section reveals cortex and pith with scattered oil canals; in the xylem, thick-walled and lignified xylem fibers appear in groups of various sizes; starch grains usually gelatinized, but rarely remaining as grains of 5 – 25 \( \mu m \) in diameter; crystals of calcium oxalate not observable.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Cnidium Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Cnidium Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Containers and storage  Containers—Well-closed containers.

Powdered Cnidium Rhizome

Cnidii Rhizoma Pulveratum

センキュウ末

Powdered Cnidium Rhizome is the powder of Cnidium Rhizome.

Description  Powdered Cnidium Rhizome occurs as a gray to light grayish brown powder. It has a characteristic odor and a slightly bitter taste.

Under a microscope <5.01>, Powdered Cnidium Rhizome reveals colorless and gelatinized starch masses, and fragments of parenchyma containing them; fragments of scalariform and reticulate vessels 15 – 30 \( \mu m \) in diameter; fragments of thick-walled and lignified xylem fibers 20 – 60 \( \mu m \) in diameter; fragments of yellow brown cork tissue; fragments of secretory tissue.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Cnidium Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Cnidium Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Cnidium Rhizome does not contain a large quantity of starch grains, stone cells, crystals of calcium oxalate or other foreign matter.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Containers and storage  Containers—Well-closed containers.

Coix Seed

Coicis Semen Pulveratum

ヨクイニン

Coix Seed is the seed of Coix lachryma-jobi Linné var. mayuen Stapf (Gramineae), from which the seed coat has been removed.

Description  Ovoid or broad ovoid seed, about 6 mm in length, and about 5 mm in width; with a slightly hollowed apex and base; dorsal side distended; ventral side longitudinally and deeply furrowed in the center; dorsal side mostly white in color and powdery; in the furrow on the ventral surface, attached brown, membranous pericarp and seed coat. Under a magnifying glass, the cross section reveals light yellow scutellum in the hollow of the ventral side. Hard in texture.

Odor, slight; taste, slightly sweet; adheres to the teeth on chewing.

Identification  To a cross-section of Coix Seed add iodine TS dropwise: a dark red-brown color develops in the endosperm, and a dark gray color develops in the scutellum.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 3.0%.

Containers and storage  Containers—Well-closed containers.

Powdered Coix Seed

Coicis Semen Pulveratum

ヨクイニン末

Powdered Coix Seed is the powder of Coix Seed.

Description  Powdered Coix Seed occurs as a brownish, grayish white to grayish yellow-white powder, and has a slight odor and a slightly sweet taste.

Under a microscope <5.01>, Powdered Coix Seed reveals starch grains, and fragments of endosperm containing them; fragments of tissue accompanied with epidermal cells of pericarp composed of yellowish and oblong cells, and fragments of parenchyma cells containing fixed oil, aleurone grains and starch grains; a very few fragments of spiral vessels. Starch grains are simple and 2-compound grains, simple grain nearly equidiameter to obtuse polygon, 10 – 20 \( \mu m \) in diameter, and have a stellate cleft-like hilum in the center. Spherical starch grains, coexisting with aleurone grains, are spherical simple grains, 3 – 7 \( \mu m \) in diameter.

Identification  Place a small amount of Powdered Coix Seed on a slide glass, add dropwise iodine TS, and examine under a microscope <5.01>: nearly equidiameter and obtuse polygonal simple starch grains, usually 10 – 15 \( \mu m \) in diame-
Condurango

Condurango Cortex

Condurango is the bark of the trunk of Marsdenia cundurango Reichenbach filius (Asclepiadaceae).

Description Tubular or semi-tubular pieces of bark, 0.1 – 0.6 cm in thickness, 4 – 15 cm in length; outer surface grayish brown to dark brown, nearly smooth and with numerous lenticels, or more or less scaly and rough; inner surface light grayish brown and longitudinally striate; fractured surface fibrous on the outer region and generally granular in the inner region.

Odor, slight; taste, bitter.

Under a microscope, a transverse section reveals a cork layer composed of several layers of thin-walled cells; primary cortex with numerous stone cell groups; secondary cortex with phloem fiber bundles scattered inside the starch sheath consisting of one-cellular layer; articulate latex tubes scattered in both cortices; parenchyma cells containing starch grains or rosette aggregates of calcium oxalate; starch grain 3 – 20 μm in diameter.

Identification Digest 1 g of pulverized Condurango in 5 mL of water, and filter: the clear filtrate becomes turbid on heating, but becomes clear again upon cooling.

Purity Foreign matter — The xylem and other foreign matter contained in Condurango do not exceed 2.0%.

Total ash <5.0% Not more than 12.0%.

Containers and storage Containers—Tight containers.

Condurango Fluidextract

Condurango Fluidextract is a brown liquid. It has a characteristic odor and a bitter taste.

Identification Mix 1 mL of Condurango Fluidextract with 5 mL of water, filter, if necessary, and heat the clear solution: turbidity is produced. However, it becomes almost clear upon cooling.

Purity Heavy metals <1.0% —Prepare the test solution with 1.0 g of Condurango Fluidextract as direct in the Fluidextracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

Containers and storage Containers—Tight containers.

Coptis Rhizome

Coptidis Rhizoma

Coptis Rhizome is the rhizome of Coptis japonica Makino, Coptis chinensis Franchet, Coptis deltoidea C.Y. Cheng et Hsiao or Coptis teeta Wallich (Ranunculaceae), from which the roots have been removed practically.

It contains not less than 4.2% of berberine [as berberine chloride (C_{20}H_{18}ClNO_{4}: 371.81)], calculated on the basis of dried material.

Description Irregular, cylindrical rhizome, 2 – 4 cm, rarely up to 10 cm in length, 0.2 – 0.7 cm in diameter, slightly curved and often branched; externally grayish yellow-brown, with ring nodes, and with numerous remains of rootlets; generally remains of petiole at one end; fractured surface rather fibrous; cork layer light grayish brown, cortex and pith yellowish brown; reddish yellow-brown, xylem is yellow to reddish yellow in color.

Odor, slight; taste, extremely bitter and lasting; it colors the saliva yellow on chewing.

Under a microscope, a transverse section of Coptis Rhizome reveals a cork layer composed of thin-walled cork cells; cortex parenchyma usually exhibiting groups of stone cells near the cork layer and yellow phloem fibers near the cambium; xylem consisting chiefly of vessels, tracheae and wood fibers; medullary ray distinct; pith large; in pith, stone cells or stone cells with thick and lignified cells are sometimes recognized; parenchyma cells contain minute starch grains.

Identification (1) To 0.5 g of pulverized Coptis Rhizome add 10 mL of water, allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid and 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) To 0.5 g of pulverized Coptis Rhizome add 20 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS in 1 mL of methanol, and use this solution as
JP XVI

the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100:7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot from the standard solution with yellow to yellow-green fluorescence show the same color tone and the same $R_f$ value.

Purity Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Coptis Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <5.01> Not more than 11.0% (6 hours).

Total ash <5.01> Not more than 4.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Assay Weigh accurately about 0.5 g of pulverized Coptis Rhizome, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (previously determine the water <2.48> in the same manner as Berberine Chloride Hydrate), dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of berberine.

Amount (mg) of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$)]

$$M_S \times A_T / A_S$$

$M_S$: Amount (mg) of Berberine Chloride RS, calculated on the anhydrous basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 345 nm).
Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).
Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.
Selection of column: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in 10 mL of methanol. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly dividing each peak.
System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of berberine is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Coptis Rhizome

Coptidis Rhizoma Pulveratum

Powdered Coptis Rhizome is the powder of Coptis Rhizome.

It contains not less than 4.2% of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$): 371.81], calculated on the basis of dried material.

Description Powdered Coptis Rhizome occurs as a yellow-brown to grayish yellow-brown powder. It has a slight odor and an extremely bitter, lasting taste, and colors the saliva yellow on chewing.

Under a microscope <5.01>, almost all elements are yellow in color; it reveals mainly fragments of vessels, tracheids and xylem fibers; parenchyma cells containing starch grains; polygonal cork cells. Usually, round to obtuse polygonal stone cells and their groups, and phloem fibers, 10 – 20 μm in diameter, and fragments of their bundles. Sometimes, polygonal and elongated epidermal cells, originated from the petiole, having characteristically thickened membranes. Starch grains are single grains 1 – 7 μm in diameter.

Identification (1) To 0.5 g of Powdered Coptis Rhizome add 10 mL of water, allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid and 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) To 0.5 g of Powdered Coptis Rhizome add 20 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Purity (1) Phellodendron bark—Under a microscope <5.01>, crystal cell rows or mucilage masses are not observable. Stir 0.5 g of Powdered Coptis Rhizome with 2 mL of water: the solution does not become gelatinous.

(2) Curcuma—Place Powdered Coptis Rhizome on a filter paper, drop diethyl ether on it, and allow to stand. Remove the powder from the filter paper, and drop 1 drop of potassium hydroxide TS: no red-purple color develops. Under a microscope <5.01>, Powdered Coptis Rhizome does...
not contain gelatinized starch or secretory cells containing yellow-red resin.

3. Arsenic - Prepare the test solution with 0.40 g of Powdered Coptis Rhizome according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying** <5.00% Not more than 11.0% (6 hours).

**Total ash** <5.00% Not more than 4.0%.

**Acid-insoluble ash** <5.00% Not more than 1.0%.

**Assay** Weigh accurately about 0.5 g of Powdered Coptis Rhizome, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (previously determined the loss on drying), add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (previously determined the loss on drying), add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution.

**Identification** To 1 g of coarse cuttings of Cornus Fruit add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01> according to the following conditions, and determine the peak areas, A1 and A5, of berberine.

\[
M_S = M_5 \times \frac{A_7}{A_3}
\]

**Containers and storage** Containers—Well-closed containers.

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**Cornus Fruit**

**Cori Fructus**

Cornus Fruit is the pulp of the pseudocarp of *Cornus officinalis* Siebold et Zuccarini (*Cornaceae*). It contains not less than 0.4% of loganin, calculated on the basis of dry material.

**Description** Flattened oblong, 1.5 – 2 cm in length, about 1 cm in width; externally dark red-purple to dark purple, lustrous, and with coarse wrinkles; a crack-like scar formed by removal of true fruit; a scar of calyx at one end, and a scar of peduncle at the other; soft in texture.

Odor, slight; taste, acid and slightly sweet.

**Identification** To 1 g of coarse cuttings of Cornus Fruit add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxy-benzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spots from the sample solution is the same with a red-purple spot from the standard solution in color tone and RI value.

**Purity (1)** Foreign matter <5.00%—The amount of its peduncles and other foreign matter contained in Cornus Fruit does no exceed 2.0%.

(2) Total BHC's and total DDT's <5.00%—Not more than 0.2 ppm, respectively.

**Extract content** <5.00% Dilute ethanol-soluble extract: not less than 35.0%.

**Assay** Weigh accurately about 1 g of fine cuttings of Cornus Fruit (separately determine the loss on drying), put in a glass-stoppered centrifuge tube, suspend in 30 mL of diluted methanol (1 in 2), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 30 mL of diluted methanol (1 in 2), and repeat the above process twice more. Combine all the extracts, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of loganin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A1 and A5, of loganin.

\[
M_S = M_5 \times \frac{A_7}{A_3}
\]
**Corydalis Tuber**

**Corydalis Tuber**

エンゴサク

Corydalis Tuber is the tuber of *Corydalis turtschaninovii* Besser forma *yanhusuo* Y. H. Chou et C. C. Hsu (*Papaveraceae*).

It contains not less than 0.08% of dehydrocorydaline (as dehydrocorydaline nitrate), calculated on the basis of dried material.

**Description**

Nearly flattened spherical, 1–2 cm in diameter, and with stem scar at one end; externally grayish yellow to grayish brown; hard in texture; fractured surface is yellow and smooth or grayish yellow-green in color and granular.

Almost odorless; taste, bitter.

**Identification**

To 2 g of pulverized Corydalis Tuber add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of methanol, a solution of ammonium acetate (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a yellow-green fluorescent spot at around Rf value 0.4 and a yellow fluorescent spot at around Rf value 0.35 appear. When spray evenly Dragendorff’s TS for spraying on the plate, air-dry, and then spray sodium nitrite TS: a brown spot appears at an Rf value of about 0.6.

**Purity**

1. Heavy metals

Proceed with 3.0 g of pulverized Corydalis Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

2. Arsenic

Prepare the test solution with 0.40 g of pulverized Corydalis Tuber according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying**

Not more than 15.0%.

**Total ash**

Not more than 3.0%.

**Assay**

Weigh accurately about 1 g of powdered Corydalis Tuber, add 30 mL of a mixture of methanol and dilute hydrochloric acid (3:1), heat under a reflux condenser on a water bath for 30 minutes, and filter after cooling. To the residue add 15 mL of a mixture of methanol and dilute hydrochloric acid (3:1), and repeat the above procedure. Combine the filtrates so obtained, add a mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of dehydrocorydaline nitrate for assay; previously dried in a desiccator (silica gel) for not less than 1 hour, dissolve in a mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL of each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A T and A S, of dehydrocorydaline.

Amount (mg) of dehydrocorydaline [as dehydrocorydaline nitrate (C22H24N2O7)]

\[ M_S = A_T / A_S \times 1/4 \]

M S: Amount (mg) of dehydrocorydaline nitrate for assay

**Operating conditions**


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: Adjust the flow rate so that the retention time of loganin is about 25 minutes.

**System suitability**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry of the peak f value 0.35 appear. When spray evenly Dragendorff’s TS for spraying on the plate, air-dry, and then spray sodium nitrite TS: a brown spot appears at an Rf value of about 0.4 and a yellow fluorescent spot at around Rf value 0.35 appear. When spray evenly Dragendorff’s TS for spraying on the plate, air-dry, and then spray sodium nitrite TS: a brown spot appears at an Rf value of about 0.6.

**Containers and storage**

Containers—Well-closed containers.
Powdered Corydalis Tuber

Corydalis Tuber Pulveratum

Powdered Corydalis Tuber is the powder of Corydalis Tuber.

It contains not less than 0.08% of dehydrocorydaline (as dehydrocorydaline nitrate), calculated on the basis of dried material.

**Description** Powdered Corydalis Tuber occurs as a greenish yellow to grayish yellow powder. Almost odorless; taste, bitter.

Under a microscope \(<0.01\), Powdered Corydalis Tuber reveals mainly, masses of gelatinized starch or light yellow to colorless parenchymatous cells containing starch grains, fragments of cork layers, light yellow stone cells, selerenchymatous cells, reticulate vessels, spiral vessels and ring vessels; starch grains observed simple grains and 2- to 3-compound grains.

**Identification** To 2 g of Powdered Corydalis Tuber add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography \(<2.07\). Spot 10 \(\mu\)L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of methanol, ammonium acetate solution (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a yellow-green fluorescent spot and a yellow fluorescent spot appear at around \(R_f\) value 0.4 and at around \(R_f\) value 0.35, respectively. Separately, spray evenly Dragendorff's TS for spraying on the plate, air-dry, and then spray sodium nitrite TS: a brown spot appears at an \(R_f\) value of about 0.6.

**Purity** (1) Heavy metals \(<1.07\)—Proceed with 3.0 g of Powdered Corydalis Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.11\)—Prepare the test solution with 0.40 g of Powdered Corydalis Tuber according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying** \(<5.01\) Not more than 15.0%.

**Total ash** \(<5.01\) Not more than 3.0%.

**Assay** Weigh accurately about 1 g of Powdered Corydalis Tuber, add 30 mL of a mixture of methanol and dilute hydrochloric acid (3:1), heat under a reflux condenser on a water bath for 30 minutes, and filter after cooling. To the residue add 15 mL of the mixture of methanol and dilute hydrochloric acid (3:1), and proceed in the same way as above. Combine the filtrate, add the mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of dehydrocorydaline nitrate for assay, previously dried in a desiccator (silica gel) for not less than 1 hour, dissolve in the mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.00\) according to the following conditions, and determine the peak areas, \(A_\text{T}\) and \(A_\text{S}\), of dehydrocorydaline.

Amount (mg) of dehydrocorydaline [as dehydrocorydaline nitrate \([\text{C}_22\text{H}_{24}\text{N}_2\text{O}_7]\)]

\[\text{Amount} = M_S \times \frac{A_\text{T}}{A_\text{S}} \times \frac{1}{4}\]

\(M_S\): Amount (mg) of dehydrocorydaline nitrate for assay

**Operating conditions**


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 17.91 g of disodium hydrogen phosphate decahydrate in 970 mL of water, and adjust to pH 2.2 with phosphoric acid. Dissolve 14.05 g of sodium perchlorate monohydrate in this solution, and add water to make exactly 1000 mL. Add 450 mL of acetonitrile, and dissolve 0.20 g of sodium lauryl sulfate in this solution.

Flow rate: Adjust the flow rate so that the retention time of dehydrocorydaline is about 24 minutes.

**System suitability**—

System performance: Dissolve 1 mg of dehydrocorydaline nitrate for assay and 1 mg of berberine chloride in 20 mL of a mixture of water and acetonitrile (20:9). When the procedure is run with 5 \(\mu\)L of this solution under the above operating conditions, berberine and dehydrocorydaline are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dehydrocorydaline is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

Crataegeus Fruit

Crataege Fructus

Crataegeus Fruit is the pseudocarp of 1) *Crataegus cuneata* Siebold et Zuccarini or 2) *Crataegus pinnatifida* Bunge var. major N. E. Brown (Rosaceae) without any treatment or cut crosswise or lengthwise.

**Description**

1) *Crataegus cuneata* Siebold et Zuccarini Nearly spherical fruits, 8 - 14 mm in diameter; externally yellow-brown to grayish brown, with fine reticulated wrinkles, remained dent of 4 - 6 mm in diameter at one end, often the base of calyx around the dent, short peduncle or scar at the other end. True fruits, usually five loculus, often split five,
Cyperus Rhizome

Identification
To 1 g of pulverized Crataegus Fruit add 5 mL of methanol, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of hyperoside for thin-layer chromatography in 20 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at a distance of about 10 cm, and air-dry with a mixture of ethyl acetate, 2-butanone, water and formic acid for thin-layer chromatography, develop the plate of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate of the sample solution and standard solution.

Loss on drying <5.01> Not more than 17.0%.

Total ash <5.01> Not more than 4.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 8.0%.

Containers and storage Containers—Well-closed containers.

Cyperus Rhizome is the rhizome of Cyperus rotundus Linné (Cyperaceae).

Description Fluoriform rhizome, 1.5 – 2.5 cm in length, 0.5 – 1 cm in diameter; externally grayish brown to grayish blackish brown, with 5 to 8 irregular ring nodes, and with hair-like fiber bundles on each node; hard in texture. The transverse section red-brown to light yellow in color, with waxy luster; thickness of cortex approximately equal to or slightly smaller than the diameter of stele. Under a magnifying glass, a transverse section reveals fiber bundles as brown spots lined in rings along circumference; here and there in the cortex, vascular bundles appear as red-brown spots, and numerous secretory cells scattered as minute yellow-brown spots; in the stele, numerous vascular bundles scattered as spots or lines.

Characteristic odor and taste.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Cyperus Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Cyperus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 3.0%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Cyperus Rhizome, provided that 1 mL of silicon resin is previously added on the sample in the flask: the volume of essential oil is not less than 0.3 mL.

Containers and storage Containers—Well-closed containers.

Powdered Cyperus Rhizome

Cyperi Rhizoma Pulveratum

コウブシ末

Powdered Cyperus Rhizome is the powder of Cyperus Rhizome.

Description Powdered Cyperus Rhizome occurs as a light red-brown powder, and has a characteristic odor and taste. Under a microscope 5.01> Powdered Cyperus Rhizome reveals fragments of polygonal parenchyma cells, scalariform vessels, and seta-like fibers; a large quantity of starch, mostly gelatinized; an extremely small number of stone cells.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Cyperus Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).
Daiokanzoto Extract / Crude Drugs

(2) Arsenic

Prepare the test solution with 0.40 g of Powdered Cyperus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope, Powdered Cyperus Rhizome does not show extremely lignified cells, except stone cells, and crystals.

Total ash <5.0%

Acid-insoluble ash <5.0%

Not more than 3.0%.

Essential oil content

Perform the test with 50.0 g of Powdered Cyperus Rhizome provided that 1 mL of silicon resin is previously added on the sample in the flask: the volume of essential oil is not less than 0.2 mL.

Containers and storage—Tight containers.

Daiokanzoto Extract

大黄甘草湯エキス

Daiokanzoto Extract contains not less than 3.5 mg of sennoside A (C42H38O20: 862.74), and not less than 9 mg and not more than 27 mg (for preparation prescribed 1 g of Glycyrrhiza) or not less than 18 mg and not more than 54 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid (C42H62O16: 822.93), per the extract prepared as directed in the Method of preparation.

Method of preparation

<table>
<thead>
<tr>
<th>1)</th>
<th>2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhubarb</td>
<td>4 g</td>
</tr>
<tr>
<td>Glycyrrhiza</td>
<td>1 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description

Daiokanzoto Extract occurs as a brown powder. It has a characteristic odor and an astringent first then slightly sweet taste.

Identification

(1) To 1.0 g of Daiokanzoto Extract add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 1 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard (Glycyrrhiza).

Purity

(1) Heavy metals

Prepare the test solution with 1.0 g of Daiokanzoto Extract as directed in Extract (4), and perform the test (not more than 30 ppm).

(2) Arsenic

Prepare the test solution with 0.67 g of Daiokanzoto Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying

Not more than 7.0% (1 g, 105°C, 5 hours).

Total ash <5.0%

Not more than 10.0%.

Assay

(1) Sennoside A—Weigh accurately about 0.2 g of Daiokanzoto Extract, add 20 mL of ethyl acetate and 10 mL of water, shake for 10 minutes, centrifuge, and remove the upper layer. To the water layer add 20 mL of ethyl acetate, shake for 10 minutes, centrifuge, and remove the upper layer. To the water layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of Sennoside A RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A1, and A5, of sennoside A in each solution.

\[
M_5 = M_5 \times A_1 / A_5 \times 1 / 4
\]

\[M_5\]: Amount (mg) of Sennoside A RS, calculated on the anhydrous basis

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (2460:540:1).

Flow rate: 1.0 mL/min (the retention time of sennoside A is about 14 minutes.)

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry
factor of the peak of sennoside A are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

2. Glycyrrhizic acid—Use the sample solution obtained in the Assay (1) as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately dehydrated and not more than 1.5% water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Thin-layer Chromatography (2.01) according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of glycyrrhizic acid in each solution.

\[
\text{Amount (mg) of glycyrrhizic acid (C}{}_{12}\text{H}{}_{6}\text{O}_{16}) = M_S \times \frac{A_T}{A_S} \times \frac{1}{2}
\]

$M_S$: Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

**Containers and storage**

Containers—Tight containers.

---

### Digenea

#### Description

Digenea is the whole algae of *Digenea simplex* C. Agardh (*Rhodomelaceae*).

**Odor, seaweed-like; taste, disagreeable and slightly salty.**

**Identification**

To 5 g of Digenea add 50 mL of water, macerate between 50°C and 60°C for 1 hour, and filter while warm. Add 50 mL of water to the residue, macerate again between 50°C and 60°C for 1 hour, and filter while warm. Evaporate all the filtrate on a water bath to about 25 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of kainic acid in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.01). Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the upper layer of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a water-saturated solution of ninhydrin in 1-butanol (1 in 500) upon the plate, and heat at 90°C for 10 minutes: the spots obtained from the sample solution and the standard solution show a light yellow color and the same *Rf* values.

**Purity**

Foreign matter (5.01)—The amount of other algae in Digenea does not exceed 20.0%.

**Loss on drying** (5.01)—Not more than 22.0%.

**Acid-insoluble ash** (5.01)—Not more than 8.0%.

**Containers and storage**

Containers—Well-closed containers.

---

### Dioscorea Rhizome

#### Description

Dioscorea Rhizome is the rhizome (rhizophore) of *Dioscorea japonica* Thunberg or *Dioscorea batatas* Decaisne (*Dioscoreaceae*), from which the periderm has been removed.

**Identification**

1. To the cut surface of Dioscorea Rhizome add dilute iodine TS dropwise: a dark blue color develops.

2. To 0.2 g of pulverized Dioscorea Rhizome add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add 0.5 mL of sulfuric acid carefully to make two layers: a red-brown to purple-brown color appears at the zone of contact.

**Purity** (1)

Heavy metals (5.07)—Proceed with 3.0 g of pulverized Dioscorea Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic (5.11)—Prepare the test solution with 0.40 g of pulverized Dioscorea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying** (5.01)—Not more than 14.0% (6 hours).
Powdered Dioscorea Rhizome

Dioscoreae Rhizoma Pulveratum

Powdered Dioscorea Rhizome is the powder of Dioscorea Rhizome.

Description

Powdered Dioscorea Rhizome occurs as nearly yellowish white to white; odorless and tasteless.

Under a microscope, Dioscorea rhizome powder reveals starch grains; fragments of parenchyma cells containing starch grains; raphides of calcium oxalate, 100 to 200 μm in length and its containing mucilage cells; ring and scalariform vessels, 15 to 35 μm in diameter; starch grain isosceles deltoid or oblong, solitary, 18 to 35 μm, hilum and striation being distinct.

Identification

To 2 g of Powdered Dioscorea Rhizome add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add carefully 0.5 mL of sulfuric acid to make two layers: a red-brown to purple-brown color develops at the zone of contact.

Purity

(1) Heavy metals

Proceed with 3.0 g of Powdered Dioscorea Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic

Prepare the test solution with 0.40 g of Powdered Dioscorea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying

Not more than 14.0% (6 hours).

Total ash

Not more than 6.0%.

Acid-insoluble ash

Not more than 0.5%.

Containers and storage

Containers—Well-closed containers.

Dolichos Seed

Dolichi Semen

Dolichos Seed is the seed of Dolichos lablab Linné (Leguminosae).

Description

Flattened ellipsoidal to flattened orbicular-ovate seed, 9 – 14 mm in length, 6 – 10 mm in width, 4 – 7 mm in thickness; externally light yellowish white to light yellow, smooth and somewhat lustrous; caruncle white, like a half-moon, protrudent at one side; hard in texture.

Almost odorless; taste, slightly sweet and acid.

Under a microscope, a transverse section reveals the outermost layer of seed coat composed of a single layer of palisade like epidermal cells coated with cuticle; beneath epidermis a single layer of sclerenchymatous and sandglass like cells; inside of the layer mentioned above parenchyma lie, the innermost portion of the parenchyma decayed; cotyledons occur inside of the seed coat; the outermost layer of cotyledon composed of a single layer of epidermal cells, inner part of cotyledon mainly parenchyma, containing aleurone grains and oil drops, and occasionally starch grains.

Identification

Put about 3 g of pulverized Dolichos Seed in a centrifuge tube, add 30 mL of methanol, shake for 10 minutes, centrifuge, and take the supernatant liquid. Evaporate the solvent of the supernatant liquid, add 30 mL of water and 50 mL of ethyl acetate to the residue, shake, and take the ethyl acetate layer. To the ethyl acetate add 10 g of anhydrous sodium sulfate, shake, and filter. Evaporate the solvent of the filtrate, add 1 mL of ethyl acetate to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography (2.03). Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and acetic acid (100) (100:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a bluish white fluorescent spot appears at an Rf value of about 0.4.

Loss on drying

Not more than 14.0% (6 hours).

Total ash

Not more than 4.5%.

Extract content

Dilute ethanol-soluble extract: not less than 9.0%.

Containers and storage

Containers—Well-closed containers.

Eleutherococcus Senticosus Rhizome

Eleutherococci senticosi Rhizoma

Eleutherococcus Senticosus Rhizome is the rhizome of Eleutherococcus senticosus Maximowicz (Acanthopanax senticosus Harms) (Araliaceae), often with root.

Description

Slightly curved subcolumnar rhizome, 15 – 30 cm in length, 1 – 2.5 cm in diameter; externally grayish brown and slightly rough; transversely cut surface light brown, cortex thin, xylem thick with a pith in center; extremely hard in texture.

Odor, slightly characteristics; tasteless or slightly sweet, astringency.

Under a microscope, a transverse section reveals the outermost layer of cortex consisting of a cork layer 3 – 7 cells thick; oil canals scattered in parenchyma; fiber bundles lined stepwise in phloem; phloem and xylem separated clearly by cambium; xylem composed of vessels, xylem fibers and xylem parenchyma; ray composed of 2 – 6 rows of cells; pith composed of parenchyma; parenchyma of cortex and ray contain ag-
Ephedra Herb, when dried, contains not less than 0.7% of total alkaloids [as ephedrine (C_{10}H_{15}NO: 165.23) and pseudoephedrine (C_{10}H_{15}NO: 165.23)].

**Description** Thin cylindrical or ellipsoidal cylinder, 0.1 – 0.2 cm in diameter; 3 – 5 cm in length of internode; light green to yellow-green; numerous parallel vertical furrows on the surface; scaly leaves at the node portion; leaves, 0.2 – 0.4 cm in length, light brown to brown in color, usually being opposite at every node, adhering at the base to form a tubular sheath around the stem. Under a magnifying glass, the transverse section of the stem appears as circle and ellipse, the outer portion grayish green to yellow-green in color, and the center filled with a red-purple substance or hollow. When fractured at internode, the outer part is fibrous and easily split vertically.

Odor, slight; taste, astringent and slightly bitter, giving a slight sensation of numbness on the tongue.

**Identification** To about 0.5 g of pulverized Ephedra Herb add 10 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Liquid Chromatography (2.01) according to the following conditions: the peak corresponds to ephedrine B shows the same retention time with that obtained with the standard solution.

**Operating conditions**
- Detector: An ultraviolet absorption photometer (wavelength: 265 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeccylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 50°C.
- Mobile phase: A mixture of water and acetonitrile (9:1).
- Flow rate: Adjust the flow rate so that the retention time of ephedrine B is about 10 minutes.

**System suitability**
- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ephedrine B are not less than 5000 and not more than 1.5, respectively.

**Purity**
1. Heavy metals <1.01>—Proceed with 3.0 g of pulverized Ephedra Herb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).
2. Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Ephedra Herb according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying** <5.01> Not more than 13.0% (6 hours).

**Total ash** <5.01> Not more than 6.0%.

**Acid-insoluble ash** <5.01> Not more than 1.0%.

**Extract content** <5.01> Dilute ethanol-soluble extract: not less than 2.5%.

**Containers and storage** Containers—Well-closed containers.

**Ephedra Herb**

**Ephedrae Herba**

マオウ

Ephedra Herb is the terrestrial stem of Ephedra sinica Stapf, Ephedra intermedia Schrenk et C.A. Meyer or Ephedra equisetina Bunge (Ephedraceae).
Epimedium Herb

Epimedii Herba

Epimedium Herb is composed of a stem and a ternate to trinervate compound leaf; leaflet ovate to broadly ovate or ovate-lanceolate, 3–20 cm in length, 2–8 cm in width, petiolule 15–70 mm in length, apex of leaflet acuminate, needle hair on margin 0.1–0.2 cm in length, base of leaflet cordate to deeply cordate, lateral leaflet asymmetry; upper surface green to green-brown, sometimes lustrous, lower surface light green to grayish green-brown, often pilose, especially on vein densely pilose, papery or coriaceous; petiole and stem cylindrical, light yellowish brown to slightly purplish and light green-brown, easily broken.

Odor, slight; taste, slightly bitter.

Under a microscope <5.01>, a transverse section of the leaf reveals 3–6 vascular bundles in midvein; mesophyll composed of upper epidermis, single-layered palisade, spongy tissue and lower epidermis; leaf margins orbiculate or oblong, sclereenchymatous; multi-cellular hairs on epidermis; 8–20 vascular bundles in petiole and 6–15 vascular bundles in petiolule. Under a microscope <5.01>, a transverse section of the stem reveals a single to several-layered hypodermis, cortex of 4–10 layers of sclerenchymatous cells, vascular bundle 13–30 in number, oblong to obovate.

Identification

To 2 g of pulverized Epimedium Herb add 20 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of icarin in thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the dark purple spot from the standard solution.

Loss on drying <5.01> Not more than 12.5% (6 hours).

Total ash <5.01> Not more than 8.5%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 17.0%.

Eucommia Bark

Eucommiae Cortex

Eucommia Bark is the bark of Eucommia ulmoides Oliver (Eucommiaceae).

Description

Eucommia Bark is a semi-tubular or plate-like bark, 2–6 mm in thickness; externally pale grayish brown to grayish brown, and rough in texture, sometimes reddish-brown due to the cork layer falling off; internally dark violet, smooth and covered with a linear pattern that runs longitudinally, silk-like threads of gutta-percha (a thermoplastic rubber-like substance) appearing when broken.

It has a faint but characteristic odor and taste.

Under a microscope <5.01>, transverse section reveals parenchymatous cells containing gutta-percha; phloem with stone-cell and fiber layers; rays in rows of 2–3 cells; calcium oxalate crystals absent.

Identification

Put 1 g of pulverized Eucommia Bark in a glass-stoppered centrifuge tube, add 10 mL of water and 20 mL of diethyl ether, shake for 15 minutes, and centrifuge. Take the diethyl ether layer so obtained, evaporate the diethyl ether on a water bath, and add 1 mL of ethanol (99.5) to the residue: colloidal substances appear.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 8.0%.

Acid-insoluble ash <5.01> Not more than 5.0%.
Extract content  〈5.01〉 Dilute ethanol-soluble extract: not less than 7.0%.

Containers and storage Containers—Well-closed containers.

Euodia Fruit

Euodiae Fructus

ゴシュユ

Euodia Fruit is the fruit of Euodia ruticarpa Hooker filius et Thomson (Evodia rutaecarpa Bentham), Euodia officinalis Dode (Evodia officinalis Dode) or Euodia bodinieri Dode (Evodia bodinieri Dode).

Description Flattened spheroidal or globular fruit, 2 – 5 mm in diameter; externally dark brown to grayish brown, with many oil sacs appearing as hollow pits, and often with peduncle, 2 – 5 mm in length, covered densely with hairs; matured pericarp split to reveal five loculi, and each loculus containing obvoid or globular seeds of a lustrous brown to blackish brown or bluish black color.

Odor, characteristic; taste, acrid, followed by a lasting bitterness.

Identification To 1.0 g of pulverized Euodia Fruit add 20 mL of methanol, heat for 5 minutes on a water bath, cool, and filter. Evaporate the filtrate to dryness, add 3 mL of dilute acetic acid to the residue, warm for 2 minutes on a water bath, cool, and filter. Perform the following tests using the filtrate as the sample solution.

(1) Spot one drop of the sample solution on a filter paper, air-dry, spray Dragendorff’s TS for spraying, and allow to stand: a yellow-red color develops.

(2) To 0.2 mL of the sample solution add 0.8 mL of dilute acetic acid. To this solution add gently 2 mL of 4-dimethylaminobenzaldehyde TS, and warm in a water bath: a purple-brown ring develops at the zone of contact.

Purity (1) Peduncle—When perform the test of foreign matter 〈5.01〉, the amount of peduncles contained in Euodia Fruit does not exceed 3.0%.

(2) Foreign matter 〈5.01〉—The amount of foreign matter other than the peduncle contained in Euodia Fruit does not exceed 1.0%.

Total ash 〈5.01〉 Not more than 10.0%.

Acid-insoluble ash 〈5.01〉 Not more than 1.5%.

Essential oil content 〈5.01〉 Perform the test with 50.0 g of pulverized Euodia Fruit: the volume of essential oil is not less than 0.7 mL.

Containers and storage Containers—Well-closed containers.

Fennel

Foeniculi Fructus

ウイキョウ

Fennel is the fruit of Foeniculum vulgare Miller (Umbelliferae).

Description Cylindrical cremocarp, 3.5 – 8 mm in length, 1 – 2.5 mm in width; externally grayish yellow-green to grayish yellow; two mericarps closely attached with each other, and with five longitudinal ridges; cremocarp often with pedicel 2 – 10 mm in length.

Characteristic odor and taste. Under a microscope 〈5.01〉, ridges near the bentral side are far protruded than those on the dorsal side; one large oil canal between each ridge, and two oil canals on the bentral side.

Identification To 0.5 g of pulverized Fennel add 10 mL of hexane, allow to stand for 5 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography 〈2.03〉. Spot 5 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a main spot with a dark purple color appears at an RF value of about 0.4.

Purity (1) Peduncle—When perform the test of foreign matter 〈5.01〉, the amount of peduncles contained in Fennel does not exceed 3.0%.

(2) Foreign matter 〈5.01〉—The amount of foreign matter other than the peduncle contained in Fennel does not exceed 1.0%.

Total ash 〈5.01〉 Not more than 10.0%.

Essential oil content 〈5.01〉 Perform the test with 50.0 g of pulverized Fennel: the volume of essential oil is not less than 7.0 mL.

Containers and storage Containers—Well-closed containers.

Powdered Fennel

Foeniculi Fructus Pulveratus

ウイキョウ末

Powdered Fennel is the powder of Fennel.

Description Powdered Fennel occurs as a greenish light brown to greenish brown, and is a characteristic odor and taste.

Under a microscope 〈5.01〉, Powdered Fennel reveals fragments of parenchyma cells of perisperm containing aleurone grain, fragments of parenchyma cells of endosperm containing fatty oil, fragments of sclerenchyma with characteristic single pits, fragments of oil canal within yellow-brown material, fragments of endocarp shown scalariform, spiral vessels, epidermis, stomata.

Identification To 0.5 g of Powdered Fennel add 10 mL of hexane, allow to stand for 5 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography 〈2.03〉. Spot 5 μL of the sample solution on a plate prepared with silica gel with fluorescent indicator for thin-layer chromatography. Then develop the
Fennel Oil

Oleum Foeniculi

ウイキョウ油

Fennel Oil is the essential oil distilled with steam from the fruit of *Foeniculum vulgare* Miller (*Umbelliferae*) or of *Illicium verum* Hooker filius (*Illiciaceae*).

**Description** Fennel Oil is a colorless to pale yellow liquid. It has a characteristic, aromatic odor and a sweet taste with a slight, bitter aftertaste.

It is miscible with ethanol (95) and with diethyl ether.

It is practically insoluble in water.

When cold, white crystals or crystalline masses may often separate from the oil.

**Identification** Dissolve 0.30 g of Fennel Oil in 20 mL of hexane, pipet 1 mL of this solution, add hexane to make exactly 10 mL, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography.

Spot 5 μL of the sample solution on a plate of silica gel with fluorescent indicator for ultraviolet light (main wavelength: 254 nm); a main spot with dark purple color appears at an *Rf* value of about 0.4.

**Total ash** <5.01> Not more than 10.0%.

**Acid-insoluble ash** <5.01> Not more than 1.5%.

**Essential oil content** <5.01> Perform the test with 50.0 g of Powdered Fennel: the volume of essential oil is not less than 0.45 mL.

**Containers and storage** Containers—Tight containers.

Forsythia Fruit

Forsythiae Fructus

レンギョウ

Forsythia Fruit is the fruit of *Forsythia suspensa* Vahl or *Forsythia viridissima* Lindley (*Oleaceae*).

**Description** Ovoid to long ovoid capsule, 1.5 – 2.5 cm in length, 0.5 – 1 cm in width, with acute apex, and sometimes with a peduncle at the base; externally light gray to dark brown, scattered with light gray and small ridged dots, and with two longitudinal furrows; a capsule dehiscing along the longitudinal furrows has the apexes bent backward; the inner surface of dehisced pericarp is yellow-brown in color, with a longitudinal partition-wall in the middle; seeds, slender and oblong, 0.5 – 0.7 cm in length, and usually with a wing.

Odor, slight; tasteless.

**Identification** (1) To 0.2 g of pulverized Forsythia Fruit add 2 mL of acetic anhydride, shake well, allow to stand for 2 minutes, and filter. To 1 mL of the filtrate add gently 0.5 mL of sulfuric acid to form two layers: a red-purple color develops at the zone of contact.

(2) To 1 g of pulverized Forsythia Fruit add 10 mL of methanol, warm on a water bath for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbin form and 1 mL of hydrochloric acid, and allow to stand: a light red to yellow-red color develops.

**Purity** (1) Branchlet—When perform the test of foreign matter <5.01>, the amount of branchlets contained in Forsythia Fruit does not exceed 5.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than branchlets contained in Forsythia Fruit does not exceed 1.0%.

**Total ash** <5.01> Not more than 5.0%.

Foeniculated Ammonia Spirit

アンモニア・ウイキョウ精

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia Water</td>
<td>170 mL</td>
</tr>
<tr>
<td>Fennel Oil</td>
<td>30 mL</td>
</tr>
<tr>
<td>Ethanol</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Medicated Spirits, with the above ingredients. A sufficient quantity of ammonia solution (28) and Purified Water or Purified Water in Containers may be used in place of Ammonia Water.

**Description** Foeniculated Ammonia Spirit is a colorless to yellow liquid, having a characteristic odor. It has a slightly sweet, pungent taste.

Specific gravity *d*<sub>30</sub>: about 0.85

**Alcohol number** <1.01> Not less than 7.8 (Method 2).

**Containers and storage** Containers—Tight containers.
Fritillaria Bulb

**Fritillariae Bulbus**

バイモ

Fritillaria Bulb is the bulb of *Fritillaria verticillata* Willdenow var. *thunbergii* Baker (*Liliaceae*).

**Description**  Fritillaria Bulb is a depressed spherical bulb, 2–3 cm in diameter, 1–2 cm in height, consisting of 2 thickened scaly leaves often separated; externally and internally white to light yellow-brown in color; inside base is in a slightly dark color; the bulb sprinkled with lime before drying is dusted with white powder; fractured surface, white in color and powdery.

Odor, slight and characteristic; taste, bitter.

Under the microscope <5.01>, a transverse section reveals the outermost layer (epidermis) to be composed of a single layer of cells; numerous vascular bundles scattered throughout the parenchyma inside of the epidermis; parenchyma filled with starch grains; starch grains are mainly simple (rarely 2- to 3-compound), 5–50 μm in diameter, narrowly ovate to ovate or triangular to obovate, stratiform figure obvious; epidermal cells and parenchymatous cells near the vessels contain solitary crystals of calcium oxalate.

**Identification**  Put 2 g of pulverized Fritillaria Bulb in a glass-stoppered centrifuge tube, add 10 mL of ammonia TS and 20 mL of a mixture of ethyl acetate and diethyl ether (1:1), shake for 20 minutes, and centrifuge. Take the upper layer, add 20 g of anhydrous sodium sulfate to the layer, shake, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (17:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: two spots of a yellow-red color appear at Rf values of about 0.4 and at 0.6.

**Purity (1)**  Heavy metals <1.07>—Proceed with 3.0 g of pulverized Fritillaria Bulb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2)  Arsenic <1.17>—Prepare the test solution with 0.40 g of pulverized Fritillaria Bulb according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying** <5.01>  Not more than 16.0% (6 hours).

**Total ash** <5.01>  Not more than 6.5%.

**Acid-insoluble ash** <5.01>  Not more than 1.0%.

**Extract content** <5.01>  Dilute ethanol-soluble extract: not less than 8.0%.

**Containers and storage**  Containers—Well-closed containers.

---

**Gambir**

**Gambir**

アセンヤク

Gambir is the dried aqueous extract prepared from the leaves and young twigs of *Uncaria gambir* Roxburgh (*Rubiaceae*).

**Description**  Brown to dark brown, brittle mass; inside light brown.

Odor, slight; taste, extremely astringent and bitter.

**Identification (1)**  To 0.2 g of pulverized Gambir add 10 mL of water, warm in a water bath for 5 minutes with occasional shaking, and filter. Cool the filtrate, and add 2 to 3 drops of gelatin TS: a white turbidity or precipitate is produced.

(2)  Shake 0.1 g of pulverized Gambir with 20 mL of dilute ethanol for 2 minutes, and filter. Mix 1 mL of the filtrate with 9 mL of dilute ethanol, and to the solution add 1 mL of vanillin-hydrochloric acid TS: a light red to red-brown color develops.

**Total ash** <5.01>  Not more than 6.0%.

**Acid-insoluble ash** <5.01>  Not more than 1.5%.

**Extract content** <5.01>  Dilute ethanol-soluble extract: not less than 70.0%.

**Containers and storage**  Containers—Well-closed containers.

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**Powdered Gambir**

**Gambir Pulveratum**

アセンヤク末

Powdered Gambir is the powder of Gambir.

**Description**  Powdered Gambir occurs as a red-brown to dark brown powder. It has a slight odor, and an extremely astringent and bitter taste.

Under a microscope <5.01>, Powdered Gambir, immersed in olive oil or liquid paraffin, consists of needle crystalline masses or yellow-brown to red-brown angular fragments, and reveals epidermal tissue and thick-walled hairs.

**Identification (1)**  To 0.2 g of Powdered Gambir add 10 mL of water, warm in a water bath for 5 minutes with occasional shaking, and filter. Cool the filtrate, and add 2 to 3 drops of gelatin TS: a white turbidity or precipitate is produced.

(2)  Shake 0.1 g of Powdered Gambir with 20 mL of dilute ethanol for 2 minutes, and filter. Mix 1 mL of the filtrate with 9 mL of dilute ethanol, and to the solution add 1
**Gardenia Fruit**

*Gardeniae Fructus*

サンシシ

Gardenia Fruit is the fruit of *Gardenia jasminoides* Ellis (Rubiaceae). It contains not less than 3.0% of geniposide, calculated on the basis of dried material.

**Description**

Nearly long ovoid to ovoid fruit, 1–5 cm in length, 1–1.5 cm in width; usually having 6, rarely 5 or 7, markedly raised ridges; calyx or its scar at one end, and sometimes peduncle at the other end; inner surface of pericarp yellow-brown, smooth and lustrous; internally divided into two loculi, containing a mass of seeds in yellow-red to dark red placenta; seed nearly circular, flat, about 0.5 cm in major axis, blackish brown or yellow-red.

Odor, slight; taste, bitter.

**Identification** (1) To 1.0 g of pulverized Gardenia Fruit, previously dried in a desiccator (silica gel) for 24 hours, add 100 mL of hot water, warm the mixture between 60°C and 70°C for 30 minutes with frequent shaking, and filter after cooling. To 1.0 mL of the filtrate add water to make 10 mL: the color of the resulting solution is yellow and is not lighter than that of the following control solution.

Control solution: Dissolve 9.8 mg of carbazochrome sodium sulfonate trihydrate in water to make exactly 10 mL. Pipet 1 mL of this solution, and add water to make exactly 50 mL.

(2) To 1.0 g of pulverized Gardenia Fruit add 20 mL of methanol, warm for 3 minutes on a water bath, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography △2.01 according to the following conditions, and determine the peak areas of geniposide, A1, and A5, of both solutions.

\[
\text{Amount (mg) of geniposide} = M_5 \times \frac{A_T}{A_S} \times 2
\]

*M5*: Amount (mg) of geniposide for assay

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (22:3). Flow rate: Adjust the flow rate so that the retention time of geniposide is about 15 minutes.

**System suitability**

System performance: Dissolve 1 mg each of geniposide for assay and caffeine in methanol to make 15 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, caffeine and geniposide are eluted in this order with the resolution between these peaks being not less than 3.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

**Containers and storage**

Containers—Well-closed containers.

**Powdered Gardenia Fruit**

*Gardeniae Fructus Pulveratus*

サンシシ末

Powdered Gardenia Fruit is the powder of Gardenia Fruit. It contains not less than 3.0% of geniposide, calculated on the basis of dried material.

**Description**

Powdered Gardenia Fruit occurs as a yellow-brown powder, and has a slight odor and a bitter taste.

Under a microscope △5.01, Powdered Gardenia Fruit reveals fragments of yellow-brown epidermis consisting of polygonal epidermal cells in surface view; unicellular hairs,
spiral and ring vessels, stone cells often containing crystals of calcium oxalate; fragments of thin-walled parenchyma containing yellow pigments, oil drops and rosette aggregates of calcium oxalate (the above elements from fruit receptacle and pericarp); fragments of large and thick-walled epidermis of seed coat, containing a red-brown substance; fragments of endosperm filled with aleuron grains (the above elements from seed).

**Identification (1)** To 1.0 g of Powdered Gardenia Fruit, previously dried in a desiccator (silica gel) for 24 hours, add 100 mL of hot water, warm the mixture between 60°C and 70°C for 30 minutes with frequent shaking, and filter after cooling. To 1.0 mL of the filtrate add water to make 10 mL: the color of the resulting solution is yellow and is not lighter than that of the following control solution.

Control solution: Dissolve 9.8 mg of carbazochrome sodium sulfonate trihydrate in water to make exactly 10 mL. Pipet 1 mL of this solution, and add water to make exactly 50 mL.

(2) To 1.0 g of Powdered Gardenia Fruit add 20 mL of methanol, warm for 3 minutes on a water bath, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and methanol (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same in color tone and Rf value.

**Loss on drying** <5.01> Not more than 13.0%.

**Total ash** <5.01> Not more than 6.0%.

**Assay** Weigh accurately about 0.5 g of Powdered Gardenia Fruit, transfer into a glass-stoppered centrifuge tube, add 40 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and take the supernatant liquid. To the residue add 40 mL of diluted methanol (1 in 2), and repeat the same procedure as above. Combine the extracts so obtained, and add diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 20 mL, use this solution as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of geniposide, A₁, and A₃, of both solutions.

\[ \text{Amount (mg) of geniposide} = M_2 \times \frac{A_1}{A_3} \times 2 \]

\[ M_2: \text{Amount (mg) of geniposide for assay} \]

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

**Gastrodiae Tuber**

Gastrodiae Tuber is an irregularly curved and flattened cylindrical to flattened fusiform tuber, 5 – 15 cm in length, 2 – 5 cm in diameter, 1 – 2 cm in thickness; externally light yellow-brown to light yellowish white; with ring nodes, and irregular longitudinal wrinkles; hard in texture; fractured surface, dark brown to yellow-brown in color, with luster, horny and gluey.

Odor, characteristic; practically tasteless.

Under a microscope 5.01>, a transverse section reveals parenchyma cells containing needle raphides of calcium oxalate; starch grain absent.

**Identification** To 1 g of pulverized Gastrodia Tuber add 5 mL of methanol, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 1 minutes: a red-purple spot appears at an Rf value of about 0.4.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Gastrodia Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Gastrodia Tuber according to Method 4, and...
perform the test (not more than 5 ppm).

Loss on drying < 0.01 Not more than 16.0% (6 hours).

Total ash < 0.01 Not more than 4.0%.

Extract content < 0.01 Dilute ethanol-soluble extract: not less than 16.0%.

Containers and storage Containers—Well-closed containers.

Gentian

Gentianae Radix

ゲンチアナ

Gentian is the root and rhizome of Gentiana lutea Linné (Gentianaceae).

Description Nearly cylindrical pieces, 10 – 50 cm in length, 2 – 4 cm in diameter; externally dark brown; the rhizome short, with fine, transverse wrinkles, and sometimes with buds and remains of leaves at the upper edge. The root longitudinally and deeply wrinkled, and more or less twisted; fractured surface yellow-brown and not fibrous, and a cambium and its neighborhood tinged dark brown.

Odor, characteristic; taste, sweet at first, later persistently bitter.

Under a microscope < 0.01, a transverse section of the root reveals several layers of collenchyma adjoined internally to 4 to 6 layers of thin-walled cork; secondary cortex of the parenchyma with irregularly distributed phloem; xylem consisting chiefly of parenchyma, with individual or clustered vessels and tracheids, and exhibiting some sieve tubes of xylem; parenchyma of the xylem and the cortex containing oil droplets, minute needle crystals of calcium oxalate and very rarely starch grains 10 – 20 μm in diameter.

Identification (1) Place 0.1 g of pulverized Gentian, previously dried in a desiccator (silica gel) for 48 hours, on a slide glass, put a glass ring 10 mm in both inside diameter and in height on it, then cover with another slide, and heat gently and gradually: light yellow crystals are sublimed on the upper glass. The crystals are insoluble in water and in ethanol (95), and soluble in potassium hydroxide TS.

(2) To 0.5 g of Pulverized Gentian add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography < 2.03>. Spot 10 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same RF value.

Purity Arsenic < 0.11—Prepare the test solution with 0.40 g of pulverized Gentian according to Method 4, and perform the test (not more than 5 ppm).

Total ash < 0.01 Not more than 6.0%.

Acid-insoluble ash < 0.01 Not more than 3.0%.

Containers and storage Containers—Well-closed containers.

Powdered Gentian

Gentianae Radix Pulverata

ゲンチアナ末

Powdered Gentian is the powder of Gentian.

Description Powdered Gentian occurs as a yellow-brown powder, and has a characteristic odor. It has a sweet taste at first, which later becomes persistently bitter.

Under a microscope < 0.01, Powdered Gentian reveals parenchyma cells containing oil droplets and minute needle crystals, vessels, tracheids, cork tissues, and crystals of calcium oxalate. Vessels are chiefly reticulate vessels and scalariform vessels, 20 – 80 μm in diameter. Starch grains are observed very rarely, in simple grains about 10 – 20 μm in diameter.

Identification (1) Place 0.1 g of Powdered Gentian, previously dried in a desiccator (silica gel) for 48 hours, on a slide glass, put a glass ring 10 mm in both inside diameter and in height on it, then cover with another slide glass, and heat gently and gradually: light yellow crystals are sublimed on the upper glass. The crystals are insoluble in water and in ethanol (95), and soluble in potassium hydroxide TS.

(2) To 0.5 g of Powdered Gentian add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography < 2.03>. Spot 10 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same RF value.

Purity (1) Arsenic < 0.11—Prepare the test solution with 0.40 g of Powdered Gentian according to Method 4, and perform the test (not more than 5 ppm).

Total ash < 0.01 Not more than 6.0%.

Acid-insoluble ash < 0.01 Not more than 3.0%.

Containers and storage Containers—Tight containers.
Gentian and Sodium Bicarbonate Powder

ゲンチアナ・重曹散

Method of preparation

<table>
<thead>
<tr>
<th>Powdered Gentian</th>
<th>Sodium Bicarbonate</th>
<th>To make 1000 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 g</td>
<td>700 g</td>
<td></td>
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</tbody>
</table>

Prepare as directed under Powders, with the above ingredients.

Description  Gentian and Sodium Bicarbonate Powder occurs as a light yellow-brown powder, and has a bitter taste.

Identification  (1) To 2 g of Gentian and Sodium Bicarbonate Powder add 10 mL of water, stir, and filter: the filtrate responds to the Qualitative Tests <1.09> (1) for bicarbonate.

(2) To 1.5 g of Gentian and Sodium Bicarbonate Powder add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 5 μL of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same Rf value.

Containers and storage  Containers—Well-closed containers.

Geranium Herb

ゲンノショウコ

Geranium Herb is the terrestrial part of Geranium thunbergii Siebold et Zuccarini (Geraniaceae).

Description  Stem with leaves opposite; stem, slender and long, green-brown; stem and leaf covered with soft hairs; leaf divided palmately into 3 to 5 lobes, and 2 - 4 cm in length, grayish yellow-green to grayish brown; each lobe oblong to obovate, and its upper margin crenate.

Odor, slight; taste, astringent.

Identification  Boil 0.1 g of Geranium Herb with 10 mL of water, filter, and to the filtrate add 1 drop of iron (III) chloride TS: a blackish blue color develops.

Purity  Foreign matter—Under a microscope <5.01>, Powdered Geranium Herb reveals no stone cells.

Containers and storage  Containers—Well-closed containers.

Ginger

Zingiberis Rhizoma

ショウキョウ

Ginger is the rhizome of Zingiber officinale Roscoe (Zingiberaceae).

Description  Irregularly compressed and often branched massive rhizome or a part of it; the branched parts are slightly curved ovoid or oblong-ovoid, 2 - 4 cm in length, and 1 - 2 cm in diameter; external surface grayish white to
light grayish brown, and often with white powder; fractured surface is somewhat fibrous, powdery, light yellowish brown; under a magnifying glass, a transverse section reveals cortex and stele distinctly divided; vascular bundles and secretions scattered all over the surface as small dark brown dots.

Odor, characteristic; taste, extremely pungent.

**Identification** To 2 g of pulverized Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 10 µL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spots from the sample solution and a green spot from the standard solution show the same color tone and $R_f$ value.

**Purity** (1) Heavy metals $<1.07>$—Proceed with 3.0 g of pulverized Ginger according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic $<1.17>$—Prepare the test solution with 0.40 g of pulverized Ginger according to Method 4, and perform the test (not more than 5 ppm).

**Total ash** $<5.01>$ Not more than 8.0%.

**Containers and storage** Containers—Well-closed containers.

**Powdered Ginger**

*Zingiberis Rhizoma Pulveratum*

ショウキョウ末

Powdered Ginger is the powder of Ginger.

**Description** Powdered Ginger occurs as a light grayish brown to light grayish yellow powder. It has a characteristic odor and an extremely pungent taste.

Under a microscope $<5.01>$, Powdered Ginger reveals mainly starch grains and parenchyma cells containing them; also, parenchyma cells containing yellow-brown to dark brown resinous substances or single crystals of calcium oxalate; fragments of fibers with distinct pits; fragments of spiral, ring and reticulate vessels, and rarely fragments of cork tissue; starch grains composed of simple, compound or half-compound grains, spherical, ovoid or globular, with abaxial hilum, usually 20 – 30 µm in long axis.

**Identification** To 2 g of Powdered Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 10 µL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spots from the sample solution and a green spot from the standard solution show the same color tone and $R_f$ value.

**Purity** (1) Heavy metals $<1.07>$—Proceed with 3.0 g of Powdered Ginger according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic $<1.17>$—Prepare the test solution with 0.40 g of Powdered Ginger according to Method 4, and perform the test (not more than 5 ppm).

**Foreign matter**—Under a microscope $<5.01>$, Powdered Ginger does not show stone cells, lignified parenchyma cells and other foreign matter.

**Total ash** $<5.01>$ Not more than 8.0%.

**Containers and storage** Containers—Well-closed containers.

**Ginseng**

*Ginseng Radix*

ニンジン

Ginseng is the root of *Panax ginseng* C. A. Meyer (*Panax schinseng* Nees) (*Araliaceae*), from which rootlets have been removed, or the root that has been quickly passed through hot water.

It contains not less than 0.10% of ginsenoside *Rg*$_1$ (*C$_{48}$H$_{72}$O$_{14}$; 801.01) and not less than 0.20% of ginsenoside *Rb*$_1$ (*C$_{54}$H$_{92}$O$_{23}$; 1109.29), calculated on the basis of dried material.

**Description** Thin and long cylindrical to fusiform root, often branching 2 to 5 lateral roots from the middle; 5 – 20 cm in length, main root 0.5 – 3 cm in diameter; externally light yellow-brown to light grayish brown, with longitudinal wrinkles and scars of rootlets; sometimes crown somewhat constricted and with short remains of rhizome; fractured surface practically flat, light yellow-brown in color, and brown in the neighborhood of the cambium.

Odor, characteristic; taste, at first slightly sweet, followed by a slight bitterness.

**Identification** (1) On a section of Ginseng add dilute iodine TS dropwise: a dark blue color is produced on the surface.

(2) To 2.0 g of pulverized Ginseng add 10 mL of water and 10 mL of 1-butanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside *Rg*$_1$ for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 5 µL of the sample solution and 2 µL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and
water (14:5:4) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution has the same color tone and RF value with the spot from the standard solution.

**Purity (1)** Heavy metals $<1.07$—Proceed with 1.0 g of pulverized Ginseng according to Method 4, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 15 ppm).

(2) Arsenic $<1.17$—Prepare the test solution with 1.0 g of pulverized Ginseng according to Method 4, and perform the test (not more than 2 ppm).

(3) Foreign matter $<5.07$—The amount of stems and other foreign matter contained in Ginseng does not exceed 2.0%.

(4) Total BHC’s and total DDT’s $<5.07$—Not more than 0.2 ppm, respectively.

**Loss on drying $<5.07$** Not more than 14.0% (6 hours).

**Total ash $<5.07$** Not more than 4.2%.

**Extract content $<5.07$** Dilute ethanol-soluble extract: not less than 14.0%.

**Assay (1)** Ginsenoside Rg1—Weigh accurately about 1.0 g of pulverized Ginseng, put in a glass-stoppered centrifuge tube, add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the procedure with the residue using 15 mL of diluted methanol (3 in 5), combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of dilute sodium hydroxide diluted methanol (3 in 5), combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of the solution under the above operating conditions, ginsenoside Rb1 and ginsenoside Rc are eluted in this order with the respective peak areas, the relative standard deviation of the peak area of ginsenoside Rg1 is not more than 1.5%.

(2) Ginsenoside Rb1—Use the sample solution obtained in (1) as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb1 RS (previously determine the water), dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 mL of the sample solution and standard solution as directed under Liquid Chromatography $<2.07$ according to the following conditions, and determine the peak areas, $A1$ and $A5$, of ginsenoside Rg1.

$$M5 = M2 \times \frac{A5}{A1}$$

When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rg1 and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

**System repeatability** When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rg1 is not more than 1.5%.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of ginsenoside Rg1 is about 25 minutes.

**System suitability**

System performance: Dissolve 1 mg each of Ginsenoside Rg1 RS and ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rg1 and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

**Containers and storage** Containers—Well-closed containers.

### Powdered Ginseng

**Ginseng Radix Pulverata**

ニンジン末

Powdered Ginseng is the powder of Ginseng. It contains not less than 0.10% of ginsenoside Rg1 $(C_{34}H_{52}O_{23})$ 801.01 and not less than 0.20% of ginsenoside Rb1 $(C_{34}H_{52}O_{23})$ 1109.29, calculated on the basis of dried material.

**Description** Powdered Ginseng occurs as a light yellowish white to light yellowish-brown powder. It has characteristic
odor and is a slight sweet taste followed by a slight bitterness.

Under a microscope <5.01>, Powdered Ginseng reveals round to rectangular parenchyma cells containing starch grains, occasionally gelatinized starch, vessels, secretory cell, sclerenchyma cell, big and thin-walled cork cell; crystals of calcium oxalate and starch. Vessel are reticulate vessel, 45 μm in diameter; scalariform vessel and spiral vessel, 15 - 40 μm in diameter. Secretory cell containing a mass of yellow glistening contents; rosette aggregate of calcium oxalate, 20 - 50 μm in diameter, and 1 - 5 μm in diameter, rarely 10 μm, in diameter. Starch grains are observed in simple grain and 2 to 4-compound grain, simple grain, simple grain, 3 - 15 μm in diameter.

**Identification** To 2.0 g of Powdered Ginseng add 10 mL of water and 10 mL of l-butanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside Rg1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (14:5:4) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol solution between these peaks being not less than 3.

**Purity**

(1) Heavy metals <5.07>—Proceed with 1.0 g of Powdered Ginseng according to Method 4, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 15 ppm).

(2) Arsenic <5.11>—Prepare the test solution with 1.0 g of Powdered Ginseng according to Method 4, and perform the test (not more than 2 ppm).

(3) Total BHC’s and total DDT’s <5.01>—Not more than 0.2 ppm, respectively.

**Loss on drying** <5.01> Not more than 13.0% (6 hours).

**Total ash** <5.01> Not more than 4.2%.

**Acid-insoluble ash** <5.01> Not more than 0.5%.

**Extract content** <5.01> Dilute ethanol-soluble extract; not less than 14.0%.

**Assay**

Ginsenoside Rg1—Weigh accurately about 1.0 g of Powdered Ginseng, put in a glass-stoppered centrifuge tube, add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the procedure with the residue using 15 mL of diluted methanol (3 in 5), combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of dilute sodium hydroxide TS, allow to stand for 30 minutes, add 3 mL of 0.1 mol/L hydrochloric acid TS and diluted methanol (3 in 5) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rg1 RS (previously determine the water), dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of ginsenoside Rg1.

Amount (mg) of ginsenoside Rg1 $(C_{42}H_{72}O_{14})$

$$S = M_S \times \frac{A_T}{A_S}$$

**Operating conditions**

- **Detector**: An ultraviolet absorption photometer (wavelength: 203 nm).
- **Column**: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature**: A constant temperature of about 30°C.
- **Mobile phase**: A mixture of water and acetonitrile (7:3).
- **Flow rate**: Adjust the flow rate so that the retention time of ginsenoside Rb1 is about 25 minutes.

**System suitability**

- **System performance**; Dissolve 1 mg each of Ginsenoside Rg1 RS and ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rg1 and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

**System repeatability**: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rg1 is not more than 1.5%.

Ginsenoside Rb1—Use the sample solution obtained in (1) as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb1 RS (previously determined its water), dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of ginsenoside Rb1.

Amount (mg) of ginsenoside Rb1 $(C_{42}H_{72}O_{14})$

$$S = M_S \times \frac{A_T}{A_S}$$

**Operating conditions**

- **Detector**: An ultraviolet absorption photometer (wavelength: 203 nm).
- **Column**: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature**: A constant temperature of about 40°C.
- **Mobile phase**: A mixture of water and acetonitrile (7:3).
- **Flow rate**: Adjust the flow rate so that the retention time of ginsenoside Rb1 is about 20 minutes.

**System suitability**

- **System performance**; Dissolve 1 mg each of Ginsenoside Rb1 RS and ginsenoside Rc in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rb1 and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Рg1 is not more than 1.5%.

Containers and storage Containers—Tight containers.

Glehnia Root and Rhizome

Glehniae Radix cum Rhizoma

Glehnia Root and Rhizome is the root and rhizome of *Glehnia littoralis* Fr. Schmidt ex Miquel (*Umbelliferae*).

**Description** Cylindrical to long conical root or rhizome, 10 – 20 cm in length, 0.5 – 1.5 cm in diameter; externally light yellow-brown to red-brown. Rhizome short, with fine ring nodes; roots having longitudinal wrinkles and numerous, dark red-brown, warty protrusions or transversely elongated protuberances. Brittle and easily breakable. A transverse section white and powdery, and under a magnifying glass, oil canals scattered as brown dots.

Odor, slight; taste, slightly sweet.

**Purity (1)** Heavy metals <1.01>—Proceed with 3.0 g of pulverized Glehnia Root and Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Glehnia Root and Rhizome according to Method 4, and perform the test (not more than 5 ppm).

**Total ash**<5.01> Not more than 6.0%.

**Acid-insoluble ash**<5.01> Not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Glycyrrhiza

Glycyrrhizae Radix

Glycyrrhiza

Glycyrrhiza is the root and stolon, with (unpeeled) or without (peeled) the periderm, of *Glycyrrhiza uralensis* Fisher or *Glycyrrhiza glabra* Linné (*Leguminosae*).

It contains not less than 2.5% of glycyrrhizic acid (C_{42}H_{26}O_{16}: 822.93), calculated on the basis of dried material.

**Description** Nearly cylindrical pieces, 0.5 – 3.0 cm in diameter, over 1 m in length. Glycyrrhiza is externally dark brown to red-brown, longitudinally wrinkled, and often has lenticels, small buds and scaly leaves; peeled Glycyrrhiza is externally light yellow and fibrous. The transverse section reveals a rather clear border between phloem and xylem, and a radial structure which often has radiating splits; a pith in Glycyrrhiza originated from stolon, but no pith from root.

Odor, slight; taste, sweet.

Under a microscope <5.01>, the transverse section reveals several layers of yellow-brown cork layers, and 1- to 3-cellular layer of cork cortex inside the cork layer; the cortex exhibiting medullary rays and obliterated sieve portions radiated alternately; the phloem exhibiting groups of phloem fibers with thick but incompletely lignified walls and surrounded by crystal cells; peeled Glycyrrhiza some times lacks periderm and a part of phloem; the xylem exhibiting large yellow vessels and medullary rays in 3 to 10 rows radiated alternately; the vessels accompanied with xylem fibers surrounded by crystal cells, and with xylem parenchyma cells; the parenchymatous pithonly in Glycyrrhiza originated from stolon. The parenchyma cells contain starch grains and often solitary crystals of calcium oxalate.

**Identification** To 2 g of pulverized Glycyrrhiza add 10 mL of a mixture of ethanol (95) and water (7:3), heat by shaking on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of Glycyrrhizinic Acid RS in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the standard solution and a dark purple spot from the standard solution show the same color tone and the same Rf value.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Glycyrrhiza according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Glycyrrhiza according to Method 4, and perform the test (not more than 5 ppm).

**Total ash**<5.01> Not more than 6.0% (6 hours).

**Acid-insoluble ash**<5.01> Not more than 2.0%.

**Extract content**<5.01> Dilute ethanol-soluble extract: not less than 2.50%.

**Assay** Weigh accurately about 0.5 g of pulverized Glycyrrhiza in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Glycyrrhizinic Acid RS (previously determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 μL each of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following conditions. De-
termine the peak areas, $A_1$ and $A_s$, of glycyrrhizic acid of each solution.

\[
A = M_s \times \frac{A_s}{A_1}
\]

$M_s$: Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: Use a column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadeclysilanized silica gel for liquid chromatography (5 to 10 mL in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of glycyrrhizic acid is about 10 minutes.

**Selection of column:** Dissolve 5 mg of Glycyrrhizic Acid RS and 1 mg of propyl parahydroxybenzoate in dilute ethanol to make 20 mL. Proceed with 20 $\mu$L of this solution under the above operating conditions. Use a column giving elution of glycyrrhizic acid and propyl parahydroxybenzoate in this order, and clearly dividing each peak.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

**Thin-layer Chromatography**

**Purity (1)**

Heavy metals $<1.0\%$—Proceed with 3.0 g of Powdered Glycyrrhiza according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic $<1.1\%$—Prepare the test solution with 0.40 g of Powdered Glycyrrhiza according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope $<5.0\%$, Powdered Glycyrrhiza shows no stone cells.

(4) Total BHC’s and total DDT’s $<5.0\%$—Not more than 0.2 ppm, respectively.

**Loss on drying** $<5.0\%$—Not more than 12.0% (6 hours).

**Total ash** $<5.0\%$—Not more than 7.0%.

**Acid-insoluble ash** $<5.0\%$—Not more than 2.0%.

**Extract content** $<5.0\%$—Dilute ethanol-soluble extract: not less than 25.0%.

**Assay** Weigh accurately about 0.5 g of Powdered Glycyrrhiza in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 $\mu$L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same $Rf$ value.

**Pipette 20 $\mu$L of the sample solution, add dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Separately, dissolve 5 mg of Glycyrrhizic Acid RS in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.0\%$. Spot 2 $\mu$L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same $Rf$ value.
Glycyrrhiza Extract

Glycyrrhiza Extract contains not less than 4.5% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93).

Method of preparation To 1 kg of fine cuttings of Glycyrrhiza or the root and stolon of Glycyrrhiza glabra Linné (Leguminosae) which meets the requirement of Glycyrrhiza add 5 L of Water, Purified Water or Purified Water in Containers, and macerate for 2 days. Filter the macerated solution through a cloth filter. Add 3 L of Water, Purified Water or Purified Water in Containers to the residue, macerate again for 12 hours, and filter through a cloth filter. Evaporate the combined filtrates until the whole volume becomes 3 L. After cooling, add 1 L of Ethanol, and allow to stand in a cold place for 2 days. Filter, and evaporate the filtrate to form a viscous extract.

Description Glycyrrhiza Extract is a brown to blackish brown, viscous extract, and has a characteristic odor and a sweet taste. It dissolves in water, forming a clear solution, or with a slight turbidity.

Identification To 0.8 g of Glycyrrhiza Extract add 10 mL of a mixture of ethanol (95) and water (7:3), shake for 2 minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Identification under Glycyrrhiza.

Purity (1) Heavy metals

Prepare the test solution with 1.0 g of Glycyrrhiza Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Insoluble matter—Dissolve 2.0 g of Glycyrrhiza Extract in 18 mL of water, and filter. To 10 mL of the filtrate add 5 mL of ethanol (95): a clear solution results.

Assay Weigh accurately about 0.15 g of Glycyrrhiza Extract, place in a glass-stoppered centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about

20 mg of Glycyrrhizic Acid RS (priviously determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in Assay under Glycyrrhiza.

Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$) = $M_S \times A_I/A_S$

$M_S$: Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

Containers and storage Containers—Well-closed containers.

Crude Glycyrrhiza Extract

カンゾウ粗エキス

Glycyrrhiza Extract contains not less than 6.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93).

Method of preparation Boil coarse powder of Glycyrrhiza or the root and stolon of Glycyrrhiza glabra Linné (Leguminosae) which meets the requirement of Glycyrrhiza with Water, Purified Water or Purified Water in Containers, filter the solution under pressure, and evaporate the filtrate.

Description Crude Glycyrrhiza Extract occurs as lustrous, dark yellow-red to blackish brown plates, rods or masses. It is comparatively brittle when cold, and the fractured surface is dark yellow-red, shell-like, and lustrous. It softens when warmed. It has a characteristic odor and a sweet taste. It dissolves in water with turbidity.

Identification To 0.6 g of Crude Glycyrrhiza Extract add 10 mL of a mixture of 95% alcohol and water (7:3), dissolve by warming if necessary, cool, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Identification under Glycyrrhiza.

Purity (1) Heavy metals

Prepare the test solution with 1.0 g of Crude Glycyrrhiza Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Water-insoluble substances—Boil 5.0 g of pulverized Crude Glycyrrhiza Extract with 100 mL of water. After cooling, filter, the mixture through a tared filter paper, wash with water, and dry the residue at 105°C for 5 hours: the mass of the residue is not more than 1.25 g.

(3) Foreign matter—The filtrate obtained in (2) does not have a strong bitter taste.

(4) Starch—To about 1 g of pulverized Crude Glycyrrhiza Extract add water to make 20 mL, shake the mixture thoroughly, and filter. Examine the insoluble substance on the filter paper under a microscope: the residue contains no starch grains.

Total ash Not more than 12.0% (1 g).

Assay Weigh accurately about 0.15 g of Crude Glycyrrhiza Extract, place in a glass-stoppered centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the extracts, add dilute ethanol to make exactly 100 mL, and use this solution

Flow rate: Adjust the flow rate so that the retention time of glycyrrhizic acid is about 10 minutes.

Selection of column: Dissolve 5 mg of Glycyrrhizic Acid RS and 1 mg of propyl parahydroxybenzoate in dilute ethanol to make 20 mL. Proceed with 20 mL of this solution under the above operating conditions. Use a column giving elution of glycyrrhizic acid and propyl parahydroxybenzoate in this order, and clearly dividing each peak.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.
as the sample solution. Separately, weigh accurately about 20 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in Assay under Glycyrrhiza.

\[
M_s = \frac{M_5 \times A_1}{A_5} 
\]

\(M_5\): Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

Containers and storage  Containers—Tight containers.

Goshajinkigan Extract

牛車腎気丸エキス

Goshajinkigan Extract contains not less than 4 mg and not more than 16 mg of loganin, not less than 6 mg and not more than 18 mg of peoniflorin (C_{23}H_{28}O_{11}; 480.46), and not less than 0.2 mg (for 6 mg and not more than 18 mg of peoniflorin and not more than 16 mg of loganin, not less than

Containers and storage  Containers—Tight containers.

Goshajinkigan Extract

Method of preparation

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description  Goshajinkigan Extract occurs as brown to blackish brown powder or viscous extract. It has slightly a characteristic odor and an acid taste.

Identification  (1) To 1.0 g of the dry extract (or 3.0 g of the viscous extract), add 10 mL of water, shake, then add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 \(\mu\)L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool; a dark-green spot is observed at an \(R_f\) value of about 0.6 (Rehmannia Root).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \(\mu\)L of the sample solution and 2 \(\mu\)L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots from the sample solution has the same color tone and \(R_f\) value with the purple spot from the standard solution (Cornus Fruit).

(3) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of alisol A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 \(\mu\)L of the sample solution and 2 \(\mu\)L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and \(R_f\) value with the purple spot from the standard solution (Alisma Rhizome).

(4) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of paconol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 \(\mu\)L of the sample solution and 2 \(\mu\)L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and \(R_f\) value with the orange spot from the standard solution (Moutan Bark).

(5) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300 mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin. Connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added
to the graduated tube. After heating under reflux for 1 hour, separate 1 mL of the hexane layer, add 0.5 mL of sodium hydroxide TS, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (E)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spots among the several spots from the sample solution has the same color tone and RF value with the yellow-orange spot from the standard solution.

(ii) To 2.0 g of dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (E)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spots among several spots from the sample solution has the same color tone and RF value with the bluish white fluorescent spot from the standard solution.

(6) To 3.0 g of dry extract (or 9.0 g of the viscous extract), add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of the spots among the several spots from the sample solution has the same color tone and RF value with the yellow-brown spot from the standard solution (Powdered Processed Aconite Root).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 0.3 g of pulverized plantago seed for thin-layer chromatography, add 1 mL of methanol, warm on a water bath for 3 minutes, centrifuge after cooling, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spots among the several spots from the sample solution has the same color tone and RF value (around 0.3) with the deep blue spot from the standard solution (Plantago Seed).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 2 g of achyranthes root for thin-layer chromatography, add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate and heat at 105°C for 5 minutes: one of the spots among the several spots from the sample solution has the same color tone and RF value (around 0.4) with the dark red spot from the standard solution (Achyranthes Root).

**Purity (1)** Heavy metals <1.07>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed in the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hyaconitine and mesaconitine)—Weigh accurately 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonicrol (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonicrol (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hyaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to...
Goshajinkigan Extract / Crude Drugs

Loading on drying

- less than 5 hours.

Perform the test with a desiccator (silica gel) for not less than 24 hours, and dissolve accurately 10 mg of loganin for assay, previously dried in a diluted methanol (1 in 2), shake for 15 minutes, filter, and dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin RS (separately determine the water), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, A₁ and A₅, of peoniflorin in each solution.

Amount (mg) of peoniflorin (C₂₃H₂₈O₁₁): $M₅ × A₁/A₅ × 1/2$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of peoniflorin is about 9 minutes).

System performance—

When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.

(2) Peoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin RS (separately determine the water), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, A₁ and A₅, of peoniflorin in each solution.

Amount (mg) of peoniflorin (C₂₃H₂₈O₁₁): $M₅ × A₁/A₅ × 1/2$

M₅: Amount (mg) of Peoniflorin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of peoniflorin is about 9 minutes).

System suitability—

When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.

(3) Total alkaloids—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the super-

Assay (1) Loganin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of loganin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, A₁ and A₅, of loganin in each solution.

Amount (mg) of loganin = $M₅ × A₁/A₅ × 1/2$

M₅: Amount (mg) of loganin for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 minutes).

System suitability—

When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.

- aconitine, jesaconitine, hypaconitine and mesaconitine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphoric buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5 respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying <2.41> The dry extract: Not more than 9.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 9.0%, calculated on the dried basis.

Assay (1) Loganin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of loganin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, A₁ and A₅, of loganin in each solution.

Amount (mg) of loganin = $M₅ × A₁/A₅ × 1/2$
natant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and the aconitum monoester alkaloids standard solution TS for assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, \(A_{TM}\), \(A_{TH}\) and \(A_{SH}\), as well as \(A_{TA}\) and \(A_{SA}\), in each solution, respectively.

\[
\text{Amount (mg) of benzoylmesaconine hydrochloride} = C_{SM} \times \frac{A_{TM}}{A_{SM}} \times 10
\]

\[
\text{Amount (mg) of benzoylhypaconine hydrochloride} = C_{SH} \times \frac{A_{TH}}{A_{SH}} \times 10
\]

\[
\text{Amount (mg) of 14-anisoylaconine hydrochloride} = C_{SA} \times \frac{A_{TA}}{A_{SA}} \times 10
\]

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhypaconine; 254 nm for 14-anisoylaconine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of benzoylmesaconine is about 15 minutes).

**System suitability—**

System performance: When the procedure is run with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the relative standard deviation of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine is not more than 1.5%.

**Containers and storage**

Containers—Tight containers.

## Gypsum

### Gypsum Fibrosum

セッコウ

Gypsum is natural hydrous calcium sulfate. It possibly corresponds to the formula \(\text{CaSO}_4\cdot\text{2H}_2\text{O}\).

**Description**

Gypsum occurs as lustrous, white, heavy, fibrous, crystalline masses, which easily split into needles or very fine crystalline powder.

It is odorless and tasteless.

It is slightly soluble in water.

**Identification**

To 1 g of pulverized Gypsum add 20 mL of water, allow to stand with occasional shaking for 30 minutes, and filter: the filtrate responds to the Qualitative Tests <1.09> (2) and (3) for calcium salt and to the Qualitative Tests <1.09> for sulfate.

**Purity**

(1) Heavy metals <1.07>—Boil 4.0 g of pulverized Gypsum with 4 mL of acetic acid (100) and 96 mL of water for 10 minutes, cool, add water to make exactly 100 mL, and filter. Perform the test using 30 mL of the filtrate as the test solution. Prepare the control solution as follows: to 4.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Gypsum according to Method 2, and perform the test (not more than 5 ppm).

**Containers and storage**

Containers—Well-closed containers.

## Exsiccated Gypsum

焼セッコウ

Exsiccated Gypsum possibly corresponds to the formula \(\text{CaSO}_4\cdot\frac{1}{2}\text{H}_2\text{O}\).

**Description**

Exsiccated Gypsum occurs as a white to grayish white powder. It is odorless and tasteless. It is slightly soluble in water, and practically insoluble in ethanol (95).

It absorbs moisture slowly on standing in air to lose its solidifying property.

When it is heated to yield an anhydrous compound at a temperature above 200°C, it loses its solidifying property.

**Identification**

Shake 1 g of Exsiccated Gypsum with 20 mL of water for 5 minutes, and filter: the filtrate responds to the Qualitative Tests <1.09> (2) and (3) for calcium salt and to the Qualitative Tests <1.09> for sulfate.

**Purity**

Alkalinity—Take 3.0 g of Exsiccated Gypsum in a glass-stoppered test tube, add 10 mL of water and 1 drop of phenolphthalein TS, and shake vigorously: no red color develops.

**Solidification**

To 10.0 g of Exsiccated Gypsum add 10 mL of water, stir immediately for 3 minutes, and allow to stand: the period until water no longer separates, when the material
is pressed with a finger, is not more than 10 minutes from the time when the water was added.

**Containers and storage** Containers—Tight containers.

### Hachimijiogan Extract

<table>
<thead>
<tr>
<th>1)</th>
<th>2)</th>
<th>3)</th>
<th>4)</th>
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</thead>
<tbody>
<tr>
<td>Rehmannia Root</td>
<td>5 g</td>
<td>5 g</td>
<td>5 g</td>
</tr>
<tr>
<td>Cornus Fruit</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Dioscorea Rhizome</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Alisma Rhizome</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
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<tr>
<td>Poria Sclerotiun</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Moutan Bark</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Cinnamon Bark</td>
<td>1 g</td>
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</table>

**Description** Hachimijiogan Extract occurs as grayish brown to blackish brown powder or viscous extract. It has a characteristic odor and a slightly bitter and acid taste.

#### Method of preparation

**Identification** (1) To 1.0 g of the dry extract (or 3.0 g of the viscous extract), add 10 mL of water, shake, then add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography and RF value with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool; a dark-green spot is observed at an RF value of about 0.6 (Rehmannia Root).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots from the sample solution has the same color tone and RF value with the purple spot from the standard solution (Cornus Fruit).

(3) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of alisol A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and RF value with the purple spot from the standard solution (Alisma Rhizome).

(4) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of paenol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and RF value with the orange spot from the standard solution (Moutan Bark).

(5) Perform the test according to the following (i) or (ii) (Cinnamon Bark).
(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300 mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate 1 mL of the hexane layer, add 0.5 mL of sodium hydroxide TS, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (E)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.05\). Spot 50 \(\mu L\) of the sample solution and 2 \(\mu L\) of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and \(R_f\) value with the yellow-orange spot from the standard solution.

(ii) To 2.0 g of dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (E)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.05\). Spot 20 \(\mu L\) of the sample solution and 2 \(\mu L\) of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 20 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm) for the yellow-orange spot among the several spots from the sample solution and the \(R_f\) value with the yellow-orange spot from the standard solution.

(6) To 3.0 g of the dry extract (or 9.0 g of the viscous extract), add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.05\). Spot 20 \(\mu L\) of the sample solution and 10 \(\mu L\) of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and \(R_f\) value with the yellow-brown spot from the standard solution (Processed Aconite Root or Powdered Processed Aconite Root).

**Purity**

1. Heavy metals \(<1.07\)—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

2. Arsenic \(<1.17\)—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

3. Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1): Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet exactly 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 \(\mu L\) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\> according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu m\) in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

**System suitability**

System performance: When the procedure is run with 20 \(\mu L\) of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 \(\mu L\) of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5 %.

**Loss on drying** \(<2.41\> The dry extract: Not more than 8.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C,
Total ash <5.0%> Not more than 10.0%, calculated on the dried basis.

**Assay (1)** Logosin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately 10 mg of logosin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of logosin in each solution.

Amount (mg) of logosin = Mₛ × A₁/A₅ × 1/2

Mₛ: Amount (mg) of logosin for assay

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of logosin is about 25 minutes).

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of logosin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of logosin is not more than 1.5%.

**System performance—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of logosin is not more than 1.5%. (3) Total alkaloids—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and the acetonitrile standard solution TS for assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A₉ and A₈, as well as A₇ and A₆, in each solution, respectively.

Amount (mg) of benzoylmesaconine hydrochloride = Cₛ₉ × A₇/A₉ × 10

Amount (mg) of benzoylhypaconine hydrochloride = Cₛ₈ × A₉/A₈ × 10

Amount (mg) of 14-anisoylaconine hydrochloride = Cₛ₆ × A₆/A₆ × 10

Cₛ₉: Concentration (mg/mL) of benzoylmesaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay

Cₛ₈: Concentration (mg/mL) of benzoylhypaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay

Cₛ₆: Concentration (mg/mL) of 14-anisoylaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of peoniflorin is about 9 minutes).

**System suitability—**

System performance: Dissolve 1 mg each of Peoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and peoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of peoniflorin is not more than 1.5%.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 minutes).

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 minutes).

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhyiacanone; 254 nm for 14-anisoylacoline).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40℃.
Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).
Flow rate: 1.0 mL per minute (the retention time of benzoylmesaconine is about 15 minutes).
System suitability—
System performance: When the procedure is run with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the relative standard deviation of the peak areas of the peak areas of benzoylmesaconine, benzoylhyiacanone and 14-anisoylacoline is not more than 1.5%.
Containers and storage—Containers—Tight containers.

Hangekobokuto Extract

Hangekobokuto Extract contains not less than 2 mg and not more than 6 mg of magnolol, not less than 4 mg (or preparation prescribed 2 g of Perilla Herb) or not less than 6 mg (or preparation prescribed 3 g of Perilla Herb) of rosmarinic acid, and not less than 0.6 mg and not more than 2.4 mg (or preparation prescribed 1 g of Ginger) or not less than 0.8 mg and not more than 3.2 mg (or preparation prescribed 1.3 g of Ginger) or not less than 0.9 mg and not more than 3.6 mg (or preparation prescribed 1.5 g of Ginger) of [6]-gingerol, per amount specified in the Method of preparation.

Method of preparation

<table>
<thead>
<tr>
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<tr>
<td>Ginger</td>
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<td>1g</td>
<td>1.3g</td>
<td>1.5g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description Hangekobokuto Extract is a light brown to blackish brown, powder or viscous extract. It has a characteristic odor and has a bitter and astringent taste first then pungent later.

Identification (1) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of magnolol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.<2.05>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spots from the sample solution has the same color tone and RF value with the dark purple spot from the standard solution (Magnolia Bark).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of 0.1 mol/L hydrochloric acid TS, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of rosmarinic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.<2.05>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (60:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride TS on the plate: one of the spots from the several spots from the sample solution has the same color tone and RF value with the dark purple spot from the standard solution (Perilla Herb).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.<2.05>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105℃ for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and RF value with the dark purple spot from the standard solution (Ginger).

Purity (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed in the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.12>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract,
(1) Magnolol—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of magnolol for assay, previously dried in a desiccator (silica gel) for not less than 1 hour, and dissolve in diluted methanol (7 in 10) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of magnolol in each solution.

Amount (mg) of magnolol = \( M_S \times A_T/A_S \times 1/8 \)

\( M_S \): Amount (mg) of magnolol for assay

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclisilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (800:200:1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 15 minutes).

**System suitability**—

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, honokiol and magnolol are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

(2) Rosmarinic acid—Conduct this procedure using light-resistant vessels. Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of rosmarinic acid for assay, previously dried in a desiccator (silica gel) for not less than 1 hour, and dissolve in diluted methanol (7 in 10) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of rosmarinic acid in each solution.

Amount (mg) of rosmarinic acid = \( M_S \times A_T/A_S \times 1/4 \)

\( M_S \): Amount (mg) of rosmarinic acid for assay

**Operating conditions**—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclisilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (800:200:1).

Flow rate: 1.0 mL per minute (the retention time of rosmarinic acid is about 11 minutes).

**System suitability**—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rosmarinic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rosmarinic acid is not more than 1.5%.

(3) [6]-Gingerol—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for assay, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of [6]-gingerol in each solution.

Amount (mg) of [6]-gingerol = \( M_S \times A_T/A_S \times 1/20 \)

\( M_S \): Amount (mg) of [6]-gingerol for assay

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeclisilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (620:380:1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 15 minutes).

**System suitability**—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operat-
ing conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

Containers and storage  Containers—Tight containers.

Hemp Fruit

Cannabis Fructus

マシンニ

Hemp Fruit is the fruit of Cannabis sativa Linné (Moraceae).

Description  Hemp Fruit is a slightly compressed void fruit, 4 – 5 mm in length, 3 – 4 mm in diameter; externally grayish green to grayish brown; pointed at one end, a scar of gynophore at the other end, and crest lines on both sides; outer surface lustrous with white mesh-like pattern; slightly hard pericarp; seed, slightly green in color and internally has grayish white albumen; 100 fruits weigh 1.6 – 2.7 g.

Practically odorless, aromatic on chewing; taste, mild and oily.

Under a microscope \(< 5.01\)>, a transverse section reveals the exocarp to be a single-layered epidermis; mesocarp composed of parenchyma, a pigment cell layer and rows of short, small cells; endocarp made up of a layer of radially elongated stone cells; seed coat comprises a tubular cell layer and spongy tissue. Inside of the seed; exosperm consists of several layers of parenchymatous cells; most of the embryo composed of parenchyma, vascular bundles occurring in the center of hypocotyls and cotyledons; embryo parenchyma contains aleurone grains and oil drops.

Identification  To 0.3 g of pulverized Hemp Fruit add 3 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography \(< 5.03\)>. Spot 5 \(\mu L\) of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate \((9:2)\) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: a dark blue-purple spot appears at an \(R_f\) value of about 0.6.

Purity  Bract—When perform the test of foreign matter \(< 5.01\), Hemp Fruit does not contain bract.

Loss on drying \(< 5.01\)  Not more than 9.0% (6 hours).

Total ash \(< 5.01\)  Not more than 7.0%.

Acid-insoluble ash \(< 5.01\)  Not more than 2.0%.

Containers and storage  Containers—Well-closed containers.

Hochuekkito Extract

補中益気湯エキス

Hochuekkito Extract contains not less than 16 mg and not more than 64 mg of hesperidin, not less than 0.3 mg and not more than 1.2 mg (for preparation prescribed 1 g of Bupleurum Root) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 2 g of Bupleurum Root) of saikosaponin b2, and not less than 12 mg and not more than 36 mg of glycyrrhizic acid \((C_{42}H_{62}O_{16}: 822.93)\), per extract prepared with the amount specified in the Method of preparation.

Method of preparation


<table>
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<tr>
<th>Method of preparation</th>
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Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 6), using the crude drugs shown above.

Description  Hochuekkito Extract occurs as a light brown to blackish brown powder or viscous extract. It has a slight odor, and a sweet and bitter taste.

Identification (1)  To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and shake. Take the 1-butanol layer, evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb1 RS in methanol to the residue, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(< 2.03\). Spot 5 \(\mu L\) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid \((100):(7.5:4:1)\) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and \(R_f\) value with the purple spot from the standard solution (Ginseng).

(2)  For preparation prescribed Atractylodes Rhizome—To 3.0 g of the dry extract (or 9.0 g of the viscous extract)
add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (2)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (10.3). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm); one of the spots among the several spots from the sample solution has the same color tone and Rf value with the bluish white fluorescent spot from the standard solution (Japanese Angelica Root).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of hesperidin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (10.3). Spot 2 μL of the sample solution and 20 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine TS on the plate, and expose to ammonia vapor: one of the spots among the several spots from the sample solution has the same color tone and Rf value with the blue spot from the standard solution (Citrus Unshiu Peel).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of saikosaponin b₁ for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (10.3). Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: one of the spots among the several spots from the sample solution has the same color tone and Rf value with the red spot from the standard solution (Bupleurum Root).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (10.3). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water
Thin-layer Chromatography

Swirl 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{<2.03> \text{[C]}} \). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Glycyrrhiza).

For preparation prescribed Ginger—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{<2.03> \text{[C]}} \). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the blue-green spot from the standard solution (Ginger).

For preparation prescribed Processed Ginger—Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect an apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for about 1 hour, separate the hexane layer, and use this as the sample solution. Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{<2.03> \text{[C]}} \). Spot 60 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the blue-green spot from the standard solution (Processed Ginger).

To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Use 3-(3-hydroxy-4-methoxyphenyl)-2-(E)-propenic acid-(E)-ferulic acid TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{<2.03> \text{[C]}} \). Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the yellow fluorescent spot from the standard solution (Cimicifuga Rhizome).

Purity (1) Heavy metals \( \text{<1.07> \text{[C]}} \)—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed in the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic \( \text{<1.11> \text{[C]}} \)—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying \( \text{<2.41> \text{[C]}} \) The dry extract: Not more than 11.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash \( \text{<5.01> \text{[C]}} \) Not more than 9.0%, calculated on the dried basis.

Assay (1) Hesperidin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add diluted tetrahydrofuran (1 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \( \text{<2.01> \text{[C]}} \) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of hesperidin in each solution.

Amount (mg) of hesperidin = \( M_S \times A_T \times A_S \times 1/20 \)

\( M_S \): Amount (mg) of hesperidin for assay

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100:82:18:1).

Flow rate: 1.0 mL per minute (the retention time of hesperidin is about 15 minutes).

System suitability—

System performance: Dissolve 1 mg each of hesperidin for assay and naringin for thin-layer chromatography in diluted methanol (1 in 2) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, naringin and hesperidin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak
area of hesperidin is not more than 1.5%.

(2) Saikosaponin b2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of saikosaponin b2 for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in 50 mL of methanol, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of saikosaponin b2.

Amount (mg) of saikosaponin b2 = \( M_S \times \frac{A_T}{A_S} \times \frac{1}{20} \)

\( M_S \): Amount (mg) of saikosaponin b2 for assay

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

**Honey**

*Mel*

ハチミツ

Honey is the saccharine substances obtained from the honeycomb of *Apis mellifera* Linné or *Apis cerana* Fabricius (Apiidae).

**Description** Honey is a light yellow to light yellow-brown, syrupy liquid. Usually it is transparent, but often opaque with separated crystals.

It has a characteristic odor and a sweet taste.

**Specific gravity** <2.50> Mix 50.0 g of Honey with 100 mL of water: the specific gravity of the solution is not less than \( \alpha^{20}_{20} 1.111 \).

**Purity** (1) Acidity—Mix 10 g of Honey with 50 mL of water, and titrate <2.50> with 1 mol/L potassium hydroxide VS (indicator: 2 drops of phenolphthalein TS): not more than 0.5 mL is required.

(2) Sulfate—Mix 1.0 g of Honey with 2.0 mL of water, and filter. To the filtrate add 2 drops of barium chloride TS: the solution does not show any change immediately.

(3) Ammonia-coloring substances—Mix 1.0 g of Honey with 2.0 mL of water, and filter. To the filtrate add 2 mL of ammonium TS: the solution does not show any change immediately.

(4) Resorcinol-coloring substances—Mix well 5 g of Honey with 15 mL of diethyl ether, filter, and evaporate the diethyl ether solution at ordinary temperature. To the residue add 1 to 2 drops of resorcinol TS: a yellow-red color may develop in the solution of resorcinol and in the residue, and a red to red-purple color which does not persist more than 1 hour.

(5) Starch or dextrin—(i) Shake 7.5 g of Honey with 15 mL of water, warm the mixture on a water bath, and add 0.5 mL of tannic acid TS. After cooling, filter, and to 1.0 mL of the filtrate add 1.0 mL of ethanol (99.5) containing 2 drops...
of hydrochloric acid: no turbidity is produced.
(ii) To 2.0 g of Honey add 10 mL of water, warm in a water bath, mix, and allow to cool. Shake 1.0 mL of the mixture with 1 drop of iodine TS; no blue, green or red-brown color develops.

(6) Foreign matter—Mix 1.0 g of Honey with 2.0 mL of water, centrifuge the mixture, and examine the precipitate microscopically. No foreign substance except pollen grains is observable.

**Total ash**<5.0% Not more than 0.4%.

**Containers and storage** Containers—Tight containers.

**Houttuynia Herb**

**Houttuyniae Herba**

ジュウヤク

Houttuynia Herb is the terrestrial part of *Houttuynia cordata* Thunberg (*Saururaceae*), collected during the flowering season.

**Description** Stem with alternate leaves and spikes; stem light brown, with longitudinal furrows and protruded nodes; when soaked in water and smoothed out, leaves wide ovate and cordate, 3–8 cm in length, 3–6 cm in width; light green-brown; margin entire, apex acuminate; petiole long, and membranous stipule at the base; spike, 1–3 cm in length, with numerous light yellow-brown achlamydeous florets, and the base enclosed by 4 long ovate, light yellow to light yellow-brown involucres.

Odor, slight; tasteless.

**Identification** Boil 2 g of pulverized Houttuynia Herb with 20 mL of ethyl acetate under a reflux condenser on a water bath for 15 minutes, and filter. Evaporate the filtrate to dryness, add 10 mL of water to the residue, warm the mixture on a water bath for 2 minutes, and, after cooling, filter. Shake well the filtrate with 20 mL of ethyl acetate in a separator, take 15 mL of ethyl acetate solution, and evaporate the solution on a water bath to dryness. Dissolve the residue in 5 mL of methanol, add 0.1 g of magnesium ribbon and 1 mL of hydrochloric acid, and allow the mixture to stand: a red-purple color develops.

**Purity** Foreign matter <5.0%—The amount of the rhizome, roots and other foreign matter contained in Houttuynia Herb is not more than 2.0%.

**Total ash**<5.0% Not more than 14.0%.

**Acid-insoluble ash**<5.0% Not more than 3.0%.

**Extract content**<5.0% Dilute ethanol-soluble extract: not more than 10.0%.

**Containers and storage** Containers—Well-closed containers.

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**Immature Orange**

**Aurantii Fructus Immaturus**

キジツ

Immature Orange is the immature fruit or the fruit cut crosswise of *Citrus aurantium* Linné var. *daidai* Makino, *Citrus aurantium* Linné or *Citrus natsudaidai* Hayata (*Rutaceae*).

**Description** Nearly spherical fruit, 1–2 cm in diameter, or semispherical, 1.5–4.5 cm in diameter; external surface, deep green-brown to brown, and without luster, with numerous small dents associated with oil sacs; the outer portion of transverse section exhibits pericarp and mesocarp about 0.4 cm in thickness, yellow-brown in color in the region contacting epidermis, and light grayish brown color in the other parts; the central portion is radially divided into 8 to 16 small loculi; each loculus is brown and indented, often containing immature seeds.

Odor, characteristic; taste, bitter.

**Identification** To 0.5 g of pulverized Immature Orange add 10 mL of methanol, boil gently for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium ribbon and 1 mL of hydrochloric acid, and allow to stand: a red-purple color develops.

**Total ash**<5.0% Not more than 7.0%.

**Containers and storage** Containers—Well-closed containers.

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**Imperata Rhizome**

**Imperatae Rhizoma**

ポウコン

Imperata Rhizome is the rhizome of *Imperata cylindrica* Beauvios (*Gramineae*), from which rootlets and scale leaves have been removed.

**Description** Long and thin cylindrical rhizome, 0.3–0.5 cm in diameter; sometimes branched; externally yellowish white, with slight longitudinal wrinkles, and with nodes at 2–3 cm intervals; difficult to break; fractured surface fibrous. Cross section irregularly round; thickness of cortex is slightly smaller than the diameter of the stele; pith often forms a hollow. Under a magnifying glass, a transverse section reveals cortex, yellowish white, and with scattered brown spots; stele, yellow-brown in color.

Odorless, and tasteless at first, but later slightly sweet.

**Identification** To 1 g of pulverized Imperata Rhizome add 20 mL of hexane, allow the mixture to stand for 30 minutes with occasional shaking, and filter. Evaporate the hexane of the filtrate under reduced pressure, dissolve the residue in 5 mL of acetic anhydride, place 0.5 mL of this solution in a test tube, and add carefully 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact, and the upper layer acquires a blue-green to blue-purple
Acid-insoluble ash

Purity (1) Rootlet and scaly leaf—When perform the test of foreign matter <5.0>1, the amount of the rootlets and scaly leaves contained in Imperata Rhizome is not more than 3.0%.

(2) Heavy metals <1.0—Proceed with 3.0 g of pulverized Imperata Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.1—Prepare the test solution with 0.40 g of pulverized Imperata Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <5.0—The amount of foreign matter other than rootlets and scaly leaves is not more than 1.0%.

Total ash <5.0> Not more than 5.0%.

Acid-insoluble ash <5.0> Not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Ipecac

Ipecacuanhae Radix

トコノ

Ipecac is the root and rhizome of Cephaelis ipecacuana H. Richard or Cephaelis acuminata Karsten (Rubiaceae).

It contains not less than 2.0% of the total alkaloids (emetine and cephaeline), calculated on the basis of dried material.

Description Slender, curved, cylindrical root, 3 – 15 cm in length, 0.3 – 0.9 cm in diameter; mostly twisted, and sometimes branched; outer surface gray, dark grayish brown, red-brown in color and irregularly annulated; when root fractured, cortex easily separable from the xylem; the cortex on the fractured surface is grayish brown, and the xylem is light brown in color: thickness of cortex up to about two-thirds of radius in thickened portion. Scales in rhizome opposite.

Odor, slight; powder irritates the mucous membrane of the nose; taste, slightly bitter and unpleasant.

Under a microscope <5.0>, the transverse section of Ipecac reveals a cork layer, consisting of brown thin-walled cork cells; in the cortex, sclerenchyma cells are absent; in the xylem, vessels and tracheids arranged alternately; parenchyma cells filled with starch grains and sometimes with raphides of calcium oxalate.

Identification To 0.5 g of pulverized Ipecac add 2.5 mL of hydrochloric acid, allow to stand for 1 hour with occasional shaking, and filter. Collect the filtrate into an evaporating dish, and add a small pieces of chlorinated lime: circumference of it turns red.

Purity Arsenic <1.1—Prepare the test solution with 0.40 g of pulverized Ipecac according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <5.0> Not more than 12.0% (6 hours).

Total ash <5.0> Not more than 5.0%.

Acid-insoluble ash <5.0> Not more than 2.0%.

Assay Weigh accurately about 0.5 g of pulverized Ipecac, in a glass-stoppered centrifuge tube, add 30 mL of 0.01 mol/L hydrochloric acid TS, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 30-mL portions of 0.01 mol/L hydrochloric acid TS. Combine all the extracts, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of emetine hydrochloride for assay, previously dried in a desiccator (reduced below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.0>1 according to the following conditions. Determine the peak areas, ATE and ATC, of emetine and cephaeline in the sample solution, and the peak area, ASE, of emetine in the standard solution.

Amount (mg) of total alkaloids (emetine and cephaeline) = MS × (ATE + (ATC × 0.971))/ASE × 0.868

MS: Amount (mg) of emetine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.0 g of sodium 1-heptane sulfonate in 500 mL of water, adjust the pH 4.0 with acetic acid (100), and add 500 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of emetine is about 14 minutes.

Selection of column: Dissolve 1 mg each of emetine hydrochloride for assay and cephaeline hydrobromide in 10 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 10 μL of this solution under the above operating conditions. Use a column giving elution of cephaeline and emetine in this order, and clearly separating each peak.

System repeatability: Repeat the test 6 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of emetine is not more than 1.5%.

Containers and storage Containers—Well-closed containers.
Powdered Ipecac

Ipecacuanhae Radix Pulverata

Powdered Ipecac is the powder of Ipecac or its powder diluted with Potato Starch. It contains not less than 2.0% and not more than 2.6% of the total alkaloids (emetine and cephaeline), calculated on the basis of dried material.

**Description**
Powdered Ipecac occurs as a light grayish yellow to light brown powder. It has a slight odor, which is irritating to the nasal mucosa, and has a somewhat bitter and unpleasant taste.

Under a microscope, Powdered Ipecac reveals starch grains and needle crystals of calcium oxalate; fragments of parenchyma cells containing starch grains or the needle crystals; substitute fibers, thin-walled cork tissue; vessels and tracheids with simple or bordered pits; a few wood fibers and wood parenchyma. Starch grains inherent in Ipecac, mainly 2~8-compound grains, rarely simple grains 4~22 μm in diameter; and needle crystals of calcium oxalate 25~60 μm in length.

**Identification**
To 0.5 g of Powdered Ipecac add 2.5 mL of hydrochloric acid, allow to stand for 1 hour with occasional shaking, and filter. Collect the filtrate into an evaporating dish, mix with 1 mL of hydrochloric acid, and add small pieces of chlorinated lime: circumference of it turns red.

**Purity**
1. Arsenic
   - Prepare the test solution with 0.40 g of Powdered Ipecac according to Method 4, and perform the test (not more than 5 ppm).
2. Foreign matter
   - Under a microscope, groups of stone cells and thick-walled fibers are not observed.

**Loss on drying**
Not more than 12.0% (6 hours).

**Total ash**
Not more than 5.0%.

**Acid-insoluble ash**
Not more than 2.0%.

**Assay**
Weigh accurately about 0.5 g of Powdered Ipecac, transfer into a glass-stoppered centrifuge tube, add 30 mL of 0.01 mol/L hydrochloric acid TS, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 30-mL portions of 0.01 mol/L hydrochloric acid TS. Combine all the extracts, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of emetine hydrochloride for assay, previously dried in a desiccator (reduced below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Perform the test with exactly 10 μL of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak areas, A_{TE} and A_{TC}, of emetine and cephaeline in the sample solution, and the peak area, A_{SE}, of emetine in the standard solution.

Amount (mg) of total alkaloids (emetine and cephaeline) per 100 mL:

\[ M_S = \left( \frac{\left( A_{TE} + (A_{TC} \times 0.971)\right)}{A_{SE} \times 0.868} \right) \]

**Operating conditions**

   - Detector: An ultraviolet absorption photometer (wavelength: 283 nm).
   - Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecysilanolized silica gel for liquid chromatography (5 to 10 μm in particle diameter).
   - Column temperature: A constant temperature of about 50°C.
   - Mobile phase: Dissolve 2.0 g of sodium 1-heptane sulfonate in 500 mL of water, adjust the pH 4.0 with acetic acid (100), and add 500 mL of methanol.
   - Flow rate: Adjust the flow rate so that the retention time of emetine is about 14 minutes.
   - Selection of column: Dissolve 1 mg each of emetine hydrochloride for assay and cephaeline hydrobromide in 10 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 10 μL of this solution under the above operating conditions. Use a column giving elution of cephaeline and emetine in this order, and clearly separating each peak.
   - System repeatability: Repeat the test 6 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of emetine is not more than 1.5%.

**Containers and storage**
Well-closed containers.

Ipecac Syrup

Ipecac Syrup is a syrup containing not less than 0.12 g and not more than 0.15 g of the total alkaloids (emetine and cephaeline) per 100 mL.

**Method of preparation**
Take coarse powder of Ipecac, prepare the fluidextract as directed under Fluidextracts using a mixture of Ethanol and Purified Water or Purified Water in Containers (3:1), and evaporate the mixture under reduced pressure or add a suitable amount of Ethanol or Purified Water or Purified Water in Containers if necessary to get a solution containing 1.7 to 2.1 g of the total alkaloids (emetine and cephaeline) per 100 mL. To 70 mL of this solution add 100 mL of Glycerin and Simple Syrup to make 1000 mL, as directed under Syrups.

**Description**
Ipecac Syrup is a yellow-brown, viscous liquid. It has a sweet taste and a bitter aftertaste.

**Identification**
Take 2 mL of Ipecac Syrup into an evaporating dish, mix with 1 mL of hydrochloric acid, and add small pieces of chlorinated lime: circumference of it turns orange.

**Purity**
Ethanol—Take exactly 5 mL of Ipecac Syrup, add exactly 5 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, pipet 5 mL of ethanol (99.5), and add water to make exactly 100 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution and water to
make 50 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the rate of peak height of ethanol to that of the internal standard, Q1 and Q2; Q1 is not larger than Q2.

Internal standard solution—A solution of acetonitrile (1 in 20).

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass-column about 3 mm in inside diameter and about 1.5 m in length, packed with ethylvinylbenzene-divinylbenzene porous co-polymer for gas chromatography (150 to 180 μm in particle diameter).
Column temperature: A constant temperature of between 105℃ and 115℃.
Carrier gas: Nitrogen.
Flow rate: Adjust the flow rate so that the retention time of ethanol is 5 to 10 minutes.
Selection of column: Proceed with 2 μL of the above operating conditions. Use a column giving elution of cephaeline and emetine in this order, and clearly separating each peak.

Assay Take exactly 5 mL of Ipecac Syrup, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of emetine hydrochloride for assay, previously dissolved in a solution of acetonitrile (1 in 20). Make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A<sub>TE</sub> and A<sub>TC</sub>, of emetine and cephaeline in the sample solution, and the peak area, A<sub>SE</sub>, of emetine in the standard solution.

Amount (mg) of total alkaloids (emetine and cephaeline) = M<sub>S</sub> × (A<sub>TE</sub> + (A<sub>TC</sub> × 0.971))/A<sub>SE</sub> × 1/2 × 0.868

M<sub>S</sub>: Amount (mg) of emetine hydrochloride for assay

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 283 nm).
Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).
Column temperature: A constant temperature of about 90℃.
Mobile phase: Dissolve 2.0 g of sodium 1-heptene sulfonate in 500 mL of water, adjust the pH to 4.0 with acetic acid (100), and add 500 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of emetine is about 14 minutes.
Selection of column: Dissolve 1 mg each of emetine hydrochloride for assay and cephaeline hydrobromide in 10 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 10 μL of this solution under the above operating conditions. Use a column giving elution of cephaeline and emetine in this order, and clearly separating each peak.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of emetine is not more than 1.5%.

Microbial limit <4.05> The acceptance criteria of TAMC and TYMC are 10<sup>3</sup> CFU/mL and 10<sup>4</sup> CFU/mL, respectively. Escherichia coli, Salmonella, Pseudomonas aeruginosa and Staphylococcus aureus are not observed.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Japanese Angelica Root

Angelicae Radix

トウキ

Japanese Angelica Root is the root of Angelica acutiloba Kitagawa or Angelica acutiloba Kitagawa var. sugiyamae Hikino (Umbelliferae), usually after being passed through hot water.

Description Thick and short main root, with numerous branched roots, nearly fusiform; 10 – 25 cm in length; externally dark brown to red-brown, with longitudinal wrinkles and horizontal protrusions composed of numerous scars of fine rootlets; fractured surface is dark brown to yellow-brown in color, and smooth; and with a little remains of leaf sheath at the crown.

Odor, characteristic; taste, slightly sweet, followed by slight pungency.

Under a microscope <5.01>, a transverse section reveals 4 to 10 layers of cork, with several layers of collenchyma inside the layer; the cortex exhibits many oil canals surrounded by secretory cells and often large hollows appear; boundary of phloem and xylem is distinct; in the xylem, numerous vessels radiate alternately with medullary rays; vessels in the outer part of the xylem are singly or in several groups, and disposed rather densely in a cuneiform pattern, but vessels in the region of the center are scattered very sparsely; starch grains are simple grains, not more than 20 μm in diameter, and rarely 2- to 5-compound grains, up to 25 μm in diameter; starch grains often gelatinized.

Purity (1) Leaf sheath—When perform the test of foreign matter <5.05>, the amount of leaf sheaths contained in Japanese Angelica Root does not exceed 3.0%.
(2) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Japanese Angelica Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).
(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Japanese Angelica Root according to Method 4, and perform the test (not more than 5 ppm).
(4) Foreign matter <5.07>—The amount of foreign matter other than leaf sheath contained in Japanese Angelica Root does not exceed 1.0%.

Total ash <5.01> Not more than 7.0%.
Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 35.0%.
Containers and storage Containers—Well-closed containers.

Powdered Japanese Angelica Root
Angelicae Radix Pulverata

Powdered Japanese Angelica Root is the powder of Japanese Angelica Root.

Description Powdered Japanese Angelica Root occurs as a light grayish brown powder. It has a characteristic odor and a slightly sweet taste with a slightly pungent aftertaste.

Under a microscope <5.01>, Powdered Japanese Angelica Root reveals starch grains or masses of gelatinized starch, duct surrounded by secretory cells; fragments, 20 – 60 μm in diameter, of scalariform cells containing oil; fragments of parenchyma containing oil; fragments of lenticular vessels with simple perforation; starch grains composed of simple grains not more than 20 μm in diameter, and rarely 2- to 3-compound grains.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Japanese Angelica Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Japanese Angelica Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Japanese Angelica Root does not show remarkably lignified sclerenchymatous cells.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 35.0%.

Containers and storage Containers—Light-resistant.

Japanese Gentian
Gentianae Scabrae Radix

Japanese Gentian is the root and rhizome of Gentiana scabra Bunge, Gentiana manshurica Kitagawa or Gentiana triflora Pallas (Gentianaceae).

Description Irregular, cylindrical, short rhizome with numerous, slender roots around, and externally yellow-brown to grayish yellow-brown. The root is 10 – 15 cm in length, about 0.3 cm in diameter, and has longitudinal, coarse wrinkles on the outer surface; flexible; fractured surface, smooth and yellow-brown in color. The rhizome is about 2 cm in length, about 0.7 cm in diameter, and has buds or short remains of stems at the top.

Odor, slight; taste, extremely bitter and lasting.

Under a microscope <5.01>, a transverse section of the young root reveals epidermis, exodermis and a few layers of primary cortex; usually, the outermost layer is endodermis consisting of characteristic cells divided into a few daughter cells, often with collenchyma of 1 to 2 layers contacting the inner side; secondary cortex having rents here and there, and irregularly scattered sieve tubes; vessels arranged rather radially in xylem, sieve tubes existing in xylem; the rhizome has a large pith, rarely with sieve tubes; parenchyma cells contain needle, plate or sand crystals of calcium oxalate and oil drops; starch grains usually absent.

Identification To 0.5 g of Powdered Japanese Gentian add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 10 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same Rf value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Japanese Gentian according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Japanese Gentian according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Containers and storage Containers—Well-closed containers.

Powdered Japanese Gentian
Gentianae Scabrae Radix Pulverata

Powdered Japanese Gentian is the powder of Japanese Gentian.

Description Powdered Japanese Gentian occurs as a grayish yellow-brown powder. It has a slight odor and a lasting, extremely bitter taste.

Under a microscope <5.01>, Powdered Japanese Gentian reveals fragments of parenchyma cells containing oil droplets and fine crystals, fragments of endodermis and exodermis divided into daughter cells with suberized membrane, and fragments of vessels. Vessels mainly consist of reticulate vessels and scalariform vessels, 20 – 30 μm in di-
Japanese Valerian / Crude Drugs

Valerianae Radix

Japanese Valerian is the root and rhizome of *Valeriana fauriei* Briquet (*Valerianaceae*).

**Description** Obvoid, short rhizome with numerous, fine and long roots; externally dark brown to grayish brown. The root, 10 – 15 cm in length, 0.1 – 0.3 cm in diameter; externally, with fine longitudinal wrinkles; brittle. The rhizome, 1 – 2 cm in length, 1 – 2 cm in diameter, with buds and remains of stem at the crown; hard in texture and difficult to break; flank of rhizome sometimes accompanied with stolons having thick and short or thin, long and extremely small, scaly leaves. Under a magnifying glass, the transverse section reveals a thick, light grayish brown cortical layer, and a grayish brown stele.

Odor, strong and characteristic; taste, slightly bitter.

**Purity** (1) Heavy metals <1.0%> — Proceed with 3.0 g of pulverized Japanese Valerian according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11%>—Prepare the test solution with 0.40 g of pulverized Japanese Valerian according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01%>, Powdered Japanese Valerian usually reveals no stone cells and fibers. No starch grains; if any, very few.

**Total ash** <5.01%> Not more than 7.0%.

**Acid-insoluble ash** <5.01%> Not more than 3.0%.

**Containers and storage** Containers—Well-closed containers.

Powdered Japanese Valerian

Valerianae Radix Pulverata

Powdered Japanese Valerian is the powder of Japanese Valerian.

**Description** Powdered Japanese Valerian occurs as a dark grayish brown powder. It is somewhat moist to the touch. It has a strong, characteristic odor and a slightly bitter taste.

Under a microscope <5.01%>, Powdered Japanese Valerian reveals starch grains and fragments of parenchyma cells containing them; fragments of pitted vessels, reticulate vessels, ring vessels, and spiral vessels; fragments of exodermis containing oil droplets and composed of cells suberized and divided into daughter cells; fragments of yellow stone cells from the rhizome and the stolon; and very rarely, some fragments of epidermis and phloem fibers. Starch grains, simple grains 10 – 20 μm in diameter and 2- to 4-compound grains; oil droplets stained red with sudan III TS.

**Purity** (1) Heavy metals <1.0%> — Proceed with 3.0 g of pulverized Japanese Valerian according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11%>—Prepare the test solution with 0.40 g of pulverized Japanese Valerian according to Method 4, and perform the test (not more than 5 ppm).

**Total ash** <5.01%> Not more than 10.0%.

**Acid-insoluble ash** <5.01%> Not more than 5.0%.

**Essential oil content** <5.01%> Perform the test with 50.0 g of pulverized Japanese Valerian provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.2 mL.

**Containers and storage** Containers—Tight containers.

Jujube

Zizyphi Fructus

Jujube is the fruit of *Zizyphus jujuba* Miller var. *inermis* Rehder (*Rhamnaceae*).

**Description** Ellipsoidal or broad ovoid fruit, 2 – 3 cm in length, 1 – 2 cm in diameter; externally reddish brown with coarse wrinkles, or dark grayish red with fine wrinkles, and...
both lustrous; both ends slightly dented, with a scar of style on one end and a scar of peduncle on the other; epicarp thin and leather; mesocarp thick, dark grayish brown in color, spongy, soft and adhesive; endocarp extremely hard, fusiform, and divided into two loculi; seeds flat and ovoid.

Orod, slight and characteristic; taste, sweet.

Purity

(1) Rancidity—Jujube has no unpleasant, rancid odor and taste.

(2) Total BHC’s and total DDT’s <5.0 ppm Not more than 0.2 ppm, respectively.

Total ash <5.0 ppm Not more than 3.0%.

Containers and storage Containers—Well-closed containers.

Jujube Seed

Zizyphi Semen

サツマイモニシ

Jujube Seed is the seed of Zizyphus jujuba Miller var. spinosa Hu ex H. F. Chou (Rhamnaceae).

Description

Jujube Seed is a compressed ovate to orbicular, lenticular seed, 5 - 9 mm in length, 4 - 6 mm in width, 2 - 3 mm in thickness, externally brown to dark red-brown, glossy; hilum at one end, charaza at the other end; seed coat slightly flexible, covering, milky white endosperm and light yellow embryo. 100 seeds weigh 3.0 - 4.5 g.

Orod, slightly oily; taste, mild and slightly oily.

Under a microscope <5.0 ppm, transverse section reveals seed coat composed of an upper epicarp, parenchyma and lower epidermis; upper epidermal cells sclerified and elongated in radial direction; lower epidermis covered with cuticle; endosperm composed of parenchyma, containing aggregated crystals of calcium oxalate, aleurone grains and starch grains; cotyledons composed of parenchyma that contains aleurone grains, starch grains and oil drops.

Identification

To 2 g of pulverized Jujube Seed add 10 mL of methanol, and heat under a reflux condenser for 10 minutes. After cooling, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 µL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a purple spot appears at an Rf value of about 0.3, which shows a yellow-green to grayish green color after spraying 1-naphthol-sulfuric acid TS on the plate and heating at 105°C for 5 minutes.

Purity

Foreign matter <5.0 ppm—Jujube Seed contains not more than 1.0% of the endocarp and other foreign matters.

Loss on drying <5.0 ppm Not more than 11.0% (6 hours).

Total ash <5.0 ppm Not more than 5.0%.

Extract content <5.0 ppm Dilute ethanol-soluble extract: not less than 9.0%.

Jujentaihoto Extract

十全大補湯エキス

Jujentaihoto Extract contains not less than 1.5 mg (for preparation prescribed 2.5 g of Ginseng) or not less than 1.8 mg (for preparation prescribed 3 g of Ginseng) of ginsenoside Rb1 (C42H62O16: 822.93), not less than 26 mg and not more than 78 mg of peonyfrolin (C23H33O11: 480.46), and not less than 8 mg and not more than 24 mg (for preparation prescribed 1 g of Glycyrrhiza) or not less than 12 mg and not more than 36 mg (for preparation prescribed 1.5 g of Glycyrrhiza) of glycyrrhizic acid (C23H28O11: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description

Juzentaihoto Extract is a light brown to blackish brown, powder or viscous extract. It has a slight odor and a sweet and bitter taste.

Identification

(1) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 15 mL of sodium hydroxide TS, centrifuge, and take the supernatant liquid. To the liquid add 10 mL of 1-butanol, shake, centrifuge, and take the 1-butanol layer. To the 1-butanol layer add 10 mL of water, shake, centrifuge, and take the 1-butanol layer. Evaporate the layer under reduced pressure, to the residue add 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb1, RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL of the sample solution and 2 µL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the
sample solution has the same color tone and Rf value with the dark brown spot from the standard solution (Ginseng).

(2) Use the sample solution obtained in (1) as the sample solution. Separately, dissolve 1 mg of astragalin IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the red-brown spot from the standard solution (Astragalus Root).

(3) (For preparation prescribed Atractylodes Rhizome) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 5 mL of diethyl ether, shake, and centrifuge. Use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the red spot from the standard solution (Atractylodes Rhizome).

(4) (For preparation prescribed Atractylodes Lancea Rhizome) Shake 5.0 g of the dry extract (or 15.0 g of the viscous extract) with 10 mL of water, add 25 mL of layer, and shake. Take the hexane layer, evaporate the hexane under reduced pressure, dissolve the residue in 2 mL of hexane, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 40 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at about Rf 0.4, and this spot shows a green-brown color after spraying 4-dimethylaminobenzaldehyde TS for spraying, heating at 105°C for 5 minutes and allow to cool (Atractylodes Lancea Rhizome).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 15 mL of water and 5 mL of 0.1 mol/L hydrochloric acid, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, then add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (Z)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the bluish white fluorescent spot from the standard solution (Chindium Rhizome; Japanese Angelica Root).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Peoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the purple spot from the standard solution (Peony Root).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehydesulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: a dark green spot is observed at about Rf 0.6 (Rehmannia Root).

(8) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination to the flask, and heat to boil under a reflux condenser. The graduated tube of the apparatus is previously filled with water to the standard line and added 2 mL of hexane. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (E)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the yellow-orange spot from the standard solution.

(ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (E)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol,
and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 20 \(\mu L\) of the sample solution and 2 \(\mu L\) of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm); one of the spot among the several spots from the sample solution has the same color tone and \(R_f\) value with the bluish white fluorescent spot from the standard solution.

(9) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquoritin in thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 \(\mu L\) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and \(R_f\) value with the yellow-brown spot from the standard solution (Glycyrrhiza).

**Purity (1)**—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

**Loss on drying** <2.41> The dry extract—Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract—Not more than 66.7% (1 g, 105°C, 5 hours).

**Total ash** <5.01> Not more than 10.0%, calculated on the dried basis.

**Assay (1)** Ginsenoside Rb1—Weigh accurately about 2 g of the dry extract (or an amount of the viscous extract, equivalent to 2 g of the dried substance), add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 15 mL of diluted methanol (3 in 5), and repeat the same procedure. Combine the supernatant liquids, add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of sodium hydroxide TS, allow to stand for 30 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 20 mL. Apply exactly 5 mL of this solution to a column (about 10 mm in inside diameter and packed with 0.36 g of octadecylsilanized silica gel for pre-treatment (55 – 105 \(\mu m\) in particle size), washed just before use with methanol and then with diluted methanol (3 in 10), and wash the column in sequence with 2 mL of diluted methanol (3 in 10), 1 mL of sodium carbonate TS and 10 mL of diluted methanol (3 in 10). Finally, elute with methanol to collect exactly 5 mL, and use this as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb1, RS (separately determine the water <2.48d>, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 \(\mu L\) each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, \(A_T\) and \(A_S\), of ginsenoside Rb1 in each solution.

\[
\text{Amount (mg) of ginsenoside Rb1 (C_{42}H_{52}O_{32})} = M_s \times A_T / A_S \times 1/5
\]

\([M_s]:\) Amount (mg) of Ginsenoside Rb1, RS, calculated on the anhydrous basis

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with carbamoyl groups bound silica gel for liquid chromatography (5 \(\mu m\) in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: A mixture of acetonitrile and water (4:1).
Flow rate: 1.0 mL per minute (the retention time of ginsenoside Rb1 is about 16 minutes).

**System suitability**—
System performance: When the procedure is run with 20 \(\mu L\) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ginsenoside Rb1 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 \(\mu L\) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb1, is not more than 1.5%.

(2) Peoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu L\) each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, \(A_T\) and \(A_S\), of peoniflorin in each solution.

\[
\text{Amount (mg) of peoniflorin (C_{23}H_{28}O_{11})} = M_s \times A_T / A_S \times 1/5
\]

\([M_s]:\) Amount (mg) of Peoniflorin RS, calculated on the anhydrous basis

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeylsilanized silica gel for liquid chromatography (5 \(\mu m\) in particle diameter).

Column temperature: A constant temperature of about 20°C.
Kakkonto Extract

Kakkonto Extract contains not less than 9 mg and not more than 27 mg (for preparation prescribed 3 g of Ephedra Herb) or not less than 12 mg and not more than 36 mg (for preparation prescribed 4 g of Ephedra Herb) of total alkaloids [ephedrine (C_{10}H_{15}NO: 165.23) and pseudoephedrine (C_{10}H_{13}NO: 165.23)], not less than 14 mg and not more than 56 mg (for preparation prescribed 2 g of Peony Root) or not less than 21 mg and not more than 84 mg (for preparation prescribed 3 g of Peony Root) of peoniflorin (C_{23}H_{35}O_{11}: 480.46), and not less than 19 mg and not more than 57 mg of glycyrrhizic acid (C_{42}H_{62}O_{16}: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

<table>
<thead>
<tr>
<th></th>
<th>1)</th>
<th>2)</th>
<th>3)</th>
<th>4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pueraria Root</td>
<td>8 g</td>
<td>4 g</td>
<td>4 g</td>
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<tr>
<td>Ephedra Herb</td>
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<td>4 g</td>
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<td>Cinnamon Bark</td>
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<tr>
<td>Glycyrrhiza</td>
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<td>2 g</td>
<td>2 g</td>
<td>2 g</td>
</tr>
<tr>
<td>Ginger</td>
<td>1 g</td>
<td>1 g</td>
<td>1 g</td>
<td>2 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description

Kakkonto Extract occurs as a light brown to blackish brown powder or viscous extract. It has a characteristic odor, and a sweet first, then hot, and slightly bitter taste.

Identification

(1) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Puerarin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the bluish white fluorescent spot from the standard solution (Pueraria Root).

(2) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ephedrine hydrochloride in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.
μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 105°C for 5 minutes; one of the spot amongst the several spots from the sample solution has the same color tone and RT value with the red-purple spot from the standard solution (Ephedra Herb).

(3) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (E)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the standard solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot amongst the several spots from the sample solution has the same color tone and RT value with the yellow-orange spot from the standard solution (Cinnamon Bark).

(4) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Peoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot amongst the several spots from the sample solution has the same color tone and RT value with the blue-green spot from the standard solution (Ginger).

Purity (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed in the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41>—The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <5.01>—Not more than 10.0%, calculated on the dried basis.

Assay (1) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.03> according to the following conditions. Determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine with the sample solution, and the peak area, A_{S}, of ephedrine with the standard solution.

\[
\text{Amount (mg) of total alkaloids [ephedrine (C_{10}H_{15}NO) and pseudoephedrine (C_{10}H_{17}NO)]} = M_5 \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819
\]

\[
M_5: \text{Amount (mg) of ephedrine hydrochloride for assay}
\]

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of sodium lauryl sulfate (1 in 130), acetonitrile and phosphoric acid (650:350:1).

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(2) Peoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, elute with water to make exactly 20 mL of eluate, and use this as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of peoniflorin in each solution.

\[
\text{Amount (mg) of peoniflorin (C_{23}H_{28}O_{11})} = M₂ \times A₁/A₅ \times 1/2
\]

M₂: Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage—Containers—Tight containers.

Kamishoyosan Extract

加味逍遥散エキス

Kamishoyosan Extract contains not less than 28 mg and not more than 84 mg of peoniflorin (C_{23}H_{28}O_{11}; 480.46), not less than 25 mg and not more than 75 mg of geniposide, and not less than 12 mg and not more than 36 mg (for preparation prescribed 1.5 g of Glycyrrhiza) or not less than 16 mg and not more than 48 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid (C_34H_{32}O_{18}; 822.93), per extract prepared with the amount specified in the Method of preparation.
Prepare a dry extract or viscous extract as directed under Extracts, according to the crude drugs shown above.

**Description** Kamishoyosan Extract occurs as a yellow-brown to blackish brown powder or viscous extract. It has slightly a characteristic odor, and a sweet, slightly hot, then bitter taste.

**Identification** (1) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (Z)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a dark purple spot is observed at an Rf value with the bluish white fluorescent spot from the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography in 1 mL of methanol, and use this solution as the sample solution. Separate the mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthyl-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the red spot from the standard solution (Atractylodes Rhizome).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b₂ for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the red spot from the standard solution (Bupleurum Root).

(3) For preparation prescribed Atractylodes Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the red spot from the standard solution (Atractylodes Lancea Rhizome).

(4) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 µL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an Rf value of about 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(5) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b₂ for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL of the sample solution and 2 µL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the red spot from the standard solution (Bupleurum Root).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 15 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of peonol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-hydroxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the red spot from the standard solution (Bupleurum Root).
several spots from the sample solution has the same color tone and Rf value with the orange spot from the standard solution (Moutan Bark).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, centrifuge, and use the supernatant liquid as the standard solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28): (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the purple spot from the standard solution (Gardenia Fruit).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde and hexane (1:1) to a distance of about 10 cm, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Glycyrrhiza).
Glycyrrhizic acid in each solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizinic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizinic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizinic acid is not more than 1.5%.

**Containers and storage**

Containers—Tight containers.

### Keishibukuryogan Extract

Keishibukuryogan Extract contains not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 3 g of Cinnamon Bark) or not less than 0.8 mg and not more than 3.2 mg (for preparation prescribed 4 g of Cinnamon Bark) of (E)-cinnamic acid, not less than 30 mg and not more than 90 mg (for preparation prescribed 3 g each of Moutan Bark and Peony Root) or not less than 40 mg and not more than 120 mg (for preparation prescribed 4 g each of Moutan Bark and Peony Root) of peoniflorin (C_{23}H_{28}O_{11}: 480.46), and not less than 21 mg and not more than 63 mg (for preparation prescribed 3 g of Peach Kernel) or not less than 28 mg and not more than 84 mg (for preparation prescribed 4 g of Peach Kernel) of amygdalin, per extract prepared with the amount specified in the Method of preparation.

#### Method of preparation

<table>
<thead>
<tr>
<th>1)</th>
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<tr>
<td>Cinnamon Bark</td>
<td>4 g</td>
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<tr>
<td>Poria Sclerotium</td>
<td>4 g</td>
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<tr>
<td>Moutan Bark</td>
<td>4 g</td>
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<tr>
<td>Peach Kernel</td>
<td>4 g</td>
</tr>
<tr>
<td>Peony Root</td>
<td>4 g</td>
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</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) using the crude drugs shown above, or prepare a dry extract by adding Light Anhydrous Silicic Acid to an extrative, prepared as directed under Extracts, according to the prescription 2), using the crude drugs shown above.

**Description**

Keishibukuryogan Extract is a light brown to
blackish brown, powder or viscous extract. It has a characteristic odor and has a taste slightly sweet first then bitter later.

**Identification (1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of \((E)-\)cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and RF value with the orange fluorescent spot from the standard solution (Peony Root).

**Purity (1)** Heavy metals <1.07>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed in the Extracts (4), and perform the test (not more than 30 ppm).

**Loss on drying <2.47>** The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

**Total ash <5.01>** Not more than 10.0%, calculated on the dried basis. However, for the dry extract prepared by adding Light Anhydrous Silicic Acid, between 9.0% and 18.0%.

**Assay (1) (E)-Cinnamic acid—Conduct this procedure using light-resistant vessels. Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of \((E)-\)cinnamic acid for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \(A_T\) and \(A_S\), of (E)-cinnamic acid in each solution.

\[
\text{Amount (mg) of (E)-cinnamic acid} = M_S \times A_T/A_S \times 1/20
\]

\(M_S\): Amount (mg) of (E)-cinnamic acid for assay

**Operating conditions—**

- Detector: An ultraviolet absorption photometer (wavelength: 273 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: A mixture of water, acetonitrile and phosphoric acid (750:250:1).
- Flow rate: 1.0 mL per minute (the retention time of (E)-cinnamic acid is about 12 minutes).

**System suitability—**
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (E)-cinnamic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (E)-cinnamic acid is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) = M_S \times \frac{A_T}{A_S}$$

$M_S$: Amount (mg) of Paeoniflorin RS, calculated on the anhydrous basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 232 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 20°C.
Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).
Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—
System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution being not less than 2.5.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Amygdalin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin (C}_{17}\text{H}_{16}\text{O}_{13}) = M_S \times \frac{A_T}{A_S}$$

$M_S$: Amount (mg) of amygdalin for assay

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 45°C.
Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).
Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Containers and storage—Containers—Tight containers.

**Koi**

Koイ

Koi is a saccharified substance obtained by hydrolysis of the starch of Zea mays Linné (Gramineae), Manihot esculenta Crantz (Euphorbiaceae), Solanum tuberosum Linné (Solanaceae), Ipomoea batatas Poir et (Convolvulaceae) or Oryza sativa Linné (Gramineae), or the seed of Oryza sativa Linné from which the seed coat is removed.

Koi is prepared by the following processes 1 or 2, and contains mainly maltose, sometimes glucose and maltotriose also.

Process 1. Saccharize starch with hydrochloric acid, oxalic acid, amylase or wort, then concentrate to dryness, and powder.

Process 2. To starch or a paste of starch prepared by adding water and heating, add hydrochloric acid, oxalic acid, amylase or wort to saccharize, and dry or concentrate.

Koi prepared by Process 1 is termed “Koi 1” and by Process 2 is termed “Koi 2”. The label states the process.

Description
Koi 1: A white crystalline powder. It is odorless and has a sweet taste.
Koi 2: Colorless or brown, clear or semi-translucent, masses or viscous liquid. It is odorless and has a sweet taste.
Identification Dissolve exact 0.50 g of Koi in a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve exact 0.02 mg of maltose hydrate in a mixture of water and methanol (1:1) to make exactly 5 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2). Spot 1.0 mL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography in equal size of circular spot each other. Develop the plate with a mixture of 2-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,3,5-triphenyl-2H-tetrazolium chloride-methanol TS for spraying on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and RF value with the orange spot from the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2).<ref>
Total ash \(<5.0\%) Not more than 0.5%.<ref>
Containers and storage Containers—Well-closed containers.

Leonurus Herb

Leonuri Herba

ヤクモノソウ

Leonurus Herb is the aerial part of Leonurus japonicus Houttuyn or Leonurus sibiricus Linné (Labiatae), collected during the flowering season.

Description Stem, leaves, and flowers usually cross sectioned, stems squaré, 0.2 – 3 cm in diameter, yellow-green to green-brown in color, covered densely with white short hairs; the pith white, a great parts of central of sections. Light in texture. Leaves opposite, petiolated, 3-dissected to 3-incised, each lobes split pinnately, and end lobes reveals linear-lanceolate, acute or acuminate, the upper surface light green, the lower surface bristle with white short hairs, grayish green. Flower, verticillate; sepal, tubular, and the upper end acerate with five lobes; light green to light green-brown in color, corolla labiate, light red-purple to light brown.

Odor, slightly; taste, slightly bitter, astringent.

Under a microscope \(<5.0\%) , a transverse section of stem reveals four ridge, a parts of the ridge of Leonurus sibiricus Linné protruding knobby. Epidermis, observed non-glandular hairs from 1 to 3 cells, glandular hairs with head of 1 to 4 celled or glandular scale with 8 cells. Each ridge parts, beneath epidermis, collenchyma developed, development of xylem fibres remarkably. Cortex composed of several layers parenchymatous cells. Collateral vascular bundle arranged in a circle. Phloem fibres observed at the outer portion of phloem. Parenchymatous cells of cortex and pith observe needle crystals or plate-like crystals of calcium oxalate.

Identification To 1 g of pulverized Leonurus Herb add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography (2). Spot 10.0 mL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of water and methanol (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying followed by immediate spraying of sodium nitrite TS on the plate: a grayish brown spot appears at an RF value of about 0.5, which color fades soon and then disappears after air-drying the plate.

Loss on drying \(<5.0\%) Not more than 12.0%.

Total ash \(<5.0\%) Not more than 10.0%.

Acid-insoluble ash \(<5.0\%) Not more than 2.0%.

Extract content \(<5.0\%) Dilute ethanol-soluble extract: not less than 12.0%.

Containers and storage Containers—Well-closed containers.

Lilium Bulb

Lilii Bulbus

ビャクゴウ

Lilium Bulb is the scaly leaves of Lilium lancifolium Thunberg, Lilium brownii F.E. Brown var. colchesteri Wilson, Lilium brownii F.E.Brown or Lilium pumilum De Candolle (Liliaceae), usually with the application of steaming.

Description Lilium Bulb reveals oblong with narrowed apex, lanceolate, or narrowly triangular boat-shaped, translucent, 1.3 – 6 cm in length, 0.5 – 2.0 cm in diameter, externally milky white to light yellow-brown occasionally purplish in color, nearly smooth; central portion somewhat thickened, circumferential portion thin, slightly waved, occasionally rolled inside; usually several lines of vascular bundles longitudinally in parallel are seen through parenchyma; hard in texture, easy to break; fractured surface horny and flat.

Odorless; taste, slightly acid and bitter.

Under a microscope \(<5.0\%) , the surface reveals epidermal
cells rectangular to almost square, stomata nearly circular, the cells adjacent to stomata mostly 4 in number. Under a microscope \( \lessdot 5.01 \), a transverse section reveals the outermost layer composed of epidermal cells covered with smooth cuticle; beneath epidermis circular to quadrangular parenchymatous cells distributed evenly, palisade tissue not observed; in parenchyma of mesophyll collateral vascular bundles extended from adaxial side to abaxial side of scaly leaves are arranged almost in a transverse line; starch grains contained in parenchymatous cells, usually gelatinized.

**Identification** To 3 g of pulverized Lilium Bulb add 10 mL of 1-butanol, shake, add 10 mL of water, shake for 30 minutes, and centrifuge. Evaporate the supernatant liquid under reduced pressure, add 1 mL of methanol to the residue, shake gently, and use the supernatant liquid so obtained as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography \( \leq 2.03 \). Spot 10 \( \mu L \) of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water \((12:2:1)\) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spotting on the plate: a yellow-brown spot appears at an \( R_f \) value of about 0.3. When examine these spots under ultraviolet light (main wavelength: 254 nm): two spots appear at an \( R_f \) value of about 0.4.

**Loss on drying** \( \leq 5.01 \) Not more than 16.0%.

**Total ash** \( \leq 5.01 \) Not more than 4.5%.

**Extract content** \( \leq 5.01 \) Dilute ethanol-soluble extract: not less than 8.0%.

**Containers and storage** Containers—Well-closed containers.

**Lindera Root**

**Linderae Radix**

シコン

Lindera Root is the root of *Lindera strychnifolia* Fernandez-Villar (Lauraceae).

**Description** Fusiform or rosary-like root, 10 – 15 cm in length, 10 – 25 mm in diameter; externally yellow-brown to brown, with a few scars of rootlets; a transverse section reveals cortex brown, xylem light yellow-brown, concentric circles and radially arranged lines brown; dense and hard in texture.

Odor, camphor-like; taste, bitter.

Under a microscope \( \leq 5.01 \), a transverse section of the root with periderm reveals a cork layer several cells thick, partially consisting of cork stone cells; cortex parenchyma sometimes contains oil cells and fibers; in xylem, vessels-xylem fibers and rays are arranged alternately; parenchymatous cells of cortex and xylem contain sandy and columnar crystals of calcium oxalate, simple starch grains 1 – 15 \( \mu m \) in diameter, and 2- to 4- compound starch grains.

**Identification** To 3 g of pulverized Lindera Root add 40 mL of hexane, and warm under a reflux condenser on a water bath for 30 minutes. After cooling, filter, to the residue add 10 mL of ammonia TS and 30 mL of a mixture of ethyl acetate and diethyl ether \((1:1)\), shake vigorously for 20 minutes, and centrifuge. Separate the supernatant liquid, add 10 g of anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate, dissolve the residue with 0.5 mL of ethanol \((99.5)\), and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography \( \leq 2.03 \). Spot 20 \( \mu L \) of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and ammonia water \((28)\) \((10:2:1)\) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a yellow-brown spot appears at an \( R_f \) value of about 0.4.

**Purity (1)** Heavy metals \( \leq 1.07 \)—Proceed with 3.0 g of pulverized Lindera Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

**Identification (2)** Arsenic \( \leq 1.11 \)—Prepare the test solution with 0.40 g of pulverized Lindera Root according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying** \( \leq 5.01 \) Not more than 14.0% (6 hours).

**Total ash** \( \leq 5.01 \) Not more than 2.5%.

**Extract content** \( \leq 5.01 \) Dilute ethanol-soluble extract: not less than 6.0%.

**Containers and storage** Containers—Well-closed containers.

**Lithospermum Root**

**Lithospermum Root**

**Lithospermum Root**

Lithospermum Root is the root of *Lithospermum erythrorhizon* Siebold et Zuccarini (Boraginaceae).

**Description** Rather slender conical root, often branched, 6 – 10 cm in length, 0.5 – 1.5 cm in diameter; externally dark purple, coarse in texture, thin and easily peeled; mostly with twisted and deep longitudinal furrows, which sometimes reach to xylem; sometimes remains of stem at the crown; easily broken; fractured surface granular and with many clefts. Under a magnifying glass, a transverse section reveals a dark purple color at the outer portion of cortex, and light brown inner portion making irregular wave; xylem yellowish in color; the center of the crown is often cracked, and the surrounding part red-purple.

Odor, slight; taste, slightly sweet.

**Identification (1)** Heat 0.5 g of pulverized Lithospermum Root in a test tube: red vapor evolves, which condenses on the wall of the upper part of the tube into red-brown oil drops.

**Identification (2)** Shake 0.5 g of pieces or powder of Lithospermum Root with 1 mL of ethanol \((95)\), and to the red solution thereby obtained add 1 drop of sodium hydroxide TS; the red color changes to blue-purple. To this solution add 1 to 2 drops of dilute hydrochloric acid: the color turns red again.
Longan Aril / Crude Drugs

Longan Arillus

Longan Aril is the aril of *Euphoria longana* Lamarck (Sapindaceae).

Description Depressed ellipsoidal aril, 1 – 2 cm in length, about 1 cm in width; yellowish red-brown to blackish brown; soft in texture and mucous; when immersed in water, bell-shaped, with the tip split in several parts.

Odor, characteristic; taste, sweet.

Under a microscope, a transverse section reveals the outmost layer composed of a single-layered epidermis, beneath this observed parenchyma consisting of depressed parenchyma cells; the innermost layer composed of slightly thick-walled epidermis; parenchyma contains red-brown to brown contents as well as solitary crystals, amorphous crystals and sand crystals of calcium oxalate.

Identification To 1 g of coarse cuttings of Longan Aril, add 10 mL of water, shake thoroughly, and filter. To 3 mL of the filtrate, add 3 mL of Fehling solution, and heat on a water bath: a red precipitate is produced.

Total ash < 0.01% Not more than 5.0%.

Extract content < 0.01% Dilute ethanol-soluble extract: Not less than 75.0%.

Containers and storage Containers—Well-closed containers.

Longgu

Fossilia Ossis Mastodi

リュウコツ

Longgu is the ossified bone of large mammal, and is mainly composed of calcium carbonate.

For Longgu used only for extracts, infusions and decoctions, the label states the restricted utilization forms.

Description Irregular masses or fragments, occasionally cylindrical masses; externally light grayish white, sometimes with grayish black or yellow-brown spots here and there; the outer part consists of a layer 2 – 10 mm in thickness, and is minute in texture, surrounding the light brown, porous portion; heavy and hard, but somewhat fragile in texture; when crushed, it changes into pieces and powder.

Odorless, tasteless, and strongly adhesive to the tongue on licking.

Identification (1) Dissolve 0.5 g of pulverized Longgu in 10 mL of dilute hydrochloric acid: it evolves a gas, and forms a slightly brownish and turbid solution. Pass the gas evolved through calcium hydroxide TS: a white precipitate is produced.

(2) The turbid solution obtained in (1) has a characteristic odor. Filter this solution and neutralize filtrate with ammonia TS: this solution responds to the Qualitative Tests for calcium salt.

(3) Dissolve 0.1 g of pulverized Longgu in 5 mL of nitric acid by warming, and add hexaammonium heptamolybdate TS: a yellow precipitate is produced.

Purity (1) Heavy metals < 0.07% — To 2.0 g of pulverized Longgu, add 5 mL of water, shake, add gradually 6 mL of hydrochloric acid, evaporate on a water bath to dryness, dissolve the residue in 50 mL of water, and filter. To 25 mL of the filtrate, add 2 mL of dilute acetic acid, 1 drop of ammonia TS and water to make 50 mL. Perform the test with this solution as the test solution. Separately, evaporate 3 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL, and use this solution as the control solution (not more than 20 ppm).

When being shown as extracts, infusions and decoctions on the label, the procedure and the limit are as follows. To 20.0 g of pulverized Longgu, add 80 mL of water, shake occasionally in a water bath, heat to make about 40 mL, allow to cool, and filter. Proceed with this solution according to Method 3, and perform the test. To the control solution, add 1.0 mL of Standard Lead Solution (not more than 0.5 ppm).

(2)Arsenic < 0.001% — Prepare the test solution with 0.20 g of pulverized Longgu according to Method 2, and perform the test (not more than 10 ppm).

When being shown the restricted utilization forms as "extracts, infusions and decoctions only", the procedure and the limit are as follows. Put 4.0 g of pulverized Longgu in a centrifuge tube, add 30 mL of water, and heat in a water bath with occasional shaking to make about 15 mL. After cooling, centrifuge,
and perform the test using the supernatant liquid as the test solution (not more than 0.5 ppm).

Containers and storage Containers—Well-closed containers.

**Powdered Longgu**

*Fossilia Ossis Mastodi Pulveratus*

リュウコツ末

Powdered Longgu is the powder of Longgu.

Description Powdered Longgu occurs as a light grayish-white to light grayish brown. It is odorless and tasteless.

Identification (1) Dissolve 0.1 g of Powdered Longgu in 5 mL of nitric acid by warming, and add hexaammonium heptamolybdate TS: a yellow precipitate is produced.

(2) Dissolve 0.5 g of Powdered Longgu in 10 mL of dilute hydrochloric acid: it evolves a gas, and forms a slightly brownish and turbid solution. Pass the gas evolved through calcium hydroxide TS: a white precipitate is produced.

(3) The turbid solution, obtained in (2), has a characteristic odor. Filter this solution, and neutralize with ammonia TS: the solution responds to the Qualitative test \(<1.09\) for calcium salt.

Purity (1) Heavy metals \(<1.07\) — To 2.0 g of Powdered Longgu add 5 mL of water, shake to mix, add carefully 6 mL of hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of water, and filter. To 25 mL of the filtrate add 2 mL of dilute acetic acid, 1 drop of ammonia TS and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: Evaporate 3 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and add water to make 50 mL (not more than 20 ppm).

(2) Arsenic \(<1.17\) — Prepare the test solution with 0.20 g of Powdered Longgu according to Method 2, and perform the test (not more than 10 ppm).

Containers and storage Containers—Well-closed containers.

**Lonicera Leaf and Stem**

*Lonicerae Folium Cum Caulis*

ニンドウ

Lonicera Leaf and Stem is the leaves and stems of *Lonicera japonica* Thunberg (*Caprifoliaceae*).

Description Leaves and opposite leaves on short stem; leaf, ovate and entire, with short petiole, 3 – 7 cm in length, 1 – 3 cm in width; upper surface greenish brown, lower surface light grayish green; under a magnifying glass, both surfaces pubescent. Stem, 1 – 4 mm in diameter; externally grayish yellow-brown to purplish brown, a transverse section of stem, round and hollow.

Almost odorless; taste, slightly astringent, followed by a litter bitterness.

Under a microscope \(<5.01\) , a transverse section of leaf reveals the outermost layer of upper and lower surfaces to be composed of a single-layered epidermis, uni-cellular non-glandular hairs and multi-cellular glandular hairs on epidermis; in midvein, several-layered collenchyma present beneath the epidermis and vascular bundles in the center; in mesophyll, palisade layer adjacent to upper epidermis, spongy tissue adjacent to lower epidermis; glandular hairs contain brown secretion, parenchymatous cells contain aggregate crystals of calcium oxalate, and occasionally starch grains.

Identification To 1 g of pulverized Lonicera Leaf and Stem add 5 mL of methanol, shake for 5 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of chlorogenic acid for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (1). Separately, dissolve 1 mg of loganin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\) . Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and RF value with the bluish white fluorescent spot from the standard solution (1). Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and RF value with the red-purple spot from the standard solution (2).

Purity Stem—Lonicera Leaf and Stem does not contains the stems larger than 5 mm in diameter.

Loss on drying \(<5.01\) Not more than 12.0% (6 hours).

Total ash \(<5.01\) Not more than 9.0%.

Acid-insoluble ash \(<5.01\) Not more than 1.0%.

Extract content \(<5.01\) Dilute ethanol-soluble extract: not less than 12.0%.

Containers and storage Containers—Well-closed containers.

**Loquat Leaf**

*Eriobotryae Folium*

ピワヨウ

Loquat Leaf is the leaf of *Eriobotrya japonica* Lindley (*Rosaceae*).

Description Loquat Leaf is an oblong to wide lanceolate leaf, 12 – 30 cm in length, 4 – 9 cm in width; pointed at the apex and wedge-shaped at the base; roughly serrate leaf with
short petiole; occasionally being cut into strips 5 – 10 mm in shorter diameter and several cm in longer diameter; upper surface green to green-brown in color, lower surface light green-brown with light brown woolly hairs; vein, light yellow-brown in color, raised out on the lower surface of the leaf.

Odor, slight; practically tasteless.

Under a microscope \(<5.01\) a transverse section of Loquat Leaf reveals thick cuticle on both surfaces; palisade tissue, mostly 4 to 5 layers with several large cells without chlorenchyma; at main vein, ring of collateral bundle partly cut by intruding fundamental tissue at xylem side, and group of fibers attaching to phloem; solitary and clustered crystals of calcium oxalate in mesophyll; woolly hair, unicellular and curved, about 25 \(\mu m\) in thickness, and up to 1.5 mm in length.

Identification To 0.3 g of pulverized Loquat Leaf add 10 mL of methanol, warm on a water bath for 5 minutes with occasional shaking, cool, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography \(<2.03\). Spot 5 \(\mu L\) of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (3:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate, heat at 105°C for 3 minutes, then spray evenly sodium nitrite TS: a dark brown principal spot appears at an RF value of about 0.5.

Purity Total BHC’s and total DDT’s \(<5.01\)—Not more than 0.2 ppm, respectively.

Loss on drying \(<5.01\) Not more than 15.0% (6 hours).

Total ash \(<5.01\) Not more than 10.0%.

Extract content \(<5.01\) Dilute ethanol-soluble extract: not less than 16.0%.

Containers and storage Containers—Well-closed containers.

Lycium Bark

Lycii Cortex

ジコッピ

Lycium Bark is the root bark of Lycium chinense Miller or Lycium barbarum Linné (Solanaceae).

Description Tubular to semitubular bark, 1 – 6 mm in thickness; externally light brown to light yellow-brown, periderm peeled easily as scale; internally grayish brown, longitudinally striate; brittle in texture; fractured surface, grayish white, not fibrous.

Odor, weak and characteristic; taste, sweet, later slightly bitter.

Under a microscope \(<5.01\), a transverse section reveals periderm composed of a cork layer of several layers of thin walled cork cells; in cortex parenchymatous cells containing sandy crystals of calcium oxalate sparsely distributed, occasionally a few fibers observed; parenchymatous cells contain starch grains, 1 – 10 \(\mu m\) in diameter; stone cells very rare.

Identification To 1.0 g of pulverized Lycium Bark add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography \(<2.03\). Spot 10 \(\mu L\) of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (3:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate, heat at 105°C for 3 minutes, then spray evenly sodium nitrite TS: a dark brown principal spot appears at an RF value of about 0.5.

Purity (1) Heavy metals \(<1.07\)—Proceed with 3.0 g of pulverized Lycium Bark according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.11\)—Prepare the test solution with 0.40 g of pulverized Lycium Bark according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying \(<5.01\) Not more than 11.5% (6 hours).

Total ash \(<5.01\) Not more than 20.0%.

Acid-insoluble ash \(<5.01\) Not more than 3.0%.

Extract content \(<5.01\) Dilute ethanol-soluble extract: not less than 10.0%.

Containers and storage Containers—Well-closed containers.

Lycium Fruit

Lycii Fructus

クロシ

Lycium Fruit is the fruit of Lycium chinense Miller or Lycium barbarum Linné (Solanaceae).

Description Fusiform fruit with acute apex, 6 – 20 mm in length, 3 – 8 mm in diameter, pericarp red to dark red, externally roughly wrinkled; under a magnifying glass, a transverse section of fruit reveals two locules containing numerous seeds; seed light brown to light yellow-brown, about 2 mm in a diameter, compressed reniform.

Odor, characteristic; taste, sweet, later slightly bitter.

Identification To 1.0 g of powdered Lycium Fruit add 5 mL of ethyl acetate, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography \(<2.03\). Spot 20 \(\mu L\) of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (10:1) to a distance of about 10 cm, and air-dry the plate: a yellow principal spot appears at an RF value of about 0.6.

Purity Foreign matter \(<5.01\)—It contains not more than 2.0% of foreign matter such as peduncle or others.

Total ash \(<5.01\) Not more than 8.0%.

Acid-insoluble ash \(<5.01\) Not more than 1.0%.

Extract content \(<5.01\) Dilute ethanol-soluble extract: not
Magnolia Bark

Magnoliae Cortex


It contains not less than 0.8% of magnolol.

**Description** Plate-like or semi-tubular bark, 2 – 7 mm in thickness; externally grayish white to grayish brown, and rough, sometimes cork layer removed, and externally red-brown; internally light brown to dark purple-brown; cut surface extremely fibrous, and light red-brown to purple-brown.

Odor, slight; taste, bitter.

Under a microscope (<5.01>, a transverse section reveals a thick cork layer or several thin cork layers, and internally adjoining the circular tissue of stone cells of approximately equal in diameter; primary cortex thin; fiber groups scattered in the pericycle; phloem fibers lined stepwise between medullary rays in the secondary cortex, and then these tissues show a latticework; oil cells scattered in the primary and secondary cortex, but sometimes observed in the narrow medullary rays.

**Identification** To 1.0 g of pulverized Magnolia Bark add 10 mL of methanol, stir for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate, spray evenly with Dragendorff’s TS: a yellow spot appears at an Rf value of about 0.3. 

**Total ash** Not more than 6.0%.

**Extract content** Dilute ethanol-soluble extract: not less than 11.0%.

**Assay** Weigh accurately about 0.5 g of pulverized Magnolia Bark add 10 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of diluted methanol (7 in 10). Combine the whole filtrates, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, dry magnolol for assay in desiccator (silica gel) for 1 hour or more. Weigh accurately about 10 mg of it, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A1 and A5, of magnolol.

\[
\text{Amount (mg) of magnolol} = M_S \times \frac{A_T}{A_S}
\]

\[
M_S = \text{Amount (mg) of magnolol for assay}
\]

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecyl-silanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: Adjust the flow rate so that the retention time of magnolol is about 14 minutes.

**System suitability**—

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

Powdered Magnolia Bark

**Magnoliae Cortex Pulveratus**

Powdered Magnolia Bark is the powder of Magnolia Bark.

It contains not less than 0.8% of magnolol.

**Description** Powdered Magnolia Bark occurs as a yellow-brown powder, and has a slight odor and a bitter taste.

Under a microscope (<5.01>, Powdered Magnolia Bark reveals starch grains and parenchyma cells containing them; stone cells of various sizes or its groups; fibers 12 to 25 μm in diameter; yellowish red-brown cork tissue; oil cells containing a yellow-brown to red-brown substance. Simple starch grains about 10 μm in diameter and 2- to 4-compound starch grains.

**Identification** To 1.0 g of Powdered Magnolia Bark add 10 mL of methanol, stir for 10 minutes, centrifuge, and perform the test with the supernatant liquid as the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate, and spray evenly with Dragendorff’s TS on the plate: a yellow spot appears at an Rf value of about 0.3.
Total ash \(<5.01\) Not more than 6.0%.

Extract content \(<5.01\) Dilute ethanol-soluble extract: not less than 11.0%.

**Assay**

Weigh accurately about 0.5 g of Powdered Magnolia Bark, add 40 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of diluted methanol (7 in 10). Combine the whole filtrates, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, dry magnolol for assay in a desiccator (silica gel) for 1 hour or more. Weigh accurately about 10 mg of it, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of magnolol.

Amount (mg) of magnolol = \( M_S \times \frac{A_T}{A_S} \)

\( M_S \): Amount (mg) of magnolol for assay

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: Adjust the flow rate so that the retention time of magnolol is about 14 minutes.

**System suitability**—

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 \( \mu L \) of this solution under the above operating conditions, honokiol and magnolol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

**Containers and storage**

Containers—Tight containers.

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**Magnolia Flower**

*Magnoliae Flos*

**シンイ**


**Description**

Magnolia Flower is a fusiform flower bud, 15 – 45 mm in length, 6 – 20 mm in diameter at central part; often having lignose peduncles on base; usually 3 bracts, externally with sparse hairs, brown to dark brown, or with dense hairs, grayish white to light yellow-brown, and the inner surface of 3 bracts smooth and dark brown in color; interior perianth of 9 pieces or 12 pieces, same size or outer three pieces are smaller; 50 – 100 stamens and numerous pistils. Brittle in texture.

**Odor**

Characteristic; taste, acrid and slightly bitter.

**Identification**

To 1 g of pulverized Magnolia Flower add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography \(<2.03\). Spot 20 \( \mu L \) of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, acetone, water and formic acid (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate: a yellow-red spot appears at an \( Rf \) value of about 0.3.

**Loss on drying** \(<5.01\) Not more than 14.0% (6 hours).

**Total ash** \(<5.01\) Not more than 5.5%.

**Acid-insoluble ash** \(<5.01\) Not more than 1.5%.

**Extract content** \(<5.01\) Dilute ethanol-extract: not less than 13.0%.

**Essential oil content** \(<5.01\) Perform the test with 50.0 g of pulverized Magnolia Flower: the volume of essential oil is not less than 0.5 mL.

**Containers and storage**

Containers—Well-closed containers.

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**Mallotus Bark**

*Malloti Cortex*

アカメガシワ

Mallotus Bark is the bark of *Mallotus japonica* Mueller Argoviensis. (Euphorbiaceae).

**Description**

Mallotus Bark is flat or semitubular pieces of bark, 1 – 3 mm in thickness; externally greenish gray to brownish gray brown in color, with a vertically stripped shape gathering numerous lenticels; internal surface light yellow-brown to grayish brown in color, and smooth with numerous fine striped lines; easy to break; slightly fibrous at fracture surface.

Mallotus Bark has a slight odor, a bitter taste and slightly astringent.

**Identification**

To 0.5 g pulverized Mallotus Bark add 10 mL of methanol, warm on a water bath for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of bergenin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\). Spot 10 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and water (100:17:13) to a distance of about 10 cm, and
Mentha Herb

Mentha Herb is the terrestrial part of Mentha arvensis Linné var. piperascens Malinvaud (Labiatae).

**Description** Stem with opposite leaves; stem, square, light brown to red-purple in color, and with fine hairs; when smoothed by immersing in water, leaf, ovate to oblong, with acute apex and base, 2–8 cm in length, 1–2.5 cm in width, margin irregularly serrated; the upper surface, light brown-yellow to light green-yellow, and the lower surface, light green to light green-yellow in color; petiole 0.3–1 cm in length. Under a magnifying glass, leaf reveals hairs, glandular hairs and scales.

It has a characteristic aroma and gives a cool feeling on keeping in the mouth.

**Identification** To 1 mL of the mixture of essential oil and xylene, obtained in the Essential oil content, add carefully 2 mL of sulfuric acid to make two layers: a deep red to red-purple in color, and with fine hairs; when shaking: Mentha Oil dissolves clearly. To the solution add 10 mL of sulfuric acid VS and 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

**Purity** Foreign matter Not more than 2.0%.

**Loss on drying** Not more than 15.0% (6 hours).

**Total ash** Not more than 12.0%.

**Acid-insoluble ash** Not more than 2.5%.

**Extract content** Dilute ethanol-soluble extract: not less than 11.0%.

**Containers and storage** Containers—Well-closed containers.

**Mentha Oil**

**Oleum Menthae Japonicae**

Mentha Oil is the essential oil which is distilled with steam from the terrestrial parts of Mentha arvensis Linné var. piperascens Malinvaud (Labiatae), and from which solids are removed after cooling.

It contains not less than 30.0% of menthol (C_{10}H_{20}O: 156.27).

**Description** Mentha Oil is a colorless or pale yellow, clear liquid. It has a characteristic, pleasant aroma and has a pungent taste, followed by a cool aftertaste.

It is miscible with ethanol (95), with ethanol (99.5), with warm ethanol (95), and with diethyl ether.

It is practically insoluble in water.

**Refractive index**<2.45> \( n_\varphi^0 \): 1.455–1.467

**Optical rotation**<2.49> \( [\alpha]_D^20 \): –17.0°––36.0° (100 mm).

**Specific gravity**<1.13> \( d_20^\circ \): 0.885–0.910

**Acid value** Not more than 1.0.

**Purity** (1) Clarity and color of solution—To 1.0 mL of Mentha Oil add 3.5 mL of diluted ethanol (7 in 10), and shake: Mentha Oil dissolves clearly. To the solution add 10 mL of ethanol (95): the solution is clear or has no more turbidity, if any, than the following control solution.

Control solution: To 0.70 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(2) Heavy metals Not more than 0.07—Proceed with 1.0 mL of Mentha Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

**Assay** Weigh accurately about 5 g of Mentha Oil, and dissolve in ethanol (95) to make exactly 20 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 10 g of \( \alpha \)-menthol for assay, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 mL each of the sample solution and standard solution as directed under Gas Chromatography according to the following conditions. Calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of menthol to that of the internal standard.

\[
\text{Amount (mg) of menthol (C}_{10}\text{H}_{20}\text{O}) = M_S \times Q_T / Q_S
\]

\(M_S\): Amount (mg) of \( \alpha \)-menthol for assay

**Internal standard solution**—A solution of \( n \)-ethyl caprylate in ethanol (95) (1 in 25).

**Operating conditions**—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter.
and about 2 m in length, packed with 25% of polyethylene glycol 6000 for gas chromatography supported on acid-washed 180 – 250 μm siliceous earth for gas chromatography.

Column temperature: A constant temperature of about 150°C.
Carrier gas: Nitrogen.
Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.
Selection of column: Proceed with 1 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and l-menthol in this order with the resolution between these peaks being not less than 5.

Containers and storage
Containers—Tight containers.
Storage—Light-resistant.

**Mentha Water**

**Method of preparation**

| Mentha Oil | 2 mL |
| Purified Water or Purified Water in Containers | a sufficient quantity |

To make 1000 mL

Prepare as directed under Aromatic Waters, with the above ingredients.

**Description** Mentha Water is a clear, colorless liquid, having the odor of mentha oil.

**Containers and storage** Containers—Tight containers.

**Moutan Bark**

**Moutan Cortex**

ボタンピ

Moutan Bark is the root bark of *Paeonia suffruticosa* Andrews (*Paeonia moutan* Sims) (*Paeoniaceae*).

It contains not less than 1.0% of paeonol.

**Description** Tubular to semi-tubular bark, about 0.5 cm in thickness, 5 – 8 cm in length, 0.8 – 1.5 cm in diameter; externally dark brown to purplish brown, with small and transversely elongated ellipsoidal scars of lateral roots, and with longitudinal wrinkles; internally, light grayish brown to purplish brown and smooth; fractured surface coarse; white crystals often attached on the internal and fractured surfaces.

Odor, characteristic; taste, slightly pungent and bitter.

**Identification** To 2.0 g of pulverized Moutan Bark add 10 mL of hexane, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot among several spots from the sample solution is similar with the spot from the standard solution in color tone and Rf value.

**Purity** (1) Xylen—When perform the test of foreign matter <5.01>, the amount of the xylem contained in Moutan Bark is not more than 5.0%.

(2) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Moutan Bark according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Moutan Bark according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <5.01>—The amount of foreign matter other than xylem contained in Moutan Bark is not exceed 1.0%.

(5) Total BHC’s and total DDT’s <5.01>—Not more than 0.2 ppm, respectively.

**Total ash** <5.01> Not more than 6.0%.

**Acid-insoluble ash** <5.01> Not more than 1.0%.

**Assay** Weigh accurately about 0.3 g of pulverized Moutan Bark, add 40 mL of methanol, heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of methanol. Combine the whole filtrates, add methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Separately, dry paeonol for assay in a desiccator (calcium chloride for drying) for more than 1 hour. Weigh accurately about 10 mg of it, dissolve in methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A1 and A5, of paeonol.

\[
\text{Amount (mg) of paeonol} = M_5 \times \frac{A_1}{A_5} \times \frac{1}{2}
\]

*M5: Amount (mg) of paeonol for assay*

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelenth: 274 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile, and acetic acid (100) (65:35:2).

Flow rate: Adjust the flow rate so that the retention time of paeonol is about 14 minutes.

Selection of column: Dissolve 1 mg of paeonol for assay and 5 mg of butyl parahydroxybenzoate in 25 mL of metha-
nol. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of paeonol and butyl parahydroxybenzoate in this order, with the resolution between these peaks being not less than 2.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of paeonol is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

## Powdered Moutan Bark

*Moutan Cortex Pulveratus*

粉末白芍

Powdered Moutan Bark is the powder of Moutan Bark.

It contains not less than 0.7% of paeonol.

**Description** Powdered Moutan Bark occurs as a light grayish yellow-brown powder. It has a characteristic odor and a slight, pungent and bitter taste.

Under a microscope \(<5.0\times\), Powdered Moutan Bark reveals starch grains and fragments of parenchyma containing them; fragments of cork tissue containing tannin; fragments of somewhat thick-walled collenchyma, medullary rays, and phloem parenchyma; rosette aggregates of calcium oxalate and also fragments of parenchyma cells containing them. Starch grains are simple or 2- to 10-compound grains, 10 – 25 μm in diameter; rosette aggregates are 20 – 30 μm in diameter.

**Identification**

1. To 2.0 g of Powdered Moutan Bark add 10 mL of hexane, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.

2. Evaporate to dryness 1 mL of the sample solution obtained in (1), dissolve the residue in ethanol (95) to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry. It exhibits maxima at around 228 nm, 274 nm and 308 nm.

**Purity**

1. Heavy metals \(<1.0\times\)—Proceed with 3.0 g of Powdered Moutan Bark according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

2. Arsenic \(<1.1\times\)—Prepare the test solution with 0.40 g of Powdered Moutan Bark according to Method 4, and perform the test (not more than 5 ppm).

3. Foreign matter—Under a microscope \(<5.0\times\), usually vessels and other thick-walled cells are not observable.

4. Total BHC’s and total DDT’s \(<5.0\times\)—Not more than 0.2 ppm, respectively.

**Total ash** \(<5.0\times\) Not more than 6.0%.

**Acid-insoluble ash** \(<5.0\times\) Not more than 1.0%.

**Assay**

Weigh accurately about 0.5 g of Powdered Moutan Bark, add 40 mL of methanol, heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of methanol. Combine the whole filtrates, add methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, dry paeonol for assay in a desiccator (calcium chloride for drying) for more than 1 hour. Weigh accurately about 10 mg of it, dissolve in methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.0\times\) according to the following conditions, and determine the peak areas, \(A_1\) and \(A_5\), of paeonol.

\[
\text{Amount (mg) of paeonol} = M_s \times A_1 / A_5 \times 1/2
\]

\(M_s\): Amount (mg) of paeonol for assay

**Operating conditions**

- Detector: An ultraviolet absorption photometer (wavelength: 274 nm).
- Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).
- Column temperature: A constant temperature of about 20°C.
- Mobile phase: A mixture of water, acetonitrile, and acetic acid (100:65:35:2).
- Flow rate: Adjust the flow rate so that the retention time of paeonol is about 14 minutes.
- Selection of column: Dissolve 1 mg of paeonol for assay and 5 mg of butyl parahydroxybenzoate in 25 mL of methanol. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of paeonol and butyl parahydroxybenzoate in this order, with the resolution between these peaks being not less than 2.
- System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of paeonol is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

## Mukoi-Daikenchuto Extract

無コウイ大建中湯エキス

Mukoi-Daikenchuto Extract contains not less than 1.8 mg of ginsenoside Rb1 (\(C_{54}H_{92}O_{23}\); 1109.29), and...
not less than 1.4 mg and not more than 4.2 mg of [6]-shogaol, per extract prepared with the amount specified in the Method of preparation.

**Method of preparation** Prepare a dry extract as directed under Extracts, with 2 g of Zanthoxylum Fruit, 3 g of Ginseng and 5 g of Processed Ginger.

**Description** Mukoi-Daikenchuto Extract is a light brown powder. It has a slight odor, and has a pungent taste.

**Identification** (1) Shake 2.0 g of Mukoi-Daikenchuto Extract with 10 mL of water, then shake with 10 mL of diethyl ether, centrifuge, and use the supernatant liquid as the sample solution. Separately, pulverize zanthoxylum fruit, shake 2.0 g with 10 mL of water, then shake with 5 mL of diethyl ether, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Prepare the test solution with 2.0 g of Mukoi-Daikenchuto Extract according to Method 3, and perform the test (not more than 1 ppm).

**Loss on drying** Not more than 5.9% (1 g, 105°C, 5 hours).

**Assay** (1) Ginsenoside Rb1.—Weigh accurately about 2 g of Mukoi-Daikenchuto Extract, add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 15 mL of diluted methanol (3 in 5), and repeat the same procedure. Combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of sodium hydroxide TS, allow to stand for 30 minutes, add 3 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 20 mL. Apply exactly 5 mL of this solution to a column [10 mm in inside diameter, packed with 0.36 g of octadecylsilanized silica gel for pre-treatment (55–105 μm in particle size), and washed just before using with methanol and then diluted methanol (3 in 10)], and wash the column in sequence with 2 mL of diluted methanol (3 in 10), 1 mL of sodium carbonate TS and 10 mL of diluted methanol (3 in 10). Finally, elute with methanol to collect exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb1 RS (separately determine the water), and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A1 and A5, of ginsenoside Rb1 in each solution.

\[
\text{Amount (mg) of ginsenoside Rb1 (C}_{38}\text{H}_{52}\text{O}_{23}) = M_s \times A_1/A_5 \times 1/5
\]

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with carboxymethyl group bound silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: A mixture of acetonitrile and water (4:1).

Flow rate: 1.0 mL per minute (the retention time of ginsenoside Rb1 is about 16 minutes).

**System suitability**

- Mobile phase: A mixture of acetonitrile and water (4:1).
- Flow rate: 1.0 mL per minute (the retention time of ginsenoside Rb1 is about 16 minutes).
- System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ginsenoside Rb1 are not less than 3000 and not more than 1.5, respectively.
- System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb1 is not more than 1.5%.
(2) [6]-Shogaol—Weigh accurately about 0.5 g of Mukoi-Daikenchuto Extract, add exactly 50 mL of diluted methanol (3 in 4), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of [6]-shogaol for assay, dissolve in diluted methanol (3 in 4) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (3 in 4) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of [6]-shogaol in each solution.

\[
M_S = \frac{S \times A_T}{A_S} \times \frac{1}{10}
\]

Amount (mg) of [6]-shogaol = $M_S$ \\
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 225 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 50°C.
Mobile phase: Dissolve 0.1 g of oxalic acid dihydrate in 600 mL of water, and add 400 mL of acetonitrile.
Flow rate: 1.0 mL per minute (the retention time of [6]-shogaol is about 30 minutes).
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-shogaol is not more than 1.5%.

Containers and storage Containers—Tight containers.

## Mulberry Bark

*Mori Cortex*

ソウハクヒ

Mulberry Bark is the root bark of *Morus alba* Linné (*Moraceae*).

**Description**

Tubular, semi-tubular or cord-like bark, 1 – 6 mm thick, often in fine lateral cuttings; externally, white to yellow-brown; in the case of the bark with periderm, its periderm is yellow-brown in color, easy to peel, with numerous longitudinal, fine wrinkles and numerous red-purple lenticels laterally elongated; inner surface, dark yellow-brown in color and flat; cross section, white to light brown in color, and fibrous.

Odor, slight; taste, slight.

Under a microscope <5.01>, a transverse section of bark with periderm reveals 5 to 12 layers of cork cells in the outer portion; phloem fibers or their bundles scattered in the cortex, arranged alternately and stepwise with phloem parenchyma; lactiferous tubes; solitary crystals of calcium oxalate; starch grains as spheroidal or ellipsoidal, simple or compound grains, simple grain 1 – 7 μm in diameter.

**Identification**

Heat 1 g of pulverized Mulberry Bark with 20 mL of hexane under a reflux condenser on a water bath for 15 minutes, and filter. Evaporate the hexane of the filtrate under reduced pressure, dissolve the residue in 10 mL of acetic anhydride, place 0.5 mL of the solution in a test tube, and add carefully 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact.

**Purity**

(1) Heavy metals <1.07>—Prepare with 3.0 g of pulverized Mulberry Bark according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 0.40 g of pulverized Mulberry Bark according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter <5.01>—The amount of the root xylem and other foreign matter is not more than 1.0%.

**Total ash** <5.01> Not more than 11.0%.

**Acid-insoluble ash** <5.01> Not more than 1.0%.

**Containers and storage**

Containers—Well-closed containers.

### Nelumbo Seed

*Nelumbis Semen*

レンニク

Nelumbo Seed is the seed of *Nelumbo nucifera* Gaertner (*Nymphaeaceae*), usually with the endocarp, sometime being removed the embryo.

**Description**

Ovoid to ellipsoidal seed, at the base a papillate protuberance surrounded with shallow depression, 1.0 – 1.7 cm in length, 0.5 – 1.2 cm in width; externally light reddish brown to light yellowish brown; projection part dark reddish brown; endocarp not lustrous and hardly peeled off; endosperm yellowish white, a green embryo in the center.

Almost odorless; taste, slightly sweet and oily, embryo is extremely bitter.

Under a microscope <5.01>, a transverse section of the seed at central portion reveals endocarp composed of parenchyma or endocarp occasionally left out; seed coat composed of epidermis and parenchyma of compressed cells; vascular bundles scattered in parenchyma; endosperm composed of epidermis and parenchyma; aggregate crystals of calcium oxalate and tannin-like substances contained in endocarp remained; parenchymatous cells of seed coat contain tannin-like substances; parenchyma of endosperm contain starch grains.

**Identification**

To 0.5 g of pulverized Nelumbo Seed add 5 mL of water, shake for 5 minutes, and centrifuge. To 0.5 mL of the supernatant liquid add 1 drop of a solution of 1-naphthol in ethanol (99.5) (1 in 5), mix, then add gently 1 mL of sulfuric acid: the solution shows a purple color.
Ting ex H. T. Chang or Notopterygium incisum (Umbelliferae).

\[\text{Description} \quad \text{Notopterygium is slightly curved, cylindrical to conical, 3 - 10 cm in length, 5 - 20 mm in diameter; rhizome occasionally branched; externally yellow-brown to dark brown. The rhizome with nearly orbicular, hollowed stem scars at the apex, sometimes having short residue of stem; externally node rising, internode short; root scars in warty processes on the node; externally root has coarse longitudinal wrinkles and lateral root scars in warty processes; light and slightly brittle in texture, easy to break. The transverse section of the rhizome reveals numerous radial cracks; cortex yellow-brown to brown; xylem light yellow to light grayish yellow; pith grayish white to light brown. Under a magnifying glass, the rhizome reveals brown, fine points of resin canals in the cortex and pith.}

\[\text{Odor, characteristic; taste, slightly acid at first, followed by a slightly pungent and slightly numbing aftertaste.}

\[\text{Under a microscope 5.01, transverse section shows the outermost layer to be composed of a cork layer several to a dozen or so cells thick; collenchyma just inside of the cork layer; oil canals scattered in cortex, large ones more than 300 \ \mu\text{m} \text{ in diameter; intercellular space occurring in radial direction in cortex; oil canals scattered in pith, large ones more than 500 \ \mu\text{m} \text{ in diameter; parenchymatous cells contain simple and 2- to 3-compound starch grains.}

\[\text{Identification To 0.3 g of pulverized Notopterygium add 3 mL of hexane in a glass-stoppered centrifuge tube, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography 2.023. Spot 10 \mu\text{L} \text{ of the sample solution on a plate of octadecylsilanized silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of methanol and water (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm); a bluish white fluorescent spot appears at an RF value of about 0.5, which shows a dark purple color under ultraviolet light (main wavelength: 254 nm).}

\[\text{Purity (1) Heavy metals 1.07—Proceed with 3.0 g of pulverized Notopterygium according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).}

\[\text{Arsenic 1.11—Prepare the test solution with 0.40 g of pulverized Notopterygium according to Method 4, and perform the test (not more than 5 ppm).}

\[\text{Loss on drying 5.01—Not more than 14.0\% (6 hours).}

\[\text{Total ash 5.01—Not more than 5.0\%.}

\[\text{Extract content 5.01—Dilute ethanol-soluble extract: not less than 14.5\%.}

\[\text{Containers and storage Containers—Well-closed containers.}

\[\text{Nuphar Rhizome}

\[\text{Nupharis Rhizoma}

\[\text{センコツ}

\[\text{Nuphar Rhizome is the longitudinally split rhizome of Nuphar japonicum De Candolle (Nymphaeaceae).}

\[\text{Description Usually, longitudinally split irregular column, twisted, bent or somewhat pressed, 20 – 30 cm in length, about 2 cm in width; the outer surface, dark brown, and the cross section, white to grayish white in color; one side shows nearly round to blunt triangular scars of petiole about 1 cm in diameter, and the other side numerous scars of roots less than 0.3 cm in diameter; light, spongy in texture, and easily broken; fractured surface flat and powdery. Under a magnifying glass, a transverse section reveals a black outer portion, and porous tissue with scattered vascular bundles in the inner portion.}

\[\text{Odor, slight; taste, slightly bitter and unpleasant.}

\[\text{Identification Boil 1 g of pulverized Nuphar Rhizome with 20 mL of methanol under a reflux condenser on a water bath for 15 minutes, cool, and filter. Evaporate the filtrate to dryness, warm the residue with 5 mL of dilute acetic acid on a water bath for 1 minute, cool, and filter. Spot 1 drop of the filtrate on a piece of filter paper, air-dry the paper, spray Dragendorff’s TS for spraying on it, and allow it to stand: a yellow-red color appears.}

\[\text{Purity (1) Petiole—When perform the test of foreign matter 5.01, the amount of the petioles contained in Nuphar Rhizome does not exceed 3.0\%.}

\[\text{Heavy metals 1.07—Proceed with 3.0 g of pulverized Nuphar Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).}

\[\text{Arsenic 1.11—Prepare the test solution with 0.40 g of pulverized Nuphar Rhizome according to Method 4, and perform the test (not more than 5 ppm).}

\[\text{Foreign matter 5.01—The amount of foreign matter other than petioles is not more than 1.0\%.}

\[\text{Loss on drying 5.01—Not more than 15.0\% (6 hours).}

\[\text{Total ash 5.01—Not more than 10.0\%.}

\[\text{Acid-insoluble ash 5.01—Not more than 1.0\%.}

\[\text{Containers and storage Containers—Well-closed containers.}
### Nutmeg

**Myristicae Semen**

ニクズク

Nutmeg is the seed of *Myristica fragrans* Houttuyn (*Myristicaceae*), usually from which the seed coat has been removed.

**Description** Ovoid-globose to ellipsoidal seeds, 1.5 – 3.0 cm in length, 1.3 – 2.0 cm in diameter; externally grayish brown, with wide and shallow longitudinal furrows and fine wrinkles; usually, grayish white to grayish yellow and slightly protruding hilum at one end, grayish brown to dark brown and slightly concave chalaza at the other end; cross section has a marble-like appearance with the dark brown thin perisperm extending irregularly into the light yellowish white to light brown endosperm.

Odor, characteristic and strong; taste, acrid and slightly bitter.

Under a microscope, a transverse section reveals perisperm composed of outer and inner layers, the outer layer composed of parenchyma containing dark red-brown contents and the inner layer composed of parenchyma containing red-brown contents with a number of large oil cells and scattered vascular bundles; in parenchyma cells of endosperm, simple or compound starch grains and aleurone grains observed.

**Identification** To 1 g of pulverized Nutmeg, add 5 mL of methanol, allow to stand for 10 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of myristicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (separately determine the loss on drying (1695)), usually from which the seed coat has been removed.

**Loss on drying** ≤5.0%

**Total ash** ≤5.0%

**Essential oil content** ≤5.0%

When the test is performed with 10.0 g of pulverized Nutmeg, the essential oil content is not less than 0.5 mL.

**Containers and storage** Containers—Well-closed containers.

### Nux Vomica

**Strychni Semen**

ホミカ

Nux Vomica is the seed of *Strychnos nux-vomica* Linné (*Loganiaceae*).

When dried, it contains not less than 1.07% of strychnine (C₂₁H₂₂N₂O₂: 334.41).

**Description** Disk, often slightly bent, 1 – 3 cm in diameter, 0.3 – 0.5 cm in thickness; externally light grayish yellow-green to light grayish brown, covered densely with lustrous appressed hairs radiating from the center to the circumference; on both sides, the margin and the central part bulged a little; the dot-like micropyyle situated at one point on the margin, and from which usually a raised line runs to the center on one side; extremely hard in texture; when cracked upon soaking in water, the seed coat thin, the interior consisting of two horny, light grayish yellow endosperms, and leaving a central narrow cavity at the center; a white embryo, about 0.7 cm in length, situated at one end between the inner surfaces of the endosperms.

Odorless; taste, very bitter and persisting.

**Identification** (1) To 3 g of pulverized Nux Vomica add 3 mL of ammonia TS and 20 mL of chloroform, macerate for 30 minutes with occasional shaking, and filter. Remove most of the chloroform from the filtrate by warming on a water bath, add 5 mL of diluted sulfuric acid (1 in 10), and warm on a water bath while shaking well until the odor of chloroform is no longer perceptible. After cooling, filter through a pledget of absorbent cotton, and add 2 mL of nitric acid to 1 mL of the filtrate: a red color develops.

(2) To the remaining filtrate obtained in (1) add 1 mL of potassium dichromate TS, and allow to stand for 1 hour: a yellow-red precipitate is produced. Collect the precipitate by filtration, and wash with 1 mL of water. Transfer a part of the precipitate to a small test tube, add 1 mL of water, dissolve by warming, cool, and add 5 drops of sulfuric acid dropwise carefully along the wall of the test tube: the layer of sulfuric acid shows a purple color which turns immediately red to red-brown.

**Total ash** ≤5.0%

**Assay** Weigh accurately about 1 g of pulverized Nux Vomica, previously dried at 60°C for 8 hours, place in a glass-stoppered centrifuge tube, and moisten with 1 mL of ammonia solution (28). To this solution add 20 mL of diethyl ether, stopper the centrifuge tube tightly, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure three times with the residue using 20-mL portions of diethyl ether. Combine all the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 10 mL of the mobile phase, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 100 mL. Filter this solution through a membrane filter with a porosity not more than 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (separately determine the loss on drying (1695)), usually from which the seed coat has been removed.
**Nux Vomica Extract**

**ホミカエキス散**

Nux Vomica Extract Powder contains not less than 0.61% and not more than 0.68% of strychnine (C₂₁H₂₂N₂O₂: 334.41).

**Method of preparation**

<table>
<thead>
<tr>
<th>Nux Vomica Extract</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch, Lactose Hydrate or their mixture</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To Nux Vomica Extract add 100 mL of Purified Water or Purified Water in Containers, then warm, and soften with stirring. Cool, add 800 g of Starch, Lactose Hydrate or their mixture little by little, and mix well. Dry, preferably at a low temperature, and dilute with a sufficient additional quantity of Starch, Lactose or their mixture to make 1000 g of the homogeneous powder.

**Description** Nux Vomica Extract Powder occurs as a yellow-brown to grayish brown powder. It has a slight, characteristic odor and a bitter taste.

**Identification** (1) To 3 g of Nux Vomica Extract Powder add 3 mL of ammonia TS and 20 mL of chloroform, macerate for 30 minutes with occasional shaking, and filter. Remove most of the chloroform from the filtrate by warming on a water bath, add 5 mL of diluted sulfuric acid (1 in 10), and not more than 30 ppm.

Nux Vomica Extract contains not less than 6.15% and not more than 6.81% of strychnine (C₂₁H₂₂N₂O₂: 334.41).

**Method of preparation** After defatting 1000 g of coarse powder of Nux Vomica with hexane, extract with the percolation method, using a mixture of 750 mL of Ethanol, 10 mL of Acetic Acid and 240 mL of Purified Water or Purified Water in Containers as the first solvent, and 70 vol% ethanol as the second solvent. Combine the extracts, and prepare the dry extract as directed under Exacts. Where, an appropriate quantity of Ethanol and Purified Water or Purified Water in Containers may be used instead of 70 vol% ethanol.

**Description** Nux Vomica Extract occurs as yellow-brown to brown powder. It has a slight characteristic odor, and an extremely bitter taste.

**Identification** Extract 0.5 g of Nux Vomica Extract with 0.5 mL of ammonia TS and 10 mL of chloroform with occasional shaking. Filter the chloroform extract, evaporate the filtrate on a water bath until most of the chloroform is removed, and proceed as directed in the Identification under Nux Vomica.

**Purity** Heavy metals <1.07>—Prepare the test solution with 1.0 g of Nux Vomica Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

**Assay** Weigh accurately about 0.2 g of Nux Vomica Extract, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to disperse the diethyl ether layer. Repeat this procedure three times with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 10 mL of the mobile phase, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 100 mL. Then, proceed as directed in the Assay under Nux Vomica.

**Internal standard solution—**A solution of barbital sodium in the mobile phase (1 in 500).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in water to make 1000 mL, and mix with acetonitrile and triethylamine (45:5:1), and adjust the mixture to pH 3.0.

Flow rate: Adjust the flow rate so that the retention time of Strychnine is about 17 minutes.

Selection of column: Proceed with 5 μL of the standard solution under the above operating conditions. Use a column giving elution of the internal standard and strychnine in this order, and clearly separating each peak.

**Containers and storage** Containers—Well-closed containers.

**Nux Vomica Extract Powder**

Nux Vomica Extract Powder occurs as yellow-brown to grayish brown powder. It has a slight, characteristic odor and a bitter taste.

**Identification** (1) To 3 g of Nux Vomica Extract Powder add 3 mL of ammonia TS and 20 mL of chloroform, macerate for 30 minutes with occasional shaking, and filter. Remove most of the chloroform from the filtrate by warming on a water bath, add 5 mL of diluted sulfuric acid (1 in 10), and not more than 30 ppm.
and warm on a water bath while shaking well until the odor of chloroform is no longer perceptible. After cooling, filter through a pledget of absorbent cotton, and add 2 mL of nitric acid to 1 mL of the filtrate: a red color develops.

(2) To the remaining filtrate obtained in (1) add 1 mL of potassium dichromate TS, and allow to stand for 1 hour: a yellow-red precipitate is produced. Collect the precipitate by filtration, and wash with 1 mL of water. Transfer a part of the precipitate to a small test tube, add 1 mL of water, dissolve by warming, cool, and add 5 drops of sulfuric acid dropwise carefully along the wall of the test tube: the layer of sulfuric acid shows a purple color which turns immediately red to red-brown.

**Assay** Weigh accurately about 2.0 g of Nux Vomica Extract Powder, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to separate the diethyl ether layer. Repeat this procedure three times with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 10 mL of the mobile phase, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 100 mL. Filter this solution through a membrane filter with a porosity not more than 0.8 µm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (separately determine the loss on drying), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Calculate the ratio, \( Q_1 \) and \( Q_0 \), of the peak area of strychnine to that of the internal standard.

\[
\text{Amount (mg) of strychnine (C}_{21}\text{H}_{22}\text{N}_{2}\text{O}_{2}) = M_S \times \frac{Q_1}{Q_0} \times \frac{1}{5} \times 0.8415
\]

\( M_S \): Amount (mg) of strychnine nitrate for assay, calculated on the dried basis

**Internal standard solution**—A solution of barbital sodium in the mobile phase (1 in 500).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadeylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: Room temperature.
Mobile phase: A mixture of a solution of potassium dihydrogen phosphate (6.8 in 1000), acetonitrile and triethylamine (45:5:1), adjusted the pH to 3.0 with phosphoric acid.
Flow rate: Adjust the flow rate so that the retention time of strychnine is about 17 minutes.
Selection of column: Proceed with 5 µL of the standard solution under the above operating conditions. Use a column giving elution of the internal standard and strychnine in this order, and clearly dividing each peak.

**Containers and storage** Containers—Tight containers.

### Nux Vomica Tincture

**ホミカチンキ**

Nux Vomica Tincture contains not less than 0.097 w/v% and not more than 0.116 w/v% of strychnine (C\(_{21}\)H\(_{22}\)N\(_2\)O\(_2\): 334.41).

**Method of preparation**

<table>
<thead>
<tr>
<th>Nux Vomica, in coarse powder</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 vol% Ethanol</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Tinctures, with the above ingredients. May be prepared with an appropriate quantity of Ethanol and Purified Water or Purified Water in Containers.

**Description** Nux Vomica Tincture is a yellow-brown liquid. It has an extremely bitter taste. Specific gravity \( d_{20}^0 \) about 0.90

**Identification** Heat 20 mL of Nux Vomica Tincture on a water bath to remove ethanol, cool, transfer to a separator, add 2 mL of ammonia TS and 20 mL of chloroform, and shake well for 2 to 3 minutes. Filter the chloroform layer through a pledget of absorbent cotton, warm the filtrate on a water bath to remove most of chloroform, and proceed as directed in the Identification under Nux Vomica.

**Alcohol number** <1.01> Not less than 6.7 (Method 2).

**Assay** Pipet 3 mL of Nux Vomica Tincture into a glass-stoppered centrifuge tube, add 10 mL of ammonia TS and 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue with 10 mL of the mobile phase, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.8 µm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (separately determine the loss on drying), and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.8 µm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (separately determine the loss on drying), and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL, and use this solution as the standard solution. Proceed with the sample solution and the standard solution as directed in the Assay under Nux Vomica.

\[
\text{Amount (mg) of strychnine (C}_{21}\text{H}_{22}\text{N}_{2}\text{O}_{2}) = M_S \times \frac{Q_1}{Q_0} \times \frac{1}{20} \times 0.8415
\]

\( M_S \): Amount (mg) of strychnine nitrate for assay, calculated on the dried basis

**Internal standard solution**—A solution of barbital sodium in the mobile phase (1 in 500).

**Containers and storage** Containers—Tight containers.
Opium Ipecac Powder

オピウムイペカ

Opium Ipecac Powder contains not less than 0.90% and not more than 1.10% of morphine \((C_{17}H_{19}NO_3)\) and not more than 1.10% of morphine \((C_{17}H_{19}NO_3)\) and not more than 1.10% of morphine \((C_{17}H_{19}NO_3)\).

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powdered Opium</td>
<td>100 g</td>
</tr>
<tr>
<td>Powdered Ipecac</td>
<td>100 g</td>
</tr>
<tr>
<td>Starch or a suitable ingredient</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Powders, with the above ingredients. Lactose Hydrate should not be used.

**Description**

Opium Ipecac Powder occurs as a light brown powder.

Orengedokuto Extract

オレンジェドウトウキ

Orengedokuto Extract contains not less than 20 mg and not more than 80 mg of berberine chloride \((C_{20}H_{18}ClNO_4)\) and not more than 240 mg of baicalin \((C_{21}H_{18}O_{11})\), and not less than 30 mg and not more than 90 mg for preparation prescribed 2 g of Gardenia Fruit.

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powdered Ipecac</td>
<td>100 g</td>
</tr>
<tr>
<td>Powdered Opium</td>
<td>100 g</td>
</tr>
<tr>
<td>Starch or a suitable ingredient</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Powders, with the above ingredients. Lactose Hydrate should not be used.

**Description**

Orengedokuto Extract occurs as a light brown powder.
Preparation of a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

**Description** Orenge dokuto Extract occurs as a yellow-brown to blackish brown, powder or viscous extract. It has a characteristic odor and a very bitter taste.

**Identification** (1) Shake 0.5 g of dry extract (or 1.5 g of the viscous extract) with 10 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of coptisine chloride for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28) and methanol (15:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the yellow fluorescent spot from the standard solution (Coptis Rhizome).

(2) Shake 0.5 g of dry extract (or 1.5 g of the viscous extract) with 5 mL of water, then add 25 mL of ethyl acetate, and shake. Separate the ethyl acetate layer, evaporate the solvent under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of limonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL of the sample solution and 5 µL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the dark purple spot from the standard solution (Gardenia Fruit).

**Purity** (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed in Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

**Loss on drying** <2.41> The dry extract: Not more than 7.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

**Total ash** <5.01> Not more than 12.0%, calculated on the dried basis.

**Assay** (1) Berberine—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract, equivalent to 0.2 g of dried substance), add exactly 50 mL of the mobile phase, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately determine the water (2.49) in the same manner as Berberine Chloride Hydrate), dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A<sub>T</sub> and A<sub>S</sub>, of berberine in each solution.

Amount (mg) of berberine chloride (C<sub>20</sub>H<sub>18</sub>ClNO<sub>4</sub>)
\[
S = \frac{M_S \times A_T}{A_S} \times \frac{1}{2}
\]

M<sub>S</sub>: Amount (mg) of Berberine Chloride RS, calculated on the anhydrous basis.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 345 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: Dissolve 3.4 g of potassium dihydrogen...
phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1). Flow rate: 1.0 mL per minute (the retention time of berberine is about 8 minutes).

**System suitability**—

System performance: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, palmatine and berberine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5%

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract equivalent to 0.1 g of dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determined), dissolve in diluted methanol (7 in 10), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, add 50 mL of diluted methanol (7 in 10) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, A1 and A3, of baicalin in each solution.

\[
M_3 = \frac{M_S \times A_1}{A_3}
\]

\[
M_2: \text{Amount (mg) of Baicalin RS, calculated on the anhydrous basis}
\]

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

**Containers and storage**—Containers—Tight containers.

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**Oriental Bezoar**

**Bezoar Bovis**

ゴオウ

Oriental Bezoar is a stone formed in the gall sac of Bos taurus Linné var. domesticus Gmelin (Bovidae).

**Description**—Spherical or massive stone, 1 - 4 cm in diameter; externally yellow-brown to red-brown; light, fragile and easily broken. Fractured surface shows yellow-brown to red-brown annular rings, often containing white granular substances or thin layers in these annular rings.

Odor, slight; taste, slightly bitter, followed by slight sweetness.

**Identification** (1) Shake 0.1 g of pulverized Oriental Bezoar with 10 mL of petroleum ether for 30 minutes, filter, and wash the residue with 10 mL of petroleum ether. Shake 0.01 g of the residue with 3 mL of acetic anhydride for 1 to 2 minutes, add a mixture of 0.5 mL of acetic anhydride and 2 drops of sulfuric acid, and shake: a yellow-red to deep red color develops, and changes through dark red-purple to dark red-brown.

(2) Shake well 0.01 g of Oriental Bezoar with 1 mL of hydrochloric acid and 10 mL of chloroform, separate the...
chloroform layer when it acquires a yellow-brown color, and shake with 5 mL of barium hydroxide TS: a yellow-brown precipitate is produced.

**Purity (1)** Synthetic dye—To 2 mg of pulverized Oriental Bezoar add 1 mL dilute hydrochloric acid: no violet color develops.

(2) Starch—To 5 mg of pulverized Oriental Bezoar add 2 mL of water, and heat on a water bath for 5 minutes. Cool and add 2 to 3 drops of iodine TS: no blue-purple color develops.

(3) Sucrose—To 0.02 g of pulverized Oriental Bezoar add 10 mL of water, shake for 15 minutes, and filter. To 1 mL of the filtrate add 2 mL of anthrone TS, and shake: no deep blue-green to dark green color develops.

**Total ash** Not more than 10.0%.

**Content of the active principle** Weigh accurately about 0.5 g of pulverized Oriental Bezoar in a flask, add 50 mL of petroleum ether, warm under a reflux condenser on a water bath for 2 hours, and filter. Place the residue along with the filter paper in the flask, add 2 mL of hydrochloric acid and 40 mL of chloroform, warm under a reflux condenser on a water bath for 1 hour, and filter into a tared flask. Wash the filter paper with a small quantity of chloroform, combine the washings with the filtrate, and distill off the chloroform. Dry the residue in a desiccator (silica gel) for 24 hours, and filter into a tared flask. Wash the filtrate with the filtrate, and distil off the chloroform. Dry the residue at 105°C for 1 hour, collect the insoluble substance by filtration, and wash it with hot water until the last washing no longer gives any reaction in Qualitative Tests for chloride. Ignite the residue and weigh: the mass of the residue does not exceed 15 mg.

**Containers and storage** Containers—Well-closed containers.

### Oyster Shell

**Ostreae Testa**

ボレイ

Oyster Shell is the shell of *Ostrea gigas* Thunberg (*Ostreidae*).

**Description** Irregularly curved, foliaceous or lamellated broken pieces. The unbroken oyster shell forms a bivalve 6–10 cm in length and 2–5 cm in width. The upper valve is flat and the lower one is somewhat hollow. Both the upper and lower edges of the valve are irregularly curved and bite with each other. The surface of the valve is externally light greenish gray-brown and internally milky in color.

Almost odorless and tasteless.

**Identification (1)** Dissolve 1 g of sample pieces of Oyster Shell in 10 mL of dilute hydrochloric acid by heating: it evolves a gas, and forms a very slightly red, turbid solution in which a transparent, thin suspended matter remains. Pass the evolved gas through calcium hydroxide TS: a white precipitate is produced.

(2) The solution obtained in (1) has a slight, characteristic odor. Filter this solution and neutralize with ammonia TS: the solution responds to the Qualitative Tests for calcium salt.

(3) Ignite 1 g of pulverized Oyster Shell: it turns blackish brown in color at first, and evolves a characteristic odor. Ignite it further: it becomes almost white.

**Purity** Barium—Dissolve 1 g of pulverized Oyster Shell in 10 mL of dilute hydrochloric acid: the solution does not respond to the Qualitative Tests (1) for barium salt.

**Containers and storage** Containers—Well-closed containers.

### Powdered Oyster Shell

**Ostreae Testa Pulverata**

ボレイ末

Powdered Oyster Shell is the powder of Oyster Shell.

**Description** Powdered Oyster Shell occurs as a grayish white powder. It is almost odorless and tasteless.

**Identification (1)** Dissolve 1 g of Powdered Oyster Shell in 10 mL of dilute hydrochloric acid by heating: it evolves a gas, and forms a very slightly red, turbid solution. Pass the gas evolved through calcium hydroxide TS: a white precipitate is produced.

(2) The solution obtained in (1) has a slight, characteristic odor. Filter this solution, and neutralize with ammonia TS: the solution responds to the Qualitative Tests for calcium salt.

(3) Ignite 1 g of Powdered Oyster Shell: it turns blackish brown in color at first evolving a characteristic odor, and becomes almost white by further ignition.

**Purity (1)** Water-soluble substances—Shake 3.0 g of Powdered Oyster Shell with 50 mL of freshly boiled and cooled water for 5 minutes, filter, and evaporate 25 mL of the filtrate to dryness. Dry the residue at 105°C for 1 hour, cool, and weigh: the mass of the residue does not exceed 15 mg.

(2) Acid-insoluble substances—To 5.0 g of Powdered Oyster Shell add 100 mL of water, and add hydrochloric acid in small portions with stirring until the solution becomes acid. Boil the acidic mixture with additional 1 mL of hydrochloric acid. After cooling, collect the insoluble substance by filtration, and wash it with hot water until the last washing no longer gives any reaction in Qualitative Tests (1) for chloride.

(3) Barium—Dissolve 1 g of Powdered Oyster Shell in 10 mL of dilute hydrochloric acid: the solution does not respond to the Qualitative Tests (1) for barium salt.

**Loss on drying** Not more than 4.0% (1 g, 180°C, 4 hours).

**Containers and storage** Containers—Tight containers.
Panax Japonicus Rhizome

Panacis Japonici Rhizoma

粉末シブニンジン

Panax Japonicus Rhizome is the rhizome of Panax japonicus C. A. Meyer (Araliaceae), usually after being treated with hot water.

**Description** Irregularly cylindrical rhizome with distinct nodes, 3 – 20 cm in length, 1 – 1.5 cm in diameter, internode 1 – 2 cm; externally light yellow-brown, with fine longitudinal wrinkles; stem scars, hollowed at the center, protruding on the upper surface, and root scars protruding as knobs on internodes; easily broken; fractured surface approximately flat, and light yellow-brown in color; horny in texture.

Odor, slight; taste, slightly bitter.

**Identification** Shake 0.5 g of pulverized Panax Japonicus Rhizome with 10 mL of methanol for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of chikusetsusaponin IV in 1 mL of methanol, and use this solution as the standard solution.

Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (5:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 5 minutes: one of several spots obtained from the sample solution shows the same color and the same RF value with the red-purple spot from the standard solution.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Panax Japonicus Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Panax Japonicus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

**Total ash <5.01>** Not more than 5.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

Containers and storage Containers—Well-closed containers.

Powdered Panax Japonicus Rhizome

Panacis Japonici Rhizoma Pulveratum

粉末シブニンジン末

Powdered Panax Japonicus Rhizome is the powder of Panax Japonicus Rhizome.

**Description** Powdered Panax Japonicus Rhizome occurs as a light grayish yellow-brown powder, and has a slight odor and a slightly bitter taste.

Under a microscope <5.01>, Powdered Panax Japonicus Rhizome reveals mainly starch grains or gelatinized starch masses, and fragments of parenchyma cells containing them; also fragments of cork tissue, somewhat thick-walled collateral phloem tissue, and reticulate vessels; rarely fragments of scalariform vessels with a simple perforation, fibers and fiber bundles, rosette aggregates of calcium oxalate, and parenchyma cells containing them; yellow to orange-yellow resin; starch grains consisting of simple grains or 2- to 4-compound grains, simple grains, 3 – 18 µm in diameter; rosette aggregates of calcium oxalate are 20 – 60 µm in diameter.

**Identification** Shake 0.5 g of Powdered Panax Japonicus Rhizome with 10 mL of methanol for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of chikusetsusaponin IV in 1 mL of methanol, and use this solution as the standard solution.

Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (5:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 5 minutes: one of several spots obtained from the sample solution shows the same color tone and the same RF value with the red-purple spot from the standard solution.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of Powdered Panax Japonicus Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Panax Japonicus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

**Total ash <5.01>** Not more than 5.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

Containers and storage Containers—Well-closed containers.

Peach Kernel

Persicae Semen

トウニン

Peach Kernel is the seed of Prunus persica Batsch or Prunus persica Batsch var. davidiana Maximowicz (Rosaceae).

It contains not less than 1.2% of amygdalin, calculated on the basis of dried material.

**Description** Flattened, asymmetric ovoid seed, 1.2 – 2.0 cm in length, 0.6 – 1.2 cm in width, and 0.3 – 0.7 cm in thickness; somewhat sharp at one end, and round at the other end with chalaza; seed coat red-brown to light brown; externally, its surface being powdery by easily detachable stone cells of
powdery; numerous vascular bundles running and rarely branching from chalaza through the seed coat, and, appearing as dented longitudinal wrinkles; when soaked in boiling water and softened, the seed coat and thin, translucent, white albumen easily separated from the cotedone; cotedone white in color.

Almost odorless; taste, slightly bitter and oily.

Under a microscope <5.0f>, the outer surface of seed coat reveals polygonal, long polygonal, or obtuse triangular stone cells on the protrusion from vascular bundles, shape of which considerably different according to the position, and their membranes almost equally thickened; in lateral view, appearing as a square, rectangle or obtuse triangle.

**Identification** To 1.0 g of ground Peach Kernel add 10 mL of methanol, immediately heat under a reflux condenser on a water bath for 10 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.0f>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:5:4) to a distance of about 10 cm, and air-dry the plate. Spray evenly thymol-sulfuric acid-methanol TS for spraying upon the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color and Rf value with the red-brown spot from the standard solution.

**Purity** (1) Rancidity—Grind Peach Kernel with boiling water: no odor of rancid oil is perceptible.
(2) Foreign matter <5.0f>—Peach Kernel does not contain broken pieces of endocarp or other foreign matter.

**Loss on drying** <5.0f> Not more than 8.0% (6 hours).

**Assay** Weigh accurately 0.5 g of ground Peach Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser on a water bath for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, filter, and use the filtrate as the sample solution. Separately, weigh approximately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0f> according to the following conditions, and determine the peak areas, A₁ and Aₙ, of amygdalin.

\[ \text{Amount (mg) of amygdalin} = M₀ \times A₁/Aₙ \times 2 \]

M₀: Amount (mg) of amygdalin for assay

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).
Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

**Powdered Peach Kernel**

*Persicae Semen Pulveratum*

Powdered Peach Kernel is the powder of the Peach Kernel.

It contains not less than 1.2% of amygdalin, calculated on the basis of dried material.

**Description** Powdered Peach Kernel occurs as a reddish-light brown to light brown powder. It is almost odorless and is oily and has slightly a bitter taste.

Under a microscope <5.0f>, Powdered Peach Kernel fragments of outer seed coat epidermis; elliptical to ovoid, containing yellow-brown compound 50 to 80 μm in diameter and stone cell; cap-like shape to ovoid, yellow-brown in color. The stone cell is element of epidermis, 50 to 80 μm in diameter and 70 to 80 μm in height, cell wall of the top, 12 to 25 μm thickness, the base 4 μm in thickness, with obvious and numerous pits. Inner seed coat, yellow-brown, irregular and somewhat long polygon, 15 to 30 μm in diameter; and fragments of cotedon and albumen containing aleurone grains and fattened oil. Aleurone grains are almost spherical grains, 5 to 10 μm in diameter.

**Identification** To 1.0 g of Powdered Peach Kernel add 10 mL of methanol, and immediately heat under a reflux condenser on a water bath for 10 minutes. After cooling, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.0f>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:5:4) to a distance of about 10 cm, and air-dry the plate. Spray evenly thymol-sulfuric acid-methanol TS for spraying on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the red-brown spot from the standard solution.
Acid-insoluble ash \(<5.01\)> Not more than 0.5%.

**Containers and storage** Containers—Tight containers.

**Assay** Weigh accurately 0.5 g of Powdered Peach Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser on a water bath for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test exactly with 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\>) according to the following conditions, and determine the peak areas, \(A_T\) and \(A_S\), of amygdalin in each solution.

Amount (mg) of amygdalin = \(M_S \times \frac{A_T}{A_S} \times 2\)

\(M_S\): Amount (mg) of amygdalin for assay

**Operating conditions—**
- **Detector:** An ultraviolet absorption photometer (wavelength: 210 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
- **Column temperature:** A constant temperature of about 45°C.
- **Mobile phase:** A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).
- **Flow rate:** 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

**System suitability—**
- **System performance:** When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.
- **System repeatability:** When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

**Containers and storage** Containers—Tight containers.

**Peony Root**

**Paeoniae Radix**

シャクヤク

Peony Root is the root of *Paeonia lactiflora* Pallas (*Paeoniaceae*).

It contains not less than 2.0% of paeoniflorin (\(C_{23}H_{28}O_{11}\); 480.46), calculated on the dried basis.

**Description** Cylindrical root, 7 - 20 cm in length, 1 - 2.5 cm in diameter; externally brown to light grayish brown, with distinct longitudinal wrinkles, with warly scars of lateral roots, and with laterally elongated lenticels; fractured surface dense in texture, light grayish brown, and with light brown radial lines in xylem.

Odor, characteristic; taste, slightly sweet at first, followed by an astringency and a slight bitterness.

**Identification**

1. Shake 0.5 g of pulverized Peony Root with 30 mL of ethanol (95) for 15 minutes, and filter. Shake 3 mL of the filtrate with 1 drop of iron (III) chloride TS: a blue-purple to blue-green color is produced, and it changes to dark blue-purple to dark green.

2. To 2 g of pulverized Peony Root add 10 mL of methanol, warm on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.01\>). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate and acetic acid (100): (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS upon the plate, and heat at 105°C for 5 minutes: one spot among the spots from the sample solution and the purple-red spot from the standard solution show the same color tone and the same \(Rf\) value.

**Purity**

1. Heavy metals \(<1.07\>—Proceed with 3.0 g of pulverized Peony Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

2. Arsenic \(<1.11\>—Prepare the test solution with 0.40 g of pulverized Peony Root according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying** \(<5.01\> Not more than 14.0% (6 hours).

**Total ash** \(<5.01\> Not more than 6.5%.

**Acid-insoluble ash** \(<5.01\> Not more than 0.5%.

**Assay** Weigh accurately about 0.5 g of Powdered Peony Root, add 40 mL of diluted methanol (9 in 10), heat under a reflux condenser on a water bath for 30 minutes, and cool. Filter the residue, add 50 mL of diluted methanol (1 in 2), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. To the residue add 50 mL of diluted methanol (1 in 2), and proceed in the same manner. Combine the filtrates, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\>) according to the following conditions. Determine the peak areas, \(A_T\) and \(A_S\), of paeoniflorin.

Amount (mg) of paeoniflorin (\(C_{23}H_{28}O_{11}\)) = \(M_S \times \frac{A_T}{A_S}\)

\(M_S\): Amount (mg) of Paeoniflorin RS, calculated on the anhydrous basis

**Operating conditions—**
- **Detector:** An ultraviolet absorption photometer (wavelength: 232 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica
gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: Adjust the flow rate so that the retention time of paeoniflorin is about 10 minutes.

**System suitability**

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

**Powdered Peony Root**

*Paeoniae Radix Pulverata*

シャクヤク末

Powdered Peony Root is the powder of Peony Root. It contains not less than 2.0% of paeoniflorin (C_{23}H_{28}O_{11}; 480.46), calculated on the dried basis.

**Description** Powdered Peony Root occurs as a light grayish brown powder, and has a characteristic odor and a slightly sweet taste at first, followed by an astringency and a slight bitterness.

Under a microscope <5.01>, Powdered Peony Root reveals starch grains and fragments of parenchyma cells containing them; fragments of cork cells, vessels, tracheids and xylem fibers; rosette aggregates of calcium oxalate, and fragments of rows of crystal cells containing them. Starch grains consist of simple grains, 5 – 25 μm in diameter, occasionally 2- to 3-compound grains.

**Identification (1)** Shake 0.5 g of Powdered Peony Root with 30 mL of ethanol (95) for 15 minutes, and filter. To 3 mL of the filtrate add 1 drop of iron (III) chloride TS, and mix: a blue-purple to blue-green color is produced, and thereafter it changes to dark blue-purple to dark green.

(2) To 2 g of Powdered Peony Root add 10 mL of methanol, warm on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate and acetic acid (100; 10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde–sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one spot among the spots from the sample solution and the purple spot from the standard solution show the same color tone and the same Rf value.

**Purity (1)** Heavy metals <1.07>—Procede with 3.0 g of Powdered Peony Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.1D>—Prepare the test solution with 0.40 g of Powdered Peony Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Peony Root does not show groups of light yellow stone cells and fibers.

**Loss on drying** <5.01> Not less than 14.0% (6 hours).

**Total ash** <5.01> Not more than 6.5%.

**Acid-insoluble ash** <5.01> Not more than 0.5%.

**Assay** Weigh accurately about 0.5 g of Powdered Peony Root, add 50 mL of diluted methanol (1 in 2), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. To the residue add 50 mL of diluted methanol (1 in 2), and proceed in the same manner. Combine the filtrates, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{T} and A_{S}, of paeoniflorin.

\[
\text{Amount (mg) of paeoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) = \frac{M_{S} \times A_{S}}{A_{T}}
\]

\[
M_{S}: \text{Amount (mg) of Paeoniflorin RS, calculated on the anhydrous basis}
\]

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: Adjust the flow rate so that the retention time of paeoniflorin is about 10 minutes.

**System suitability**—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.
**Perilla Herb**

*Perillae Herba*

ソヨウ

Perilla Herb is the leaves and the tips of branches of *Perilla frutescens* Britton var. *acuta* Kudo or *Perilla frutescens* Britton var. *crispa* Decaisne (Labiatae).

It contains not less than 0.08% of perillaldehyde, calculated on the basis of dried material.

**Description** Usually, contracted and wrinkled leaves, often with thin stems. Both surfaces of the leaf are brownish purple, or the upper surface is grayish green to brownish green, and the lower surface is brownish purple in color. When smoothed by immersion in water, the lamina is ovate to obcordate, 5–12 cm in length, 5–8 cm in width; the apex, acuminate; the margin, serrate; the base, broadly cuneate; petiole, 3–5 cm in length; cross sections of stem and petiole, square. Under a magnifying glass, hairs are observed on both surfaces of the leaf, but abundantly on the vein and sparsely on other parts; small glandular hairs are observed on the lower surface.

Odor, characteristic; taste slightly bitter.

**Identification** To 0.6 g of pulverized Perilla Herb, add 10 mL of diethyl ether, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of perillaldehyde for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for liquid chromatography (5 μm in particle diameter). Develop the plate with a mixture of hexane and ethyl acetate (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid-ethanol TS for spray on the lower surface. Sprays uniformly with a colorless to red-purple color (Rf value with the red-purple spot from 0.23 to 0.30). The area of the red-purple spot from 0.23 to 0.30 is not more than 1.5 (5.01). Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography (<2.0×) according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of perillaldehyde.

Amount (mg) of perillaldehyde = $M_S \times A_T/A_S \times 1/20$

$M_S$: Amount (mg) of perillaldehyde for assay

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: 1.0 mL per minute.

**System suitability**—

System performance: Dissolve 1 mg of (E)-asarone in the standard solution to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, perillaldehyde and (E)-asarone are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of perillaldehyde is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

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**Peucedanum Root**

*Peucedani Radix*

ゼンコ

Peucedanum Root is the root of 1) *Peucedanum praeruptorum* Dunn or 2) *Angelica decursiva* Maximowicz (Umbelliferae).

**Description** 1) *Peucedanum praeruptorum* Dunn

Slender obconical to cylindrical root, occasionally dichotomized at the lower part 3–15 cm in length, 0.8–1.8 cm in diameter at the crown; externally light brown to dark brown; ring-node-like wrinkles numerous at the crown, sometimes with hair-like remains of petioles; the root having somewhat deep longitudinal wrinkles and scars of cutting off of lateral roots; transverse section surface light brown to whitish in color; brittle in texture.

Odor, characteristic; taste, slightly bitter.

Under a microscope, a transverse section reveals the outermost layer composed of a cork layer, inner tangential walls of some cork cells thickened; collenchyma just inside of the cork layer; in cortex numerous oil canals scattered and intercellular air spaces observed; occasionally phloem fibers...
observed at the terminal portion of phloem; vessels and scattered oil canals in xylem; starch grains in parenchyma, 2 to 10 several-compound grains.

2) *Angelica decursiva* Franchet et Savatier

Similar to 1), but without hair-like remains of petioles at the crown.

Under a microscope $<5.01^\circ$, a transverse section reveals, similar to 1), but cell wall of cork cells not thickened, phloem fibers not observed at the terminal portion of phloem, nor oil canals observed in xylem.

**Identification (1) Peucedanum praeruptorum** Dunn—To 1 g of pulverized *Peucedanum* Root add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of nodakenin ($\pm$)-praeruptorin A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (12:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and $R_f$ value with the blue-purple fluorescent spot from the standard solution.

(2) *Angelica decursiva* Franchet et Savatier—To 1 g of pulverized *Peucedanum* Root add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of nodakenin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03^\circ$. Spot 10 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and hexane (3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and $R_f$ value with the blue-purple fluorescent spot from the standard solution.

**Loss on drying** $<5.01^\circ$ Not more than 13.0%.

**Total ash** $<5.01^\circ$ Not more than 7.0%.

**Acid-insoluble ash** $<5.01^\circ$ Not more than 2.0%.

**Extract content** $<5.01^\circ$ Dilute ethanol-soluble extract: not less than 20.0%.

**Containers and storage** Containers—Well-closed containers.

**Pharbitis Seed**

*Pharbitidis* *Semen*

ケンゴシ

Pharbitis Seed is the seed of *Pharbitis nil* Choisy (*Convolvulaceae*).

**Phelloendron Bark**

*Phellodendri* *Cortex*

オウパク

Phelloendron Bark is the bark of *Phellodendron amurense* Ruprecht or *Phellodendron chinense* Schneider (*Rutaceae*), from which the periderm has been removed.

It contains not less than 1.2% of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], calculated on the basis of dried material.

**Description** Flat or rolled semi-tubular pieces of bark, 2 – 4 mm in thickness; externally grayish yellow-brown to grayish brown, with numerous traces of lenticel; the internal surface yellow to dark yellow-brown in color, with fine vertical lines, and smooth; fractured surface fibrous and bright yellow. Under a magnifying glass, the transverse section of Phellodendron Bark reveals a thin and yellow outer cortex, scattered with stone cells appearing as yellow-brown dots; inner cortex thick; primary medullary rays expanding its width towards the outer end, the phloem appearing as a nearly triangular part between these medullary rays in secondary cortex, and many secondary medullary rays radiating and gathering to the tip of the triangle; brown phloem fiber bundles lined in tangential direction, crossed over the secondary medullary rays, and then these tissues show a latticework.

Odor, slight; taste, extremely bitter; mucilaginous; it colors the saliva yellow on chewing.

**Identification (1)** To 1 g of pulverized Phellodendron Bark add 10 mL of diethyl ether, allow to stand for 10 minutes with occasional shaking, and filter to remove the diethyl ether. Collect the powder on the filter paper, add 10 mL of ethanol (95), allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid, add 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.
(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.07>$. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same Rt value.

(3) Stir up pulverized Phellodendron Bark with water: the solution becomes gelatinous owing to mucilage.

**Loss on drying** $<5.01>$ Not more than 11.0% (6 hours).

**Total ash** $<5.01>$ Not more than 7.5%.

**Acid-insoluble ash** $<5.01>$ Not more than 0.5%.

**Assay** Weigh accurately about 0.5 g of pulverized Phellodendron Bark, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately determine the water $<2.40>$ in the same manner as Berberine Chloride Hydrate), dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.07>$ according to the following conditions, and determine the peak areas, $A_1$, and $A_5$, of berberine.

\[
\text{Amount (mg) of berberine [as berberine chloride} \quad (C_{25}H_{18}ClNO_4) \]
\[
= M_2 \times \frac{A_1}{A_5}
\]

$M_2$: Amount (mg) of Berberine Chloride RS, calculated on the anhydrous basis

**Operating conditions—**

**Detector:** An ultraviolet absorption photometer (wavelength: 345 nm).

**Column:** A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 mm in particle diameter).

**Column temperature:** A constant temperature of about 40°C

**Mobile phase:** Dissolve 3.4 g of potassium dihydrogenphosphate and 1.7 g of sodium laurel sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

**Flow rate:** Adjust the flow rate so that the retention time of berberine is about 10 minutes.

**Selection of column:** Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in 10 mL of methanol. Perform the test with 20 mL of this solution under the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly separating each peak.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions the relative deviation of the peak area of berberine is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

---

**Powdered Phellodendron Bark**

**Phellodendri Cortex Pulveratus**

Powdered Phellodendron Bark is the powder of Phellodendron Bark. It contains not less than 1.2% of berberine [as berberine chloride ($C_{25}H_{18}ClNO_4$: 371.81)], calculated on the basis of dried material.

**Description** Powdered Phellodendron Bark occurs as a bright yellow to yellow powder. It has a slight odor and an extremely bitter taste, is mucilaginous, and colors the saliva yellow on chewing. Under a microscope $<5.01>$, Powdered Phellodendron Bark reveals fragments of yellow, thick-walled fiber bundles or fibers, and fibers often accompanied by crystal cell rows; fewer groups of stone cells together with idioblasts; fragments of parenchyma cells containing starch grains and oil droplets; fragments of medullary ray and phloem; mucilage cells and mucilage masses. Numerous solitary crystals of calcium oxalate, 7–20 μm in diameter; starch grains, simple grains and 2- to 4-compound grains, simple grain, 2–6 μm in diameter; oil droplets, stained red with sudan III TS.

**Identification** (1) To 1 g of Powdered Phellodendron Bark add 10 mL of diethyl ether, allow to stand for 10 minutes with occasional shaking, and filter to remove the diethyl ether. Collect the powder on the filter paper, add 10 mL of ethanol (95), allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid, add 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.07>$. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same Rt value.

(3) Stir up Powdered Phellodendron Bark with water: the solution becomes gelatinous owing to mucilage.

**Purity** Curcuma—Place Powdered Phellodendron Bark on filter paper, drop diethyl ether on it, and allow to stand. Take the powder off the filter paper, and drip 1 drop of po-
tassium hydroxide TS: no red-purple color develops. Under a microscope <5.01>, Powdered Phellodendron Bark does not contain gelatinized starch or secretory cells containing yellow-red resin.

**Loss on drying <5.01>** Not more than 11.0% (6 hours).

**Total ash <5.01>** Not more than 7.5%.

**Acid-insoluble ash <5.01>** Not more than 0.5%.

**Assay** Weigh accurately about 0.5 g of Powdered Phellodendron Bark, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To obtained residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately determine the water <2.48>) in the same manner as Berberine Chloride Hydrate, dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of berberine.

\[
M_2: \text{Amount (mg) of Berberine Chloride RS, calculated on the anhydrous basis}
\]

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogenphosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in 10 mL of methanol. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly dividing each peak.

System repeatability: When repeat the test 5 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

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**Compound Phellodendron Powder for Cataplasm**

パップ用複方オウバク散

**Method of preparation**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powdered Phellodendron Bark</td>
<td>660</td>
</tr>
<tr>
<td>Powdered Gardenia Fruit</td>
<td>325</td>
</tr>
<tr>
<td>d- or dl-Camphor</td>
<td>10</td>
</tr>
<tr>
<td>dl- or l-Menthol</td>
<td>5</td>
</tr>
<tr>
<td><strong>To make</strong></td>
<td>1000</td>
</tr>
</tbody>
</table>

Prepare as directed under Powders, with the above ingredients.

**Description** Compound Phellodendron Powder for Cataplasm occurs as a yellow-brown powder, having a characteristic odor.

**Identification** Shake thoroughly 0.2 g of Compound Phellodendron Powder for Cataplasm with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.01>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the yellow to yellow-green fluorescent spot from the standard solution (phellodendron bark).

**Containers and storage** Containers— Tight containers.

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**Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder**

オウバク・タンナルビン・ビスマス散

Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder contains not less than 12.9% and not more than 16.3% of bismuth (Bi: 208.98).

**Method of preparation**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powdered Phellodendron Bark</td>
<td>300</td>
</tr>
<tr>
<td>Albumin Tannate</td>
<td>300</td>
</tr>
<tr>
<td>Bismuth Subnitrate</td>
<td>200</td>
</tr>
<tr>
<td>Scopolia Extract</td>
<td>10</td>
</tr>
<tr>
<td>Starch, Lactose Hydrate or their mixture</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td><strong>To make</strong></td>
<td>1000</td>
</tr>
</tbody>
</table>

Prepare as directed under Powders, with the above ingredients. Scopolia Extract Powder may be used in place of Scopolia Extract.
**Description**  Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder is brownish yellow in color, and has a bitter taste.

**Identification**  (1) Shake thoroughly 0.1 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100:7:2:1) to a distance of about 10 cm, air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot from the standard solution show the same color tone and the same Rf value (phellodendron bark).

(2) To 0.3 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder add 20 mL of ethanol (95), heat in a water bath for 3 minutes with shaking, cool, and filter. To 10 mL of the filtrate add 1 drop of iron (III) chloride TS: a blue-green color is produced. Allow to stand: a bluish black precipitate is produced (albumin tannate).

(3) To 0.3 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder add 10 mL of diluted pyridine (1:7:2:1) to a distance of about 10 cm, air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot from the standard solution show the same color tone and the same Rf value (phellodendron bark).

(4) To 0.5 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder add 5 mL of dilute hydrochloric acid and 10 mL of water, warm, shake thoroughly, and filter. The filtrate responds to the Qualitative Tests <1.09> for bismuth salt.

**Assay**  Weigh accurately about 0.7 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder, shake well with 10 mL of water and 20 mL of diluted nitric acid (1 in 3), add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 10 mL of the filtrate, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.23 g of bismuth nitrate pentahydrate, add 20 mL of diluted nitric acid (1 in 3) and water to make exactly 100 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution according to Atomic Absorption Spectrophotometry <2.22> under the following conditions. On the other hand, determine the absorbance \( A_S \) of the solution prepared in the same manner using 20 mL of diluted nitric acid (1 in 3) instead of the standard solution.

\[
M = \frac{A_T - A_S}{A_S - A_0} \times 0.4308
\]

**Containers and storage**  Containers—Well-closed containers.

---

**Picrasma Wood**

**Picrasmae Lignum**

ニガキ

Picrasma Wood is the wood of *Picrasma quassioides* Bennet (Simaroubaceae).

**Description**  Light yellow chips, slices or short pieces of wood; a transverse section reveals distinct annual rings and thin medullary rays; tissue dense in texture. Odorless; taste, extremely bitter and lasting.

Under a microscope <5.01>, it reveals medullary rays consisting of 1 - 5 cells wide for transverse section, and 5 - 50 cells high for longitudinal section; vessels of spring wood up to about 150 μm in diameter, but those of autumn wood only one-fifth as wide; vessels, single or in groups, scattered in the xylem parenchyma; membrane of wood fibers extremely thickened; medullary rays and xylem parenchyma cells contain rosette aggregates of calcium oxalate and starch grains. Vivid yellow or red-brown, resinous substance often present in the vessels.

**Purity**  Foreign matter <5.01>—The amount of foreign matter contained in Picrasma Wood does not exceed 1.0%.

**Total ash**  <5.01>  Not more than 4.0%.

**Containers and storage**  Containers—Well-closed containers.

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**Powdered Picrasma Wood**

**Picrasmae Lignum Pulveratum**

ニガキ末

Powdered Picrasma Wood is the powder of Picrasma Wood.

**Description**  Powdered Picrasma occurs as a grayish white to light yellow powder. It is odorless, and has an extremely bitter and lasting taste.

Under a microscope <5.01>, Powdered Picrasma reveals fragments of vessels of various sizes, xylem fibers and xylem parenchyma cells; fragments of medullary rays containing starch grains; all tissues lignified; a few crystals of calcium oxalate observed. Starch grains are 5 to 15 μm in diameter.

**Total ash**  <5.01>  Not more than 4.0%.

**Acid-insoluble ash**  <5.01>  Not more than 1.0%.

**Containers and storage**  Containers—Well-closed containers.
Pinellia Tuber

*Pinelliae Tuber*

ハンゲ

Pinellia Tuber is the tuber of *Pinellia ternata* Breitenbach (*Araceae*), from which the cork layer has been removed.

**Description** Slightly flattened spherical to irregular-shaped tuber; 0.7 – 2.5 cm in diameter and 0.7 – 1.5 cm in height; externally white to grayish yellow-white; the upper end dentated, where the stem has been removed, with root scars dentated as numerous small spots on the circumference; dense in texture; cross section white and powdery.

Almost odorless; tasteless at first, slightly mucous, but leaving a strong acrid taste.

Under a microscope <5.0\(^{-1}\), a transverse section reveals mainly tissue of parenchyma filled with starch grains, and scattered with a few mucilage cells containing raphides of calcium oxalate. Starch grains mostly 2- to 3-compound grains, usually 10 – 15 \(\mu\)m in diameter, and simple grains, usually 3 – 7 \(\mu\)m in diameter; raphides of calcium oxalate 25 – 150 \(\mu\)m in length.

**Purity** (1) Rhizome of Arisaema species and others—Under a microscope <5.0\(^{-1}\), no mucilage canal is revealed on the outer layer of cortex.

(2) Heavy metals <1.0\(^{-1}\)—Proceed with 3.0 g of pulverized Pinellia Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.1\(^{-1}\)—Proceed with the test solution with 0.40 g of pulverized Pinellia Tuber according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying** <5.0\(^{-1}\) Not more than 14.0% (6 hours).

**Total ash** <5.0\(^{-1}\) Not more than 3.5%.

**Containers and storage** Containers—Well-closed containers.

Plantago Herb

*Plantaginis Herba*

シャゼンソウ

Plantago Herb is the entire plant of *Plantago asiatica* Linné (*Plantaginaceae*), collected during the flowering season.

**Description** Usually wrinkled and contracted leaf and spike, grayish green to dark yellow-green in color; when soaked in water and smoothed out, the lamina is ovate to orbicular-ovate, 4 – 15 cm in length, 3 – 8 cm in width; apex acute, and base sharply narrowed; margin slightly wavy, with distinct parallel veins; glabrous or nearly glabrous; petiole is rather longer than the lamina, and its base is slightly expanded with thin-walled leaf-sheath; scape is 10 – 50 cm in length, one-third to one-half of the upper part forming the spike, with dense florets; the lower part of inflorescence often shows pyxidia; roots usually removed, but, if any, fine roots are closely packed.

Odor, slight; tasteless.

**Identification** To 2.0 g of pulverized Plantago Herb add 10 mL of methanol, warm on a water bath for 3 minutes, cool, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.0\(^{-1}\>). Spot 10 \(\mu\)L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride TS on the plate: a dark blue spot appears at the *Rf* value about 0.5.

**Total ash** <5.0\(^{-1}\) Not more than 15.0%.

**Acid-insoluble ash** <5.0\(^{-1}\) Not more than 4.0%.

**Extract content** <5.0\(^{-1}\) Dilute ethanol-soluble extract: not less than 14.0%.

**Containers and storage** Containers—Well-closed containers.

Plantago Seed

*Plantaginis Semen*

シャゼンシ

Plantago Seed is the seed of *Plantago asiatica* Linné (*Plantaginaceae*).

**Description** Flattened ellipsoidal seed, 2 – 2.5 mm in length, 0.7 – 1 mm in width, 0.3 – 0.5 mm in thickness; externally brown to yellow-brown and lustrous. Under a magnifying glass, the surface of the seed is practically smooth, with the dorsal side protruding like a bow, and with the ventral side somewhat dentated; micropyle and raphe not observable. 100 seeds weigh about 0.05 g.

Odorless; taste, slightly bitter and mucous.

Under a microscope <5.0\(^{-1}\), a transverse section reveals a seed coat consisting of three layers of epidermis composed of cells containing mucilage, a vegetative layer, and a pigment layer of approximately equidiameter cells; in the interior, endosperm thicker than seed coat, enclosing two cotyledons.

**Identification** (1) To 1 g of Plantago Seed add 2 mL of warm water, and allow the mixture to stand for 10 minutes: the seed coat swells to discharge mucilage.

(2) Boil gently 1 g of Plantago Seed with 10 mL of dilute hydrochloric acid for 2 minutes, and filter. Neutralize the filtrate with sodium hydroxide TS, to 3 mL of this solution add 1 mL of Fehling’s TS, and warm the mixture: a red precipitate is produced.

**Purity** Foreign matter <5.0\(^{-1}\)—The amount of foreign matter contained in Plantago Seed does not exceed 2.0%.

**Total ash** <5.0\(^{-1}\) Not more than 5.5%.

**Acid-insoluble ash** <5.0\(^{-1}\) Not more than 2.0%.

**Containers and storage** Containers—Well-closed containers.
Platycodon Root

Platycodi Radix

キキョウ

Platycodon Root is the root of Platycodon grandiflorum A. De Candolle (Campanulaceae).

Description Irregular, somewhat thin and long fusiform to conical root, often branched; externally grayish brown, light brown or white; main root 10–15 cm in length, 1–3 cm in diameter; the upper end, with dented scars of removed stems; the neighborhood, with fine lateral wrinkles and longitudinal furrows and also slightly constricted; the greater part of the root, except the crown, covered with coarse longitudinal wrinkles, lateral furrows and lenticel-like lateral lines; hard in texture, but brittle; fractured surface not fibrous, often with cracks. Under a magnifying glass, a transverse section reveals cambium and its neighborhood often brown in color; cortex slightly thinner than xylem, almost white and with scattered cracks; xylem white to light brown in color, and the tissue slightly denser than cortex.

Odor, slight; tasteless at first, later acrid and bitter.

Identification (1) Boil 0.5 g of pulverized Platycodon Root with 10 mL of water for a while, allow to cool, and shake the mixture vigorously: a lasting fine foam is produced.

(2) Warm 0.2 g of pulverized Platycodon Root with 2 mL of acetic anhydride on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add carefully 0.5 mL of sulfuric acid: a lasting fine foam is produced.

Purity (1) Heavy metals <1.0>—Prepare with 3.0 g of Powdered Platycodon Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.1>—Prepare the test solution with 0.40 g of pulverized Platycodon Root according to Method 4, and perform the test (not more than 5 ppm).

Foreign matter—Under a microscope <5.0>, Powdered Platycodon Root does not show fibers, stone cells or other foreign matter.

Total ash <5.0> Not more than 4.0%.

Acid-insoluble ash <5.0> Not more than 1.0%.

Extract content <5.0> Dilute ethanol-soluble extract: not less than 25.0%.

Containers and storage Containers—Well-closed containers.

Powdered Platycodon Root

Platycodi Radix Pulverata

キキョウ末

Powdered Platycodon Root is the powder of Platycodon Root.

Description Powdered Platycodon Root occurs as a light grayish yellow to light grayish brown powder. It has a slight odor, and is tasteless at first, later acrid and bitter.

Under a microscope <5.0>, Powdered Platycodon Root reveals numerous fragments of colorless parenchyma cells; fragments of reticulate vessels and scalariform vessels; fragments of sieve tubes and laticiferous tubes; fragments of cork layer are sometimes observed. Usually, starch grains are not observed, but very rarely simple grain.

Identification (1) Boil 0.5 g of Powdered Platycodon Root with 10 mL of water for a while, allow to cool, and shake the mixture vigorously: a lasting fine foam is produced.

(2) Warm 0.2 g of Powdered Platycodon Root with 2 mL of acetic anhydride on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add carefully 0.5 mL of sulfuric acid to make two layers: a red to red-brown color develops at the zone of contact, and the upper layer acquires a blue-green to green color.

Purity (1) Heavy metals <1.0>—Prepare with 3.0 g of Powdered Platycodon Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.1>—Prepare the test solution with 0.40 g of Powdered Platycodon Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.0>, Powdered Platycodon Root does not show fibers, stone cells or other foreign matter.

Total ash <5.0> Not more than 4.0%.

Acid-insoluble ash <5.0> Not more than 1.0%.

Extract content <5.0> Dilute ethanol-soluble extract: not less than 25.0%.

Containers and storage Containers—Well-closed containers.

Platycodon Fluidextract

キキョウ流エキス

Method of preparation Take coarse powder of Platycodon Root, and prepare the fluidextract as directed under Fluidextracts using 25 vol% ethanol. An appropriate quantity of Ethanol and Purified Water or Purified Water in Containers may be used in place of 25 vol% ethanol.

Description Platycodon Fluidextract is a red-brown liquid. It is miscible with water, producing slight turbidity. It has a mild taste at first, followed by an acrid and bitter taste.

Identification (1) Shake vigorously 0.5 mL of Platycodon Fluidextract with 10 mL of water: a lasting fine foam is produced.

(2) Dissolve 1 drop of Platycodon Fluidextract in 2 mL of acetic anhydride and add gently 0.5 mL of sulfuric acid: a red to red-brown color develops at the zone of contact.

Purity (1) Heavy metals <1.0>—Prepare the test solution with 1.0 g of Platycodon Fluidextract as directed in the Fluidextracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Starch—Mix 1 mL of Platycodon Fluidextract with 4
mL of water, and add 1 drop of dilute iodine TS: no purple or blue color develops.

Content of the active principle Transfer exactly 5 mL of Platyctodon Fluid extract to a tared beaker, evaporate to dryness on a water bath, and dry at 105°C for 5 hours: the mass of the residue is not less than 0.50 g.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Pogostemon Herb

Pogostemoni Herba

 образом

Pogostemon Herb is the terrestrial part of Pogostemon cablin Bentham (Labiatae).

Description Stems with opposite leaves, leaves wrinkled and shrieveled. When smoothed by immersion in water, leaves are obovate to ovate-oblong, 2.5 – 10 cm in length, 2.5 – 7 cm in width, with obtusely serrate margins and petioles at the cuneate bases; the upper surface of leaves dark brown, the lower surface grayish brown, both sides covered densely with hairs. Stems are square, solid, grayish green, covered with grayish to yellowish white hairs; the pith broad, whitish, spongy. Under a magnifying glass, leaf reveals hairs, glandular hairs and glandular scales.

Odor, distinct; taste, slightly bitter.

Under a microscope (5.01), a transverse section of petiole reveals central portion of the adaxial side protruding remarkably, with collenchyma cells beneath epidermis; vascular bundles at the center divided into two groups. Under a microscope (5.01), a transverse section of the midvein of lamina reveals the adaxial side protruding remarkably, with collenchyma cells beneath epidermis; vascular bundles at the center arranged in fan-shape. Under a microscope (5.01), a transverse section of stem reveals several-cells-layered collenchyma beneath epidermis, occasionally with cork layer developed; beneath cortex, collateral vascular bundles arranged in a circle, phloem fibers in groups observed at the outer portion of phloem; oil droplets observed in parenchymat cells of cortex, needle, solitary or columnar crystals of calcium oxalate in parenchyma cells of pith.

Identification To 0.5 g of pulverized Pogostemon Herb, add 5 mL of methanol, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography (2.03). Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 105°C; a red spot appears at an Rf value of about 0.4.

Loss on drying (5.01) Not more than 15.0% (6 hours).

Total ash (5.01) Not more than 13.0%.

Acid-insoluble ash (5.01) Not more than 3.0%.

Essential oil content (5.01) When the test is performed with 50.0 g of pulverized Pogostemon Herb in a flask with 1 mL of silicon resin added, the essential oil content is not less than 0.3 mL.

Containers and storage Containers—Well-closed containers.

Polygala Root

Polygalae Radix

オジャ

Polygala Root is the root of Polygala tenuifolia Willdenow (Polygalaceae).

Description Thin, long and bent, cylindrical or tubular root; main root, 10 – 20 cm in length, 0.2 – 1 cm in diameter, sometimes with one to several lateral roots; externally light grayish brown, with coarse longitudinal wrinkles, and with deep lateral furrows cracked to some degree here and there; brittle, and fractured surface not fibrous; margin of the transverse section irregularly undulate; cortex, comparatively thick, with large cracks here and there; xylem usually round to elliptical, light brown in color, and often tears in a wedge-like shape.

Odor, slight; taste, slightly acrid.

Identification (1) Shake vigorously 0.5 g of pulverized Polygala Root with 10 mL of water: a lasting fine foam is produced.

(2) To 0.5 g of pulverized Polygala Root add 2 mL of acetic anhydride. After shaking well, allow to stand for 2 minutes, and filter. To the filtrate add carefully 1 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact, and the upper layer acquires a light blue-green to brown color.

Purity (1) Stem—When perform the test of foreign matter (5.01), the amount of the stems contained in Polygala Root does not exceed 10.0%.

(2) Heavy metals (1.07)—Prepare with 3.0 g of pulverized Polygala Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic (1.11)—Prepare the test solution with 0.40 g of pulverized Polygala Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter (5.01)—The amount of foreign matter other than the stems is not more than 1.0%.

(5) Total BHC’s and total DDT’s (5.01)—Not more than 0.2 ppm, respectively.

Total ash (5.01) Not more than 6.0%.

Containers and storage Containers—Well-closed containers.
Powdered Polygala Root

*Polygalae Radix Pulverata*

オンジ末

Powdered Polygala Root is the powder of Polygala Root.

**Description** Powdered Polygala Root occurs as a light grayish yellow-brown powder. It has a slight odor and a slightly acrid taste.

Under a microscope <5.01>, Powdered Polygala Root reveals fragments of cork layers, pitted vessels, reticulate vessels and tracheids; fragments of xylem fibers and xylem parenchyma cells with a small number of simple pits; fragments of parenchyma cells containing substances such as oil droplets, rosette aggregates and solitary crystals of calcium oxalate. Oil drop-like contents stained red with sudan III TS.

**Identification** (1) Shake vigorously 0.5 g of Powdered Polygala Root with 10 mL of water: a lasting fine foam is produced.

(2) To 0.5 g of Powdered Polygala Root add 2 mL of acetic anhydride. After shaking well, allow to stand for 2 minutes, and filter. To the filtrate add carefully 1 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact, and the upper layer acquires a light blue-green to brown color.

**Purity** (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Polygala Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Polygala Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Polygala Root does not show stone cells or starch grains.

(4) Total BHC’s and total DDT’s <5.01>—Not more than 0.2 ppm, respectively.

**Total ash** <5.01> Not more than 6.0%.

**Containers and storage** Containers—Well-closed containers.

Polygonatum Rhizome

*Polygonati Rhizoma*

オウセイ

Polygonatum Rhizome is the rhizome of *Polygonatum falcatum* A. Gray, *Polygonatum sibiricum* Redouté, *Polygonatum kingianum* Collett et Hemsley or *Polygonatum cyrtomonema* Hua (*Liliaceae*), usually after being steamed.

**Description** Irregularly cylindrical rhizome, 3 – 10 cm in length, 0.5 – 3 cm in diameter; or irregular massive rhizome, 5 – 10 cm in length, 2 – 6 cm in diameter, occasionally branched; both rhizomes with many cyclic nodes and longitudinally striate; externally yellow-brown to blackish brown; stem scars, round, concave at their center, and protuberant on the upper surface; root scars on the lower surface; cut surface flat and horny.

Odor, slight; taste, slightly sweet.

Under a microscope <5.01>, a transverse section of the rhizome reveals epidermis coated with cuticle; inside of epidermis parenchyma lie; numerous vascular bundles and mucilage cells scattered in parenchyma; vascular bundles collateral or amphivasal concentric; mucilage cells contain raphides of calcium oxalate.

**Identification** (1) To 0.5 g of fine cutted Polygonatum Rhizome add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add gently 0.5 mL of sulfuric acid: a red-brown color appears at the zone of contact.

(2) To 1.0 g of fine cutted Polygonatum Rhizome add 10 mL of dilute hydrochloric acid, boil gently for 2 minutes, and filter. Neutralize the filtrate with sodium hydroxide TS. To 3 mL of this solution add 1 mL of Fehling’s TS, and warm: red precipitates appear.

**Purity** (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Polygonatum Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Polygonatum Rhizome according to Method 4, and perform the test (not more than 5 ppm).

**Total ash** <5.01> Not more than 5.0%.

**Acid-insoluble ash** <5.01> Not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

Polygonum Root

*Polygoni Multiflori Radix*

カシュウ

Polygonum Root is the root of *Polygonum multiflorum* Thunberg (*Polygonaceae*), often being cut into round slices.

**Description** Polygonum Root is nearly fusiform, 10 – 15 cm in length, 2 – 5 cm in diameter; externally red-brown to dark brown; roughly wrinkled; a cross section light red-brown or light grayish brown, with numerous abnormal vascular bundles scattering irregularly around the large vascular bundles near center; heavy and hard in texture.

Odor, slight and characteristic; taste, astringent and slightly bitter.

Under a microscope <5.01>, transverse section reveals the outermost layer to be several cells thick and composed of cork; cork cells contain brown substances; cortex composed of parenchyma; abnormal vascular bundles, exhibiting a ring of cambium; xylem lies inside of the cambium, and phloem outside; fibers lie outside the phloem; central portion of root lignified; parenchymatous cells contain aggregated crystals.
of calcium oxalate, and both simple and 2- to 8-compound starch grains; navel of starch grain obvious.

Identification To 1 g of pulverized Polygonum Root add 10 mL of methanol, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 2 mL of methanol, and use this as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography \(<2.03\>\). Spot 5 \(\mu\)L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, water, methanol and acetic acid (100) (200:10:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a fluorescent bluish white spot appears at an \(Rf\) value of about 0.3.

Purity (1) Heavy metals \(<1.07\>)—Proceed with 3.0 g of pulverized Polygonum Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.17\>)—Prepare the test solution with 0.40 g of pulverized Polygonum Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying \(<5.01\>) Not more than 14.0% (6 hours).

Total ash \(<5.01\>) Not more than 5.5%.

Extract content \(<5.01\>) Dilute ethanol-soluble extract: not less than 17.0%.

Containers and storage Containers—Well-closed containers.

### Polyergus Sclerotium

Polyergus

チョレイ

Polyergus Sclerotium is the sclerotium of *Polyergus umbellatus* Fries (*Polyporaceae*).

Description Irregularly shaped mass, usually 5 – 15 cm in length; externally blackish brown to grayish brown, with numerous dents and coarse wrinkles; breakable; fractured surface rather soft and cork-like, and almost white to light brown in color, and a white speckled pattern on the inner region; light in texture.

Odorless and tasteless.

Identification Warm, while shaking, 0.5 g of Powdered Polyergus Sclerotium with 5 mL of acetone on a water bath for 2 minutes, filter and evaporate the filtrate to dryness. Dissolve the residue in 5 drops of acetic anhydride, and add 1 drop of sulfuric acid: a red-purple color develops, and immediately changes to dark green.

Purity (1) Heavy metals \(<1.07\>)—Proceed with 3.0 g of Powdered Polyergus Sclerotium according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.17\>)—Prepare the test solution with 0.40 g of Powdered Polyergus Sclerotium according to Method 4, and perform the test (not more than 5 ppm).

Total ash \(<5.01\>) Not more than 16.0%.

Acid-insoluble ash \(<5.01\>) Not more than 4.0%.

Containers and storage Containers—Tight containers.

### Poria Sclerotium

Poria

ブクリョウ

Poria Sclerotium is the sclerotium of *Wolfiporia cocos* Ryvarden et Gilbertson (*Poria cocos* Wolf) (*Polyporaceae*), from which usually the external layer has been mostly removed.

Description Mass, about 10 – 30 cm in diameter, up to 0.1 – 2 kg in mass; usually it appears as broken or chipped pieces; white or slightly reddish white; sclerotium with remaining outer layer is dark brown to dark red-brown in
color, coarse, which fissures; hard in texture, but brittle.

Almost odorless, tasteless, and slightly mucous.

**Identification (1)** Warm 1 g of pulverized Poria Sclerotium with 5 mL of acetone on a water bath for 2 minutes with shaking, and filter. Evaporate the filtrate to dryness, dissolve the residue in 0.5 mL of acetic anhydride, and add 1 drop of sulfuric acid: a light red color develops, which changes immediately to dark green.

(2) To a section or powder of Poria Sclerotium add 1 drop of iodine TS: a deep red-brown color is produced.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of Poria Sclerotium according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Poria Sclerotium according to Method 4, and perform the test (not more than 5 ppm).

**Total ash** <5.01> Not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

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**Powdered Poria Sclerotium**

*Poria Pulveratum*

ブクリョウ末

Powdered Poria Sclerotium is the powder of Poria Sclerotium.

**Description** Powdered Poria Sclerotium occurs as a white to grayish white powder. It is almost odorless and tasteless, but is slightly mucous.

Under a microscope <5.01>, Powdered Poria Sclerotium reveals colorless and transparent hyphae strongly refracting light, and fragments of false tissue consisting of granules and mucilage plates. Thin hyphae, 2–4 μm in diameter; thick ones, usually 10–20 μm, up to 30 μm.

**Identification (1)** Warm 1 g of Powdered Poria Sclerotium with 5 mL of acetone on a water bath for 2 minutes with shaking, and filter. Evaporate the filtrate to dryness, dissolve the residue in 0.5 mL of acetic anhydride, and add 1 drop of sulfuric acid: a light red color develops, which changes immediately to dark green.

(2) To Powdered Poria Sclerotium add 1 drop of iodine TS: a deep red-brown color is produced.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of Powdered Poria Sclerotium according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Poria Sclerotium according to Method 4, and perform the test (not more than 5 ppm).

**Total ash** <5.01> Not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

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**Processed Aconite Root**

*Processi Aconiti Radix*

ブシ

Processed Aconite Root is the tuberous root of *Aconitum carmichaeli* Debeaux or *Aconitum japonicum* Thunberg (*Ranunculaceae*) prepared by the following processes.

**Process 1**: Autoclaving. [Processed Aconite Root 1]

**Process 2**: Heating or autoclaving after rinsing in salt or rock salt solution. [Processed Aconite Root 2]

**Process 3**: Treating with calcium hydroxide after rinsing in salt solution. [Processed Aconite Root 3]

Processed Aconite Root 1, Processed Aconite Root 2 and Processed Aconite Root 3 contain the total alkaloid [as benzoyl aconin (C_{32}H_{45}NO_{10} 603.70) of not less than 0.7% and not more than 1.5%, not less than 0.1% and not more than 0.6%, and not less than 0.5% and not more than 0.9%, calculated on the dried bases, respectively.

The label indicates the treating process.

**Description** Processed Aconite Root 1: Cut pieces irregularly polygonal, less than 10 mm in diameter; externally dark grayish brown to blackish brown; hard in texture; cut surface flat, light brown to dark brown, usually horny and lustrous.

Odor, weak and characteristic.

Under a microscope <5.01>, transverse and longitudinal sections reveal pitted, scaraliform, reticulate and spiral vessels; starch grains in parenchymatous cells usually gelatinized but sometimes not gelatinized; starch grains, simple, spherical or ellipsoidal, 2–25 μm in diameter, or 2- to a dozen or so- compound, hilum of starch grain distinct.

Processed Aconite Root 2: Nearly obconical root, 15–30 mm in length, 12–16 mm in diameter, slices cut longitudinally or transversely, 20–60 mm in length, 15–40 mm in width, and 200–700 μm in thickness, or cut pieces irregularly polygonal, less than 12 mm in diameter; externally light brown to dark brown or yellowish brown; hard in texture, usually without wrinkles; cut surface flat, light brown to dark brown or yellowish white to light yellow-brown, usually horny, semi-transparent and lustrous.

Odor, weak and characteristic.

Under a microscope <5.01>, transverse and longitudinal sections reveal metaderm, primary cortex, endodermis, secondary cortex, cambium, and xylem; primary cortex contains oblong to oblong-square sclerenchymatous cells, 30–75 μm in short axis, 60–150 μm in long axis; endodermis single layered, endodermal cells elongated in tangential direction; cambium, star shaped or irregular polygons to orbicular; a group of vessel in xylem v-shaped; sometimes isolated ring of cambium appears in secondary cortex or in pith; vessels, pitted, scaraliform, reticulate and spiral; starch grains in parenchymatous cells gelatinized.

Processed Aconite Root 3: Cut pieces irregularly polygonal, less than 5 mm in diameter; externally grayish brown; hard
in texture; cut surface flat, light grayish brown to grayish white, not lustrous.

Odor, weak and characteristic.

Under a microscope \(<5.0\times\) transverse and longitudinal sections reveal pitted, scaraliform, reticulate and spiral vessels; starch grains, simple, spherical or ellipsoid, 2–25 \(\mu\text{m}\) in diameter, or 2–to a dozen or so- compound, hilum of starch grain distinct.

**Identification** To 3 g of pulverized Processed Aconite Root add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and take the ether layer. Evaporate the ether layer to dryness under reduced pressure, dissolve the residue in 1 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.0\times\). Spot 10 \(\mu\text{L}\) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia water (28:40:32) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate, air-dry the plate, and spray evenly sodium nitrite TS: one of the spot among the several spots from the sample solution has the same color tone and \(Rf\) value with the yellow-brown spot from the standard solution.

**Purity** (1) Heavy metals \(<1.0\times\)—Proceed with 3.0 g of pulverized Processed Aconite Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.1\times\)—Prepare the test solution with 0.40 g of pulverized Processed Aconite Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately about 0.5 g of pulverized Processed Aconite Root, put in a glass-stoppered centrifuge tube, suspend in 3.0 mL of water by shaking, and add 1.0 mL of ammonia TS and 20 mL of diethyl ether. Stopper tightly the tube, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all extracts, evaporate to dryness under reduced pressure below 40°C, and dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetoxitride (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 \(\mu\text{L}\) each of the sample solution and aconitum diester alkaloids standard solution for purity as directed under Liquid Chromatography \(<2.0\times\) according to the following conditions, and determine the heights of the peaks corresponding to aconitine, \(H_{TA}\) and \(H_{SA}\), jesaconitine, \(H_{SJ}\) and \(H_{SJ}\), hypaconitine, \(H_{TH}\) and \(H_{TH}\), and mesaconitine, \(H_{TM}\) and \(H_{SM}\), respectively, and calculate the amounts of them by the following formulæ: the amounts of aconitine, jesaconitine, hypaconitine and mesaconitine per g calculated on the dried basis are not more than 60 \(\mu\text{g}\), 60 \(\mu\text{g}\), 280 \(\mu\text{g}\) and 140 \(\mu\text{g}\), respectively, and the total amount of them is not more than 450 \(\mu\text{g}\).

**Crude Drugs / Processed Aconite Root**

Amount (\(\mu\text{g}\)) of aconitine (\(C_{34}H_{47}NO_{11}\)) 
\[= \frac{C_{SA}}{M} \times \frac{H_{TA}}{H_{SA}} \times 10\]

Amount (\(\mu\text{g}\)) of jesaconitine (\(C_{34}H_{46}NO_{12}\)) 
\[= \frac{C_{SJ}}{M} \times \frac{H_{SJ}}{H_{SM}} \times 10\]

Amount (\(\mu\text{g}\)) of hypaconitine (\(C_{34}H_{45}NO_{13}\)) 
\[= \frac{C_{SH}}{M} \times \frac{H_{TH}}{H_{SH}} \times 10\]

Amount (\(\mu\text{g}\)) of mesaconitine (\(C_{34}H_{44}NO_{14}\)) 
\[= \frac{C_{SM}}{M} \times \frac{H_{TM}}{H_{SM}} \times 10\]

**System suitability**—

System performance: When the procedure is run with 20 \(\mu\text{L}\) of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5, respectively.

System repeatability: To 1 mL of aconitum diester alkaloids standard solution for purity add a mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17)

Flow rate: Adjust the flow rate so that the retention time of mesaconitine is about 31 minutes.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeylsilanized silica gel for liquid chromatography (5 \(\mu\text{m}\) in particle diameter).

Column temperature: A constant temperature of about 40°C

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: Adjust the flow rate so that the retention time of mesaconitine is about 31 minutes.

**System suitability**—

System performance: When the procedure is run with 20 \(\mu\text{L}\) of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5, respectively.

System repeatability: To 1 mL of aconitum diester alkaloids standard solution for purity add a mixture of phosphate buffer solution for processed aconite root and acetoxitride (1:1) to make 10 mL. When the test is repeated 6 times with 20 \(\mu\text{L}\) of this solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

**Loss on drying** \(<5.0\times\) Not more than 15.0% (6 hours).

**Total ash** \(<5.0\times\)

Processed Aconite Root 1: Not more than 4.0%.
Processed Aconite Root 2: Not more than 12.0%.
Processed Aconite Root 3: Not more than 19.0%.

**Acid-insoluble ash** \(<5.0\times\) Not more than 0.9%.

**Assay** Weigh accurately about 2 g of pulverized Processed Aconite Root, put in a glass-stoppered centrifuge tube, and add 1.6 mL of ammonia TS and 20 mL of diethyl ether. Stopper tightly the tube, shake for 30 minutes, centrifuge,
and separate the ether layer. To the residue add 0.8 mL of ammonia TS and 20 mL of diethyl ether, and proceed as above. Repeat this process more three times. Combine all extracts, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL of ethanol (99.5), add 30 mL of freshly boiled and cooled water, and titrate \( \text{C}_2\text{H}_5\text{OH} \) with 0.01 mol/L hydrochloric acid VS until the color of the solution changes from green to gray-blue through blue-green (indicator: 3 drops of methyl red-methylene blue TS). Perform a blank determination and make any necessary correction.

Each mL of 0.01 mol/L hydrochloric acid VS = 6.037 mg of total alkaloid [as benzoylaconine \( \text{C}_{32}\text{H}_{45}\text{NO}_{10} \)]

Containers and storage Containers—Well-closed contains. Exposed Processed Aconite Root 2 occurs as a light grayish white powder. It has a characteristic odor.

**Powdered Processed Aconite Root**

*Processi Aconiti Radix Pulverata*

PWSh

Powdered Processed Aconite Root is the powder of Processed Aconite Root prepared by the process 1 or process 2, the powder of Processed Aconite Root prepared by process 1, or the powder of Processed Aconite Root prepared by the process 1 to which Corn Starch or Lactose Hydrate is added.

**Process 1:** Autoclaving. [Powdered Processed Aconite Root 1]

**Process 2:** Heating or autoclaving after rinsing in salt or rock salt solution. [Powdered Processed Aconite Root 2]

Powdered Processed Aconite Root 1 and Powdered Processed Aconite Root 2 contain the total alkaloid [as benzoyl aconin \( \text{C}_{32}\text{H}_{45}\text{NO}_{10} \): 603.70] of not less than 0.4% and not more than 1.2%, and not less than 0.1% and not more than 0.3%, calculated on the dried bases, respectively.

The label indicates the treating process.

**Description** Powdered Processed Aconite Root 1: Powdered Processed Aconite Root occurs as a light grayish brown powder. It has a characteristic odor.

Under a microscope \( \text{SA} \), Powered Processed Aconite Root 1 reveals gelatinized starch masses or starch grains and parenchymatous cells containing them, fragments of red-brown metaderm, fragments of pitted, scaraliform, reticulate and spiral vessels; also square to oblong-square sclerenchymatous cells, 30 – 150 \( \mu \text{m} \) in diameter, 100 – 250 \( \mu \text{m} \) in length, cell wall of sclerenchymatous cells, 6 – 12 \( \mu \text{m} \) in thickness.

Powdered Processed Aconite Root 2: Powdered Processed Aconite Root 2 occurs as a light yellowish white powder. It has a characteristic odor.

Under a microscope \( \text{SA} \), Powered Processed Aconite Root 2 reveals gelatinized starch masses and parenchymatous cells containing them, fragments of red-brown metaderm, fragments of pitted, scaraliform, reticulate and spiral vessels; also square to oblong-square sclerenchymatous cells, 30 – 150 \( \mu \text{m} \) in diameter, 100 – 250 \( \mu \text{m} \) in length, cell wall of sclerenchymatous cells, 6 – 12 \( \mu \text{m} \) in thickness.

**Identification** To 3 g of Powdered Processed Aconite Root add 2 mL of ammonia TS and 20 mL of diethyl ether, shake for 10 minutes, and centrifuge. Evaporate the ether layer to dryness under reduced pressure, dissolve the residue in 1 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{C}2\text{.03} \). Spot 10 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia water (28-40:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate, air-dry the plate, and spray evenly sodium nitrite TS: one of the spots among the several spots from the sample solution has the same color tone and \( Rf \) value with the yellow-brown spot from the standard solution.

**Purity** (1) Heavy metals \( \text{C}1\text{.07} \)—Proceed with 3.0 g of Powdered Processed Aconite Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \( \text{C}1\text{.17} \)—Prepare the test solution with 0.40 g of Powdered Processed Aconite Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately about 0.5 g of Powdered Processed Aconite Root, put in a glass-stoppered centrifuge tube, suspend in 3.0 mL of water by shaking, and add 1.0 mL of ammonia TS and 20 mL of diethyl ether. Stopper tightly the tube, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process two times. Combine all extracts, evaporate to dryness under reduced pressure below 40°C, and dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 \( \mu \text{L} \) each of the sample solution and aconitum diester alkaloids standard solution for purity as directed under Liquid Chromatography \( \text{C}2\text{.01} \) according to the following conditions, and determine the heights of the peaks corresponding to aconitine, \( H_{\text{TA}} \) and \( H_{\text{SA}} \), jesaconitine, \( H_{\text{TA}} \) and \( H_{\text{SA}} \), hypaconitine, \( H_{\text{TA}} \) and \( H_{\text{SA}} \), and mesaconitine, \( H_{\text{TA}} \) and \( H_{\text{SA}} \), respectively, and calculate the amounts of them by the following formulae: the amounts of aconitine, jesaconitine, hypaconitine and mesaconitine per g calculated on the dried basis are not more than 55 \( \mu \text{g} \), 40 \( \mu \text{g} \), 55 \( \mu \text{g} \) and 120 \( \mu \text{g} \), respectively, and the total amount of them is not more than 230 \( \mu \text{g} \).

Amount (\( \mu \text{g} \)) of aconitine \( \text{C}_{32}\text{H}_{45}\text{NO}_{10} \)= \( C_{\text{SA}}/M \times H_{\text{TA}}/H_{\text{SA}} \times 10 \)

Amount (\( \mu \text{g} \)) of jesaconitine \( \text{C}_{32}\text{H}_{45}\text{NO}_{10} \)= \( C_{\text{SA}}/M \times H_{\text{TA}}/H_{\text{SA}} \times 10 \)
Amount (µg) of hypaconitine (C₃₃H₄₅NO₁₀)
= Cₛ₃H/M × Hₛ₃H/₁₀

Amount (µg) of mesaconitine (C₃₃H₄₆NO₁₁)
= Cₛ₃M/M × Hₛ₃M/₁₀

Cₛ₃A: Concentration (µg/mL) of aconitine for purity in aconitum diester alkaloids standard solution for purity
Cₛ₃J: Concentration (µg/mL) of jesaconitine for purity in aconitum diester alkaloids standard solution for purity
Cₛ₃M: Concentration (µg/mL) of hypaconitine for purity in aconitum diester alkaloids standard solution for purity
Cₛ₃M: Concentration (µg/mL) of mesaconitine for purity in aconitum diester alkaloids standard solution for purity
M: Amount (g) of the sample, calculated on the dried basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A temperature constant of about 40°C.
Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).
Flow rate: Adjust the flow rate so that the retention time of mesaconitine is about 31 minutes.

System suitability—
System performance: When the procedure is run with 20 µL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5, respectively.
System repeatability: To 1 mL of aconitum diester alkaloids standard solution for purity add a mixture of phosphate buffer solution for processed aconite root and acetone (1:1) to make 10 mL. When the test is repeated 6 times with 20 µL of this solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying <5.0% Not more than 11.0% (6 hours).
Total ash ≤ 0.01%Powdered Processed Aconite Root 1: Not more than 4.0%.
Powdered Processed Aconite Root 2: Not more than 7.0%.
Acid-insoluble ash <0.01% Not more than 0.7%.

Assay Weigh accurately about 2 g of Powdered Processed Aconite Root, put in a glass-stoppered centrifuge tube, and add 1.6 mL of ammonia TS and 20 mL of diethyl ether. Stopper tightly the tube, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 0.8 mL of ammonia TS and 20 mL of diethyl ether, and proceed as above. Repeat this process more three times. Combine all extracts, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL of ethanol (99.5), add 30 mL of freshly boiled and cooled water, and titrate with 0.01 mol/L hydrochloric acid VS until the color of the solution changes from green to gray-blue through blue-green (indicator: 3 drops of methyl red-methylene blue TS). Perform a blank determination and make any necessary correction.

Each mL of 0.01 mol/L hydrochloric acid VS = 6.037 mg of total alkaloid (as benzoylaconine (C₃₂H₄₅NO₁₀))

Containers and storage Containers—Well-closed containers.

Processed Ginger

Zingiberis Processum Rhizoma

カンキョウ

Processed Ginger is the rhizome of Zingiber officinale Roscoe (Zingiberaceae), after being passed through hot water or being steamed.

Description Irregularly compressed and often branched massive rhizome; branched parts slightly curved ovoid or oblong-ovoid, 2 – 4 cm in length, and 1 – 2 cm in diameter; external surface grayish yellow to grayish yellow-brown, with wrinkles and ring node; fractured surface brown to dark brown, transparent and horny; under a magnifying glass, a transverse section reveals cortex and stele distinctly divided; vascular bundles scattered throughout the surface.

Odor, characteristic; taste, extremely pungent.

Under a microscope <5.0%, a transverse section reveals cork layer, cortex and stele in this order from the outside; cortex and stele, divided by a single-layered endodermis, composed of parenchyma, vascular bundles scattered and surrounded by fiber bundles; oil cells contain yellow oil-like substances, scattered in parenchyma; parenchymatous cells contain solitary crystals of calcium oxalate, and gelatinized starch.

Identification To 2 g of pulverized Processed Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution (1). To the residue add 5 mL of methanol, proceed in the same manner as above, and use so obtained solution as the sample solution (2). Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (1). Separately, dissolve 1 mg of Sucrose in 2 mL of methanol, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 µL each of the sample solution (1) and standard solution (1) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray even 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution (1) has the same color tone and Rf value with the green spot from the standard solution (1). Spot 10 µL each of the sample solution (2) and
standard chromatography, develop the plate with a mixture of 1-
butanol, water and acetic acid (100) (8:5:3) to a distance of
about 10 cm, and air-dry the plate. Spray evenly 1,3-
naphthalenediol TS on the plate, and heat at 105°C for 5
minutes: one of the spot among the several spots from the
sample solution (2) has the same color tone and Rf value
with the red-purple spot from the standard solution (2).

Purity Arsenic \(<1.11>\) Prepare the test solution with
0.40 g of pulverized Processed Ginger according to Method
4, and perform the test (not more than 5 ppm).

Loss on drying \(<5.01>\) Not more than 15.0% (6 hours).

Total ash \(<5.01>\) Not more than 6.5%.

Acid-insoluble ash \(<5.01>\) Not more than 1.5%.

Extract content \(<5.01>\) Dilute ethanol-soluble extract: not
less than 8.0%.

Containers and storage Containers—Well-closed contain-
ers.

Prunella Spike

Prunellae Spica

カゴソウ

Prunella Spike is the spike of Prunella vulgaris Linné var. *ilacina* Nakai (*Labiatae*).

Description Spikes in nearly cylindrical and wheat ear-like
shape, 3 – 6 cm in length, 1 – 1.5 cm in diameter, externally
grayish brown; spikes composed of a floral axis having
numerous bracts and calyces; corollas often remaining on
the upper part; a calyx usually enclosing four mericarps;
bract, cordonate to eccentric, and exhibiting white hairs on
the vein, as on the calyx; light in texture.

Almost odorless and tasteless.

Purity (1) Stem—When perform the test of foreign mat-
ter \(<5.01>\), the amount of the stems contained in Prunella
Spike does not exceed 5.0%.

(2) Foreign matter \(<5.01>\) —The amount of foreign matter
other than the stems contained in Prunella Spike does not
exceed 1.0%.

Total ash \(<5.01>\) Not more than 13.0%.

Acid-insoluble ash \(<5.01>\) Not more than 5.0%.

Containers and storage Containers—Well-closed contain-
ers.

Pueraria Root

Puerariae Radix

カッコン

Pueraria Root is the root of *Pueraria lobata* Ohwi
(*Leguminosae*), from which periderm has been re-
moved.

It contains not less than 2.0% of puerarin (*C_{21}H_{20}O_{9*}:
416.38), calculated on the basis of dried material.

Description Usually cut into small pieces of irregular hexa-
gons of about 0.5 cm cube, or cut into longitudinally plate-
like pieces 20 – 30 cm in length, 5 – 10 cm in width, and
about 1 cm in thickness; externally light grayish yellow to
grayish white; transverse section showing concentric annu-
late ring or part of it formed by abnormal growth of cambium.
Under a magnifying glass, phloem light grayish yellow in
color; in xylem, numerous vessels appearing as small dots;
medullary rays slightly dented; vertical section showing lon-
gitudinal patterns formed alternately by fibrous xylem and
parenchyma; easily breakable lengthwise, and its section ex-
tremely fibrous.

Odorless; taste, at first slightly sweet, followed by a slight
bitterness.

Under a microscope \(<5.01>\), a transverse section reveals
fiber bundles accompanied by crystal cells in phloem; dist-
inct vessels and xylem fibers in xylem; starch grains
numerous in parenchyma, mainly composed of polygonal
simple grains, rarely 2- to 3-compound grains, 2 – 18 \(\mu\)m,
mostly 8 – 12 \(\mu\)m, in size, with hilum or cleft in the center,
and also with striae.

Identification To 2 g of pulverized Pueraria Root add 10
mL of methanol, shake for 3 minutes, filter, and use the fil-

trate as the sample solution. Separately, dissolve 1 mg of
Puerarin RS in 1 mL of methanol, and use this solution as
the standard solution. Perform the test with these solutions
as directed under Thin-layer Chromatography \(<2.03>\). Spot 2
\(\mu\)L each of the sample solution and standard solution on a
plate of silica gel for thin-layer chromatography. Develop
the plate with a mixture of ethyl acetate, methanol and water
(12:2:1) to a distance of about 10 cm, and air-dry the plate.
Examine under ultraviolet light (main wavelength: 365 nm):
one of the spot among the several spots from the sample
solution has the same color tone and Rf value with the bluish
white fluorescent spot from the standard solution.

Purity (1) Heavy metals \(<1.07>\) —Proceed with 3.0 g of
pulverized Pueraria Root according to Method 3, and per-
form the test. Prepare the control solution with 3.0 mL of
Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.11>\) —Prepare the test solution with 0.40 g
of pulverized Pueraria Root according to Method 4, and per-
form the test (not more than 5 ppm).

Loss on drying \(<5.01>\) Not less than 13.0% (6 hours).

Total ash \(<5.01>\) Not more than 6.0%.

Assay Weigh accurately about 0.3 g of pulverized Pueraria
Root, add 30 mL of diluted methanol (1 in 2), and heat
under a reflex condenser on a water bath for 30 minutes,
cool, and filter. To the residue add 50 mL of diluted metha-


nol (1 in 2), and perform as the same as above. Combine the
filtrates, add diluted methanol (1 in 2) to make exactly 100
mL, and use this solution as the standard solution. Separately,
weigh accurately about 10 mg of Puerarin RS (separately
determine the water), add diluted methanol (1 in 2) to make
exactly 100 mL, and use this solution as the sample solution.
Perform the test with exactly 10 \(\mu\)L each of the sample solu-
tion and standard solution as directed under Liquid Chroma-
tography \(<2.01>\) according to the following conditions, and
Quercus Bark

Quercus Cortex

ボクソク

Quercus Bark is the bark of Quercus acutissima Carruthers, Quercus serrata Murray, Quercus mongolica Fischer ex Ledebour var. crispa Ohashi or Quercus variabilis Blume (Fagaceae).

Description Plate-like or semi-tubular pieces of bark, 5–15 mm in thickness; externally grayish brown to dark brown, with thick periderm and longitudinal coarse splits; internally brown to light brown, with longitudinal ridges, the transverse section brown to light brown, white small spots composed of stone cells in groups observed sporadically.

Almost odorless, tasteless.

Under a microscope <5.0×>, a transverse section reveals a cork layer with scattered cork stone cells; in secondary cortex fiber bundles lined almost stepwise, large groups of stone cells arranged irregularly; in parenchyma aggregated crystals of calcium oxalate scattered; adjacent to stone cells and fiber cells, cells containing solitary crystals of calcium oxalate observed, and these cells form crystal cell rows in a longitudinal section.

Identification To 2 g of pulverized Quercus Bark, add 10 mL of ethyl acetate, shake for 10 minutes, and centrifuge to remove ethyl acetate. Add 10 mL of acetone to the residue, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>.

Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): Two consecutive fluorescent spots in different colors are observed at Rf value of about 0.4. Then, spray evenly diluted sulfuric acid on the plate, heat at 105°C. Examine under ultraviolet light (main wavelength: 365 nm): one of these spots produces fluorescence.

Total ash <5.0%> Not more than 8.5%.

Acid-insoluble ash <5.0%> Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Red Ginseng

Ginseng Radix Rubra

コウジン

Red Ginseng is the root of Panax ginseng C. A. Meyer (Panax schinseng Nees) (Araliaceae), after being steamed.

It contains not less than 0.10% of ginsenoside Rg1 (C42H72O14: 801.01) and not less than 0.20% of ginsenoside Rb1 (C44H92O23: 1109.29), calculated on the basis of dried material.

Description Thin and long cylindrical to fusiform root, often branching out into 2 to 5 lateral roots from the middle; 5–25 cm in length, main root 0.5–3 cm in diameter; externally light yellow-brown to red-brown, and translucent and with longitudinal wrinkles; crown somewhat constricted, and sometimes with short remains of stem; fractured surface flat; horny and hard in texture.

Odor, characteristic; taste, at first slightly sweet, followed by a slight bitterness.

Identification (1) To 0.2 g of pulverized Red Ginseng add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add gently 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact.

(2) To 2.0 g of pulverized Red Ginseng add 10 mL of water and 10 mL of 1-butanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside Rg1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (14:5:4) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol
TS for spraying on the plate, and heat at 105°C for 10 minutes: one of the spots among the several spots obtained from the sample solution has the same color tone and RI value with the spot from the standard solution.

**Purity (1)** Heavy metals $<1.0\%$—Proceed with 1.0 g of pulverized Red Ginseng according to Method 4, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 15 ppm).

(2) Arsenic $<0.1\%$—Prepare the test solution with 1.0 g of pulverized Red Ginseng according to Method 4, and perform the test (not more than 2 ppm).

(3) Foreign matter $<3.0\%$—The amount of stems and other foreign matter contained in Red Ginseng does not exceed 2.0%.

(4) Total BHC’s and total DDT’s $<5.0\%$—Not more than 0.2 ppm, respectively.

**Loss on drying** $<5.0\%$ Not more than 15.5% (6 hours).

**Total ash** $<5.0\%$ Not more than 4.5%.

**Extract content** $<5.0\%$ Dilute ethanol-soluble extract: not less than 18.0%.

**Assay (1)** Ginsenoside Rg$_1$—Weigh accurately about 1 g of pulverized Red Ginseng, put in a glass—stopped centrifuge tube, add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the procedure with the residue using 15 mL of diluted methanol (3 in 5), combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of 0.1 mol/L hydrochloric acid TS and diluted methanol (3 in 5) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rg$_1$ RS (separately determine the water) dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of ginsenoside Rg$_1$.

\[
A_T/A_S = S \times M_S
\]

$M_S$: Amount (mg) of Ginsenoside Rg$_1$, calculated on the anhydrous basis

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of ginsenoside Rg$_1$ is about 25 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rg$_1$ RS and ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, ginsenoside Rg$_1$ and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rg$_1$ is not more than 1.5%.

**System performance**—

System performance: Dissolve 1 mg each of Ginsenoside Rb$_1$ RS and ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, ginsenoside Rb$_1$ and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb$_1$ is not more than 1.5%.

**Containers and storage**—

Containers—Well-closed containers.

**Rehmannia Root**

**Rehmanniae Radix**

シオウ

Rehmannia Root is the root of *Rehmannia glutinosa* Liboschitz var. purpurea Makino or *Rehmannia glutinosa* Liboschitz (Scrophulariaceae), with or without application of steaming.

**Description** Thin and long, usually fusiform root, 5 – 10 cm in length, 0.5 – 3.0 cm in diameter, often broken or markedly deformed in shape; externally yellow-brown to blackish brown, with deep, longitudinal wrinkles and constrictions; soft in texture and mucous; transverse section
yellow-brown to blackish brown, and cortex darker than xylem in color; pith hardly observable.

Odor, characteristic; taste, slightly sweet at first, followed by a slight bitterness.

Under a microscope <5.01>, a transverse section reveals 7 to 15 layers of cork; cortex composed entirely of parenchyma cells; outer region of cortex with scattered cells containing brown secretes; xylem practically filled with parenchyma; vessels radially lined, mainly reticulate vessels.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Rehmannia Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm). (2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Rehmannia Root according to Method 4, and perform the test (not more than 5 ppm).

**Total ash** <5.01> Not more than 6.0%.

**Acid-insoluble ash** <5.01> Not more than 2.5%.

**Containers and storage** Containers—Well-closed containers.

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**Rhubarb**

**Rhei Rhizoma**


It contains not less than 0.25% of sennosides A (C_{42}H_{36}O_{20}: 862.74), calculated on the basis of dried material.

**Description** Ovoid, oblong-ovoid or cylindrical rhizome, often cut crosswise or longitudinally, 4 – 10 cm in diameter, 5 – 15 cm in length. In the case of Rhubarb without most part of cortex, the outer surface is flat and smooth, yellow-brown to light brown in color, and sometimes exhibiting white, fine reticulations; thick and hard in texture. In the case of Rhubarb with cork layer, externally dark brown or reddish black, and with coarse wrinkles; rough and brittle in texture. The fractured surface of Rhubarb is not fibrous; transverse section grayish brown, light grayish brown or brown in color, having patterns of blackish brown tissue complicated with white and light brown tissues; near the cambium, the patterns often radiate, and in pith, consist of whirrs of tissues radiated from the center of a small brown circle 1 – 3 mm in diameter and arranged in a ring or scattered irregularly.

Odor, characteristic; taste, slightly astringent and bitter; when chewed, gritty between the teeth, and coloring the saliva yellow.

Under a microscope <5.01>, the transverse section reveals mostly parenchyma cells; small abnormal cambium-rings scattered here and there in the pith; the cambium-rings produce phloem inside and xylem outside, accompanied with 2 to 4 rows of medullary rays containing brown-colored substances, and the rays run radiately from the center of the ring towards the outside forming whirrs of tissues; parenchyma cells contain starch grains, brown-colored substances or crystal druses of calcium oxalate.

**Identification** To 2 g of pulverized Rhubarb add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 by adding 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, add 30 mL of tetrahydrofuran, shake for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A RS in 4 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 40 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography at 10 mm along the initial line. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100:40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same RI value.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Rhubarb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm). (2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Rhubarb according to Method 4, and perform the test (not more than 5 ppm).

**Total ash** <5.01> Not more than 13.0% (6 hours).

**Loss on drying** <5.01> Not more than 13.0%.

**Extract content** <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

**Assay** Weigh accurately about 0.5 g of pulverized Rhubarb, add exactly 50 mL of a solution of sodium hydrogen carbonate (1 in 1000), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A RS, (separately determine the water) dissolve in a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 50 mL. Pipet 5 mL of this solution, add a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following
Powdered Rhubarb

Rhei Rhizoma Pulveratum

Powdered Rhubarb is the powder of Rhubarb. It contains not less than 0.25% of sennoside A (C_{42}H_{38}O_{20}: 862.74), calculated on the basis of dried materials.

Description Powdered Rhubarb occurs as a brown powder. It has a characteristic odor and a slightly astringent and bitter taste; is gritty between the teeth and colors the saliva yellow on chewing.

Under a microscope \( \times 50 \), Powdered Rhubarb reveals starch grains, dark brown substances or druses of calcium oxalate, fragments of parenchyma cells containing them, and reticulate vessels. The starch grains are spherical, simple, or 2- to 4-compound grains. Simple grain, 3 – 18 \( \mu \)m in diameter, rarely 30 \( \mu \)m; crystal druses of calcium oxalate, 30 – 60 \( \mu \)m in diameter, sometimes exceeding 100 \( \mu \)m.

Identification To 2 g of Powdered Rhubarb add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 with 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, add 30 mL of tetrahydrofuran, shake for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A RS in 4 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \times 0.3 \) \( \times 0.3 \). Spot 40 \( \mu \)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography at 10 mm along the initial line. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same \( R_f \) value.

Purity (1) Heavy metals \( \times 0.07 \) — Proceed with 3.0 g of Powdered Rhubarb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \( \times 1.11 \) — Prepare the test solution with 0.40 g of Powdered Rhubarb according to Method 4, and perform the test (not more than 5 ppm).

(3) Raponticin — To 0.5 g of Powdered Rhubarb add exactly 10 mL of ethanol (95), heat on a water bath under a reflux condenser for 10 minutes, and filter. Perform the test as directed under Thin-layer Chromatography \( \times 0.25 \), using the filtrate as the sample solution. Spot 10 \( \mu \)L of the sample solution on a plate of silica gel for thin-layer chromatography \( \times 0.25 \). Develop the plate with a mixture of isopropyl ether, methanol and 1-butanol (26:7:7) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): no spot with blue-purple fluorescence is observed at an \( R_f \) value between 0.3 and 0.6, though a bluish white fluorescence may appear.

Loss on drying \( \times 0.01 \) — Not more than 13.0% (6 hours).

Total ash \( \times 0.01 \) — Not more than 13.0%.

Acid-insoluble ash \( \times 0.01 \) — Not more than 2.0%.

Extract content \( \times 0.01 \) — Dilute ethanol-soluble extract: not less than 30.0%.

Assay Weigh accurately about 0.5 g of Powdered Rhubarb, add exactly 50 mL of a solution of sodium hydrocarbonate (1 in 1000), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A RS (separately determine the water), dissolve in a solution of sodium hydrocarbonate (1 in 1000) to make exactly 50 mL. Pipet 5 mL of this solution, add a solution of sodium hydrocarbonate (1 in 1000) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu \)L of each of the sample solution and standard solution as directed under Liquid Chromatography \( \times 0.25 \). According to the following conditions, and determine the peak areas, \( A_1 \) and \( A_5 \), of sennoside A.

\[
\text{Amount (mg) of sennoside A (C}_{42}\text{H}_{38}\text{O}_{20}) = M_S \times A_1 / A_5 \times 1/4
\]

Operating conditions—
Column: A stainless steel column 4 – 6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).
Column temperature: A constant temperature of about 40\(^\circ\)C.
Mobile phase: A mixture of diluted acetic acid (100) (1 in 80) and acetonitrile (4:1).
Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 15 minutes.
System suitability—
System performance: Dissolve 1 mg each of Sennoside A RS and naringin for thin-layer chromatography in a solution of sodium hydrogen carbonate (1 in 1000) to make 10 mL. When the procedure is run with 20 \( \mu \)L of this solution under the above operating conditions, sennoside A and naringin are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Containers and storage — Well-closed containers.
Operating conditions—


Column: A stainless steel column about 4 – 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 80) and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of Sennoside A RS and naringin for thin-layer chromatography in a solution of sodium hydrogen carbonate (1 in 1000) to make 10 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, sennoside A and naringin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Compounds Rhubarb and Senna Powder

Powdered Rhubarb and Senna Powder has a characteristic odor and a bitter taste.

Identification To 2 g of Compound Rhubarb and Senna Powder add 50 mL of water, warm on a water bath for 30 minutes, and filter. Add 2 drops of dilute hydrochloric acid to the filtrate, shake with two 20-mL portions of diethyl ether, and remove the diethyl ether layer. Add 5 mL of hydrochloric acid to the aqueous layer, and heat it on a water bath for 30 minutes. Cool, shake with 20 mL of diethyl ether, take the diethyl ether layer, add 10 mL of sodium hydroxide TS, and shake: the aqueous layer is red in color.

Containers and storage Containers—Well-closed containers.

Rikkunshito Extract

Rikkunshito Extract contains not less than 2.4 mg of ginsenoside Rb1 (C_{54}H_{92}O_{23}: 1109.29), not less than 16 mg and not more than 48 mg of hesperidin, and not less than 8 mg and not more than 24 mg of glycyrrhizic acid (C_{42}H_{62}O_{16}: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>1)</th>
<th>2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginseng</td>
<td>4 g</td>
<td>4 g</td>
</tr>
<tr>
<td>Atractylodes Rhizome</td>
<td>4 g</td>
<td>—</td>
</tr>
<tr>
<td>Atractylodes Lancea Rhizome</td>
<td></td>
<td>4 g</td>
</tr>
<tr>
<td>Poria Scleroti um</td>
<td>4 g</td>
<td>4 g</td>
</tr>
<tr>
<td>Pinellia Tuber</td>
<td>4 g</td>
<td>4 g</td>
</tr>
<tr>
<td>Citrus Unshiu Peel</td>
<td>2 g</td>
<td>2 g</td>
</tr>
<tr>
<td>Jujube</td>
<td>2 g</td>
<td>2 g</td>
</tr>
<tr>
<td>Glycyrrhiza</td>
<td>1 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Ginger</td>
<td>0.5 g</td>
<td>0.5 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Rikkunshito Extract is a light brown to blackish brown, powder or viscous extract. It has an odor and a sweet and bitter taste.

Identification (1) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb1 RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 0.1 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the purple spot from the standard solution (Ginseng).

(2) For preparation prescribed Atractylodes Rhizome) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of atracylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 5 μL each of the sample solution and standard solution
on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spots among the several spots from the sample solution has the same color tone and Rf value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 25 mL of hexane, and shake. Take the hexane layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of hexane, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.07>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at Rf value about 0.4, and this spot shows green-brown color after spraying 4-dimethylaminobenzaldehyde TS for spraying, heating at 105°C for 5 minutes and allow to cool (Atractylodes Lancea Rhizome).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of hesperidin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.06>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in an ammonia gas: one of the several spots from the sample solution has the same color tone and Rf value with the blue spot from the standard solution (Citrus Unshiu Peel).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 30 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the spots among the several spots from the sample solution has the same color tone and Rf value with the blue-green spot from the standard solution (Ginger).

Purity (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> The dry extract—Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract—Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 9.0%, calculated on the dried basis.

Assay (1) Ginsenoside Rb1—Weigh accurately about 2 g of the dry extract (or an amount of the viscous extract, equivalent to 2 g of the dried substance), add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 15 mL of diluted methanol (3 in 5), repeat the same procedure. Combine the supernatant liquids, add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of sodium hydroxide TS, allow to stand for 30 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 20 mL. Apply exactly 5 mL of this solution to a column (about 10 mm in inside diameter and packed with 0.36 g of octadeccylsilanized silica gel for pre-treatment (55 – 105 μm in particle size), washed just before use with methanol and then with diluted methanol (3 in 10), and wash the column in sequence with 2 mL of diluted methanol (3 in 10), 1 mL of sodium carbonate TS and 10 mL of diluted methanol (3 in 10). Finally, elute with methanol to collect exactly 5 mL, and use this as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb1 RS (separately determine the water), and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the sample solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, A1 and A2, of ginsenoside Rb1 in each solution.

Amount (mg) of ginsenoside Rb1 (C54H92O23)

\[ W = W_S \times A_1/A_2 \times 1/5 \]

Mg: Amount (mg) of Ginsenoside Rb1 RS, calculated on
the anhydrous basis

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with carbamoyl groups bound silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: A mixture of acetonitrile and water (4:1).

Flow rate: 1.0 mL per minute (the retention time of ginsenoside Rb₁ is about 16 minutes).

**System suitability**—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ginsenoside Rb₁ are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb₁ is not more than 1.5%.

(2) **Hesperidin**—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of Hesperidin RS (separately dried in a desiccator (silica gel) for liquid chromatography (5 μm in particle diameter)).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and water (1:1) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution.

Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₅, of glycyrrhizic acid in each solution.

**Amount (mg) of hesperidin (C₂₀H₁₅O₇)**

\[
M₅ = \frac{S_A}{S_M} \times 1/20
\]

M₅: Amount (mg) of hesperidin for assay

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100:82:18:1).

Flow rate: 1.0 mL per minute (the retention time of hesperidin is about 15 minutes).

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage—Tight containers.

**Rose Fruit**

**Rosae Fructus**

エイジツ

Rose Fruit is the pseudocarp of fruit of *Rosa multiflora* Thunberg (*Rosaceae*).

**Description** The pseudocarp, spherical, ellipsoidal or spheroidal, 5 – 9.5 mm in length, 3.5 – 8 mm in diameter; the external surface red to dark brown in color, smooth and lustrous; often with peduncle about 10 mm in length at one end, and with pentagonal remains of calyx without sepal at the other end; internal wall of receptacle covered densely with silvery hairs; the interior containing 5 – 10 mature nuts;
the nut, irregularly angular ovoid, about 4 mm in length, about 2 mm in diameter; external surface, light yellow-brown; obtuse at one end, and slightly acute at the other.

Odor, slight; taste of receptacle, sweet and acid, and of nut, mucilaginous, astringent, bitter, and slightly acid taste.

Identification  Boil gently 1 g of pulverized Rose Fruit with 20 mL of methanol for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbon form and 0.5 mL of hydrochloric acid, and allow the mixture to stand: a light red to red color develops.

Purity  Foreign matter <5.0%—The amount of the peduncle and other foreign matter contained in Rose Fruit is not more than 1.0%.

Total ash <5.0%  Not more than 6.0%.

Containers and storage  Containers—Well-closed containers.

**Powdered Rose Fruit**

*Rosae Fructus Pulveratus*

エイジツ末

Powdered Rose Fruit is the powder of Rose Fruit.

Description  Powdered Rose Fruit occurs as a grayish yellow-brown powder. It has a slight odor, and has a slightly mucilaginous, astringent, bitter, and slightly acid taste.

Under a microscope <5.0%, Powdered Rose Fruit reveals fragments of extremely thick-walled hairs 35 – 70 μm in diameter, fragments of epidermis and hypodermis containing brown tannin masses, fragments of thin-walled fundamental tissue containing grayish brown substances, fragments of fine vessels, and solitary or twin crystals or rosette aggregates of calcium oxalate (components of receptacle); fragments of sclerenchyma, fiber groups, fine vessels, and fragments of epidermis containing brown tannin and mucilage (components of pericarp); fragments of endosperm composed of polygonal cells containing aleuron grains and fatty oil, fragments of outer epidermis composed of polygonal cells containing tannin, and fragments of inner epidermis composed of elongated cells having wavy lateral walls (components of seed).

Identification  Boil gently 1 g of Powdered Rose Fruit with 20 mL of methanol for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbon form and 0.5 mL of hydrochloric acid, and allow the mixture of stand: a light red to red color develops.

Total ash <5.0%  Not more than 6.0%.

Containers and storage  Containers—Well-closed containers.

**Rosin**

*Resina Pini*

ロジン

Rosin is the resin obtained from the exudation of plants of *Pinus* species (*Pinaceae*) from which essential oil has been removed.

Description  Rosin occurs as a light yellow to light brown, glassily transparent, brittle mass, the surfaces of which are often covered with a yellow powder. The fractured surface is shell-like and lustrous.

It has a slight odor.

It melts easily, and burns with a yellow-brown flame.

It is freely soluble in ethanol (95), in acetic acid (100) and in diethyl ether.

A solution of Rosin in ethanol (95) is acidic.

Acid value <1.13  150 – 177

Total ash <5.0%  Not more than 0.1%.

Containers and storage  Containers—Well-closed containers.

**Royal Jelly**

*Apilac*

ローヤルゼリー

Royal Jelly is the viscous liquid or its dried substance secreted by the secreting gland on the head of *Apis mellifera* Linné or *Apis cerana* Fabricius (*Apidae*).

It contains not less than 4.0% and not more than 8.0% of 10-hydroxy-2-(E)-decenoic acid, calculated on the basis of dried material.

Description  Slightly viscous liquid or powder, milky white to light yellow in color. Odor, characteristic; taste, astringent and acid.

Identification  To a portion of Royal Jelly, equivalent to 0.2 g of dried substance, add 5 mL of water, 1 mL of dilute hydrochloric acid and 10 mL of diethyl ether, shake for 15 minutes, and centrifuge. Take the diethyl ether layer, evaporate under reduced pressure, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 2 mg of 10-hydroxy-2-(E)-decenoic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of 1-propanol and ammonia solution (28:72) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution has the same color tone and RF value with the dark purple spot from the standard solu-
Purity (1) Heavy metals $<1.07$—Proceed with a portion of Royal Jelly, equivalent to 1.0 g of the dried substance, according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Arsenic $<1.17$—Prepare the test solution with an amount of Royal Jelly, equivalent to 0.40 g of the dried substance according to Method 3, and perform the test (not more than 5 ppm).

Loss on drying $<5.01$ The slightly viscous liquid: Not less than 57.0% and not more than 77.0% (6 hours).

The powder: Not less than 7.0% and not more than 13.0% (6 hours).

Total ash $<5.01$ Not more than 4.0%, calculated on the dried basis.

Acid-insoluble ash $<5.01$ Not more than 0.5%, calculated on the dried basis.

Assay Weigh accurately a portion of Royal Jelly, equivalent to 0.2 g of the dried substance, add 20 mL of methanol, treat with ultrasonic waves for 30 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet 2 mL of the supernatant, add exactly 2 mL of the internal standard solution, then add 25 mL of water and methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of 10-hydroxy-2-(E)-decenoic acid for assay, dissolve in methanol to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 2 mL of the internal standard solution, then add 25 mL of water and methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01$ according to the following conditions, and calculate the ratios, $Q_1$ and $Q_5$, of the peak area of 10-hydroxy-2-(E)-decenoic acid to that of the internal standard.

\[
\text{Amount (mg) of 10-hydroxy-2-(E)-decenoic acid} = M_5 \times \frac{Q_5}{Q_5} \times \frac{3}{4}
\]

\[
M_5: \text{Amount (mg) of 10-hydroxy-2-(E)-decenoic acid for assay}
\]

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 5000).

Operating conditions—
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 50°C. 
Mobile phase: A mixture of water, methanol and phosphoric acid (550:450:1).
Flow rate: Adjust the flow rate so that the retention time of 10-hydroxy-2-(E)-decenoic acid is about 10 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, 10-hydroxy-2-(E)-decenoic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 10-hydroxy-2-(E)-decenoic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—At not exceeding 10°C.

**Ryokeijutsukanto Extract**

苓桂朮甘湯エキス

Ryokeijutsukanto Extract contains not less than 1 mg and not more than 4 mg of (E)-cinnamic acid, and not less than 21 mg and not more than 63 mg of glycyrrhizic acid (C$_{22}$H$_{22}$O$_{12}$: 822.93), per a dried extract prepared as directed in the Method of preparation.

**Method of preparation**

<table>
<thead>
<tr>
<th></th>
<th>1)</th>
<th>2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poria Sclerotium</td>
<td>6 g</td>
<td>6 g</td>
</tr>
<tr>
<td>Cinnamon Bark</td>
<td>4 g</td>
<td>4 g</td>
</tr>
<tr>
<td>Atractylodes Rhizome</td>
<td>3 g</td>
<td>—</td>
</tr>
<tr>
<td>Atractylodes Lancea Rhizome</td>
<td>—</td>
<td>3 g</td>
</tr>
<tr>
<td>Glycyrrhiza</td>
<td>2 g</td>
<td>2 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

**Description** Ryokeijutsukanto Extract occurs as a brown to blackish brown powder or viscous extract. It has an odor, and a sweet first then bitter taste.

**Identification (1)** To 1.0 g of dry extract (3.0 g for viscous extract) of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (E)-cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane, ethyl acetate, formic acid and water (60:40:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); one of the spot among the several spots from the sample solution has the same color tone and RF value with the blue-purple spot from the standard solution (Cinnamon Bark).

(2) For preparation prescribed Atractylodes Rhizome—To 1.0 g of dry extract (3.0 g for viscous extract) of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as
the sample solution. Separately, dissolve 1 mg of atracyloneolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 5 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of dry extract (6.0 g for viscous extract) of Ryokejutsukanto Extract add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography (2.03). Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an Rf value of about 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(4) To 1.0 g of dry extract (3.0 g for viscous extract) of Ryokejutsukanto Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 5 μL of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Glycyrrhiza).

Purity (1) Heavy metals (1.02)—Prepare the test solution with 1.0 g of dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) of Ryokejutsukanto Extract as directed in the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic (1.11)—Prepare the test solution with 0.67 g of dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) of Ryokejutsukanto Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying (2.41) Dry extract: Not more than 8.5% (1 g, 105°C, 5 hours).

Viscous extract: Not more than 66.7% (1 g 105°C, 5 hours).

Total ash (5.01) Not more than 8.0%, calculated on the dried basis.

Assay (1) (E)-Cinnamic acid—Conduct this procedure using light-resistant vessels. Weigh accurately about 0.5 g of dry extract (for viscous extract an amount equivalent to about 0.5 g as dried substance) of Ryokejutsukanto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of (E)-cinnamic acid for assay, previously dried in a desiccator (silica gel) for more than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, A1 and A5, of (E)-cinnamic acid in each solution.

Amount (mg) of (E)-cinnamic acid

\[ M_5 \times A_1 / A_5 \times 1 / 20 \]

M5: Amount (mg) of (E)-cinnamic acid for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeccylsilsanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (750:250:1).

Flow rate: 1.0 mL per minute (the retention time of (E)-cinnamic acid is about 12 minutes). System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (E)-cinnamic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (E)-cinnamic acid is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of dry extract (for viscous extract an amount equivalent to about 0.5 g as dried substance) of Ryokejutsukanto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, A1 and A5, of glycyrrhizic acid in each solution.

Amount (mg) of glycyrrhizic acid (C22H22O16)

\[ M_5 \times A_1 / A_5 \times 1 / 2 \]
Safflower

Carthami Flos

サフラ

Safflower is the tubulous flower of Carthamus tinctorius Linné (Compositae) without any treatment or with most of the yellow pigment removed, and sometimes with pressed into a flat slab.

Description
Red to red-brown corolla, yellow style and stamen, rarely mixed with immature ovary; total length about 1 cm; corolla, tubular and with 5 lobes; 5 stamens surrounding long pistil; pollen grains yellow and approximately spherical, about 50 μm in diameter, with fine protrusions on the surface. The pressed slab, about 0.5 cm in thickness, consists of a collection of numerous corollas.

Odor, characteristic; taste, slightly bitter.

Identification
Boil 0.2 g of Safflower with 10 mL of dilute ethanol under a reflux condenser for 15 minutes, and after cooling, filter. Place 3 mL of the filtrate in a small glass vessel about 3 cm in both internal diameter and height, hang a piece of filter paper, 20 mm by 300 mm, so that one end of the filter paper reaches the bottom of the vessel, and allow the paper to soak up the liquid for 1 hour. Transfer and immediately hang the paper in another glass vessel of the same type, containing 3 mL of water, and allow the paper to soak up the water for 1 hour: most of the upper part of the paper is colored light yellow, and the lower portion, light red.

Purity
Foreign matter ≤<1.0%>—The amount of ovaries, stems, leaves and other foreign matter contained in Safflower does not exceed 2.0%.

Total ash ≤<0.1%> Not more than 18.0%.

Containers and storage
Containers—Well-closed containers.
Storage—Light-resistant.

Saffron

Crocus

サフラ

Saffron is the stigma of Crocus sativus Linné (Iridaceae).

Description
Thin cord-like stigma, externally dark yellow-red to red-brown, 1.5 – 3.5 cm in length, tripartite or separate; the end of parite part widened and the other end narrowed gradually.

Odor, strong and characteristic; taste, bitter; colors the saliva yellow on chewing.

Under a microscope ≤<0.01>, when softened by immersion in water, the upper end has numerous tubular protrusions about 150 μm in length, with a small number of pollen grains.

Identification
Add 1 drop of sulfuric acid to Saffron: the color changes to dark blue which gradually turns red-brown through purple.

Purity
(1) Aniline dyes—Shake 0.05 g of Saffron with 10 mL of chloroform: the solution is colorless, or only slightly yellow.

(2) Glycerol, sugar or honey—Saffron has no sweet taste. Press it between two pieces of paper: no spot is left on the paper.

(3) Yellow style—When perform the test of foreign matter ≤<0.01>, the yellow style in Saffron does not exceed 10.0%.

Loss on drying ≤<0.01> Not more than 12.0% (6 hours).

Total ash ≤<0.01> Not more than 7.5%.

Content of the active principle
Crocin—Dry Saffron in a desiccator (silica gel) for 24 hours, and powder. To exactly 0.100 g of the powder add 150 mL of warm water, warm the mixture between 60°C and 70°C for 30 minutes with frequent shaking, cool, and filter. Pipet 1 mL of the filtrate, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve exactly 98 mg of carbazochrome sodium sulfonate trihydrate in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution at 438 nm as directed under Ultraviolet-visible Spectrophotometry ≤<2.24>: the absorbance of the sample solution is larger than that of the standard solution.

Containers and storage
Containers—Well-closed containers.
Storage—Light-resistant.
Saibokuto Extract
柴朴湯エキス

Saibokuto Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b2, not less than 90 mg and not more than 270 mg of baicalin (C21H108O11: 446.36), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid (C23H22O16: 822.93), per extract prepared with the amount specified in the Method of preparation.

**Method of preparation**

<table>
<thead>
<tr>
<th>1)</th>
<th>2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupleurum Root</td>
<td>7 g</td>
</tr>
<tr>
<td>Pinellia Tuber</td>
<td>6 g</td>
</tr>
<tr>
<td>Poria Sclerotium</td>
<td>5 g</td>
</tr>
<tr>
<td>Scutellaria Root</td>
<td>3 g</td>
</tr>
<tr>
<td>Magnolia Bark</td>
<td>3 g</td>
</tr>
<tr>
<td>Jujube</td>
<td>3 g</td>
</tr>
<tr>
<td>Ginseng</td>
<td>3 g</td>
</tr>
<tr>
<td>Glycyrrhiza</td>
<td>2 g</td>
</tr>
<tr>
<td>Perilla Herb</td>
<td>2 g</td>
</tr>
<tr>
<td>Ginger</td>
<td>1 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

**Description**

Saibokuto Extract is a light yellow to blackish brown, powder or viscous extract, having a slightly odor and a slight sweet first, then a bitter taste.

**Identification**

(1) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 0.5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes: one of the spots obtained from the sample solution has the same color tone and Rf value with the purple spot from the standard solution (Bupleurum Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the diethyl ether of the layer under reduced pressure, add to the residue 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Scutellaria Root).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the diethyl ether of the layer under reduced pressure, add to the residue 2 mL of diethyl ether, and use this as the sample solution. Separately, dissolve 1 mg of magnolol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the dark purple spot from the standard solution (Magnolia Bark).

(4) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb1 RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the purple spot from the standard solution (Ginseng).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of 0.1 mol/L hydrochloric acid TS, add 25 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the diethyl ether of the layer under reduced pressure, add to the residue 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of
rosmarinic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and RF value with the dark purple spot from the standard solution (Perilla Herb).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the diethyl ether of the layer under reduced pressure, add to the residue 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 µL of the sample solution and 5 µL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and RF value with the blue-green spot from the standard solution (Ginger).

**Purity (1)** Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Examinations (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.1D>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

**Loss on drying <2.4F>** The dry extract—Not more than 9.0% (1 g, 105°C, 5 hours).

The viscous extract—Not more than 66.7% (1 g, 105°C, 5 hours).

**Total ash <5.0F>** Not more than 9.0%, calculated on the dried basis.

**Assay (1)** Saikosaponin b₂—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of saikosaponin b₂ for assay, previously dried in a desiccator (silica gel) for 24 hours or more, and dissolve in 50 mL of methanol, add water to make exactly 100 mL. Pipet 10 mL of this solution, and add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A₁ and A₂, of saikosaponin b₂ in each solution.

Amount (mg) of saikosaponin b₂ = Mₛ × A₁/A₂ × 1/20

Mₛ: Amount (mg) of saikosaponin b₂ for assay

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:5).
Flow rate: 1.0 mL per minute (the retention time of saikosaponin b₂ is about 12 minutes).

**System suitability—**
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b₂ are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b₂ is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A₁ and A₂, of baicalin in each solution.

Amount (mg) of baicalin (C₂₁H₁₈O₁₁) = Mₛ × A₁/A₂ × 1/4

Mₛ: Amount (mg) of Baicalin RS, calculated on the anhydrous basis

**Operating conditions—**
Detector: An ultraviolet absorption photometer (wavelength: 277 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).
Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

**System suitability—**
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in diluted methanol (1 in 2) to accurately about 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, \( A_S \) and \( A_T \), of glycyrrhizic acid in each solution.

\[
\text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) = MS \times \frac{A_T}{A_S} \times 1/2
\]

\( M_S \): Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeceansilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (I3.7).
Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage—Containers—Tight containers.

**Saikokeishito Extract**
柴胡桂枝湯エキス

Saikokeishito Extract contains not less than 1.5 mg and not more than 6 mg of saikosaponin b2, not less than 60 mg and not more than 180 mg of baicalin (C_{14}H_{10}O_{5}; 446.36), not less than 17 mg and not more than 51 mg for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid (C_{12}H_{20}O_{12}; 822.93), per extract prepared with the amount specified in the Method of preparation.

### Method of preparation

<table>
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<tr>
<th></th>
<th>1)</th>
<th>2)</th>
<th>3)</th>
<th>4)</th>
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<tr>
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<td>4 g</td>
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<td>Scutellaria Root</td>
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<td>2 g</td>
<td>2 g</td>
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</tr>
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<td>Ginseng</td>
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<td>2 g</td>
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<tr>
<td>Cinnamon Bark</td>
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<td>2.5 g</td>
<td>2.5 g</td>
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</tr>
<tr>
<td>Glycyrrhiza</td>
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<td>1.5 g</td>
<td>1.5 g</td>
<td>2 g</td>
</tr>
<tr>
<td>Ginger</td>
<td>0.5 g</td>
<td>1 g</td>
<td>1 g</td>
<td>1 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

**Description** Saikokeishito Extract is a yellow-brown to blackish brown, powder or viscous extract, having a slightly odor and a slight sweet first, then a bitter and slightly pungent taste.

**Identification** (1) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-buthanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethyl alcohol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and \( R_f \) value with the red spot from the standard solution (Bupleurum Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the layer under reduced pressure, add to the residue 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and \( R_f \) value with the yellow-brown spot from the standard solution (Scutellaria Root).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-buthanol, shake, centrifuge, and use the supernatant liquid as the sam-
Ple solution. Separately, dissolve 1 mg of Paeoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.0\%\). Spot 5 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dimethylaminobenzaldehyde TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and \(R_f\) value with the purple spot from the standard solution (Peony Root).

(4) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb1 RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.0\%\). Spot 10 \(\mu\)L of the sample solution and 2 \(\mu\)L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and \(R_f\) value with the yellow-orange spot among the several spots obtained from the sample solution (Ginseng).

(5) Perform the test according to the following (i) or (ii).

(Cinnamon Bark)

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for about 1 hour, separate the hexane layer, and use this solution as the sample solution. Separately, dissolve 1 mg of \((E)\)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.0\%\). Spot 50 \(\mu\)L of the sample solution and 2 \(\mu\)L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots obtained from the sample solution has the same \(R_f\) value with the purple spot from the standard solution.

(ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of \((E)\)-2-methoxyacinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.0\%\). Spot 20 \(\mu\)L of the sample solution and 2 \(\mu\)L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and \(R_f\) value with the bluish white fluorescent spot from the standard solution.

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.0\%\). Spot 5 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and \(R_f\) value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add to the residue 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of \([6]\)-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.0\%\). Spot 10 \(\mu\)L of the sample solution and 5 \(\mu\)L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and \(R_f\) value with the blue-green spot from the standard solution (Ginger).

Purity (1) Heavy metals \(<1.0\%\)—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic \(<1.1\%\)—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying \(<2.4\%\) The dry extract—Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract—Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash \(<5.0\%\) Not more than 10.0%, calculated on the dried basis.

Assay (1) Saikosaponin b2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of saikosaponin b2 for assay, previ-
Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), dissolve in methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water), dissolve in methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of baicalin in each solution.

Amount (mg) of baicalin in each solution.

\[
M_S \times \frac{A_T}{A_S} \times \frac{1}{20}
\]

\( M_S \): Amount (mg) of Baicalin RS, calculated on the anhydrous basis.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).
Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water), dissolve in methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of paeoniflorin in each solution.

Amount (mg) of paeoniflorin

\[
M_S \times \frac{A_T}{A_S} \times \frac{1}{2}
\]

\( M_S \): Amount (mg) of Paeoniflorin RS, calculated on the anhydrous basis.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 232 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (μm in particle diameter).
Column temperature: A constant temperature of about 20°C.
Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).
Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—
System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution being not less than 2.5.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(4) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard...
solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the peak areas, \( A_1 \) and \( A_2 \), of glycyrrhizic acid in each solution.

\[
M_S = \frac{M_S}{A_1/A_0 \times 1/2}
\]

\( M_S \): Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

**Containers and storage** Containers—Tight containers.

### Saireito Extract

柴苓湯エキス

Saireito Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b2, not less than 80 mg and not more than 240 mg of baicalin (C₂₁H₁₈O₁₁: 446.37), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid (C₁₈H₂₂O₁₆: 822.93), per a dried extract prepared as directed in the Method of preparation.

<table>
<thead>
<tr>
<th>Method of preparation</th>
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</tr>
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<tbody>
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<td>Pinellia Tuber</td>
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<tr>
<td>Ginger</td>
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<td>1 g</td>
</tr>
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<td>Scutellaria Root</td>
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</tr>
<tr>
<td>Jujube</td>
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<tr>
<td>Ginseng</td>
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<td>Atractylodes Lancea Rhizome</td>
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<td>3 g</td>
</tr>
<tr>
<td>Cinnamon Bark</td>
<td>3 g</td>
<td>2 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

**Description** Saireito Extract occurs as a light yellow-brown powder. It has slightly a characteristic odor, and a sweet, then bitter taste.

**Identification (1)** To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b₂ for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 9 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and \( R_f \) value with the red spot from the standard solution (Bupleurum Root).

(2) To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 15 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and \( R_f \) value with the red spot from the standard solution (Ginger).

(3) To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this
solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\leq 2.0 \%$. Spot 20 $\mu$L of the sample solution and 2 $\mu$L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, air-dry the plate, and spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and RF value with the yellow-brown spot from the standard solution (Scutellaria Root).

(4) To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb$_1$ RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\leq 2.0 \%$. Spot 10 $\mu$L of the sample solution and 2 $\mu$L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and RF value with the purple spot from the standard solution (Ginseng).

(5) To 2.0 g of Saireito Extract add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\leq 2.0 \%$. Spot 10 $\mu$L of the sample solution and 2 $\mu$L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and RF value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(6) To 2.0 g of Saireito Extract add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of alisols A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\leq 2.0 \%$. Spot 40 $\mu$L of the sample solution and 2 $\mu$L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and RF value with the purple spot from the standard solution (Alisma Rhizome).

(7) For preparation prescribed Atractylodes Rhizome—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\leq 2.0 \%$. Spot 5 $\mu$L of each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plat, heat at 105°C for 5 minutes, examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and RF value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(8) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography $\leq 2.0 \%$. Spot 20 $\mu$L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an RF value of about 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylamino-benzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(9) To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (E)-cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\leq 2.0 \%$. Spot 40 $\mu$L of the sample solution and 2 $\mu$L of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane, ethyl acetate, formic acid and water (60:40:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and RF value with the dark purple spot from the standard solution (Cinnamon Bark).

Purity (1) Heavy metals $\leq 0.07 \%$—Prepare the test solution with 1.0 g of Saireito Extract as directed in Extract (4), and perform the test (not more than 0.3 ppm).

(2) Arsenic $\leq 1.1 \%$—Prepare the test solution with 0.67 g of Saireito Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying $\leq 2.4 \%$ Not more than 10.0% (1 g, 105°C,
5 hours).

**Total ash** $<0.01$> Not more than 9.0%.

**Assay** (1) Saikosaponin b$_2$—Weigh accurately about 0.5 g of Saireito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of saikosaponin b$_2$ for assay, previously dried in a desiccator (silica gel) for more than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of saikosaponin b$_2$ in each solution.

Amount (mg) of saikosaponin b$_2$ = $M_S \times A_T/A_S \times 1/20$

$M_S$: Amount (mg) of saikosaponin b$_2$ for assay

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3)

Flow rate: 1.0 mL/min. (the retention time of saikosaponin b$_2$ is about 12 minutes.)

**System suitability**—
System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b$_2$ are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b$_2$ is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of Saireito Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water), dissolve in diluted methanol (7 in 10) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of baicalin in each solution.

Amount (mg) of baicalin (C$_{21}$H$_{18}$O$_{11}$) = $M_S \times A_T/A_S \times 1/2$

$M_S$: Amount (mg) of Baicalin RS, calculated on the anhydrous basis

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6)

Flow rate: 1.0 mL/min. (the retention time of baicalin is about 10 minutes.)

**System suitability**—
System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of Saireito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and the standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of glycyrrhizic acid in each solution.

Amount (mg) of glycyrrhizic acid (C$_{42}$H$_{62}$O$_{16}$) = $M_S \times A_T/A_S \times 1/2$

$M_S$: Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7)

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid is about 12 minutes.)

**System suitability**—
System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

**Containers and storage** Containers—Tight containers.
### Saposhnikovia Root and Rhizome

*Saposhnikovia Radix*

ボウフウ

Saposhnikovia Root and Rhizome is the root and rhizome of *Saposhnikovia divaricata* Schischkin (*Umbelliferae*).

**Description** Long and narrow, conical rhizome and root, 15 - 20 cm in length, 0.7 - 1.5 cm in diameter; externally light brown; rhizome reveals dense crosswise wrinkles like ring nodes, and sometimes reveals brown and hair-like remains of leaf sheath; the root reveals many longitudinal wrinkles and scars of rootlets; in a transverse section, cortex is grayish brown in color and reveals many lacunae, and xylem is yellow in color.

Odor, slight; taste, slightly sweet.

**Identification** To 1 g of pulverized Saposhnikovia Root and Rhizome, add 5 mL of methanol, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of 4'-O-glucosyl-5-O-methylvisamminol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water (10:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among several spots from the sample solution has the same ultraviolet absorbance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among several spots from the sample solution has the same ultraviolet absorbance of about 10 cm.

**Purity**

1. **Heavy metals**
   - Proceed with 3.0 g of pulverized Saposhnikovia Root and Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).
   - Arsenic
     - Prepare the test solution with 0.40 g of pulverized Saposhnikovia Root and Rhizome according to Method 4, and perform the test (not more than 5 ppm).
   - Foreign matter
     - The amount of stems and other foreign matter is not more than 2.0%.
   - **Total ash**
     - Not more than 7.0%.
   - **Acid-insoluble ash**
     - Not more than 1.5%.
   - **Extract content**
     - Dilute ethanol-soluble extract: not less than 20.0%.

**Containers and storage** Containers—Well-closed containers.

### Sappan Wood

*Sappan Lignum*

ソボク

Sappan Wood is the duramen of *Caesalpinia sappan* Linné (*Leguminosae*).

**Description** Chips, slices or short pieces of wood; yellowish red to grayish yellow-brown, sometimes with light brown to grayish white splint woods; hard in texture; a transverse section shows a pattern like annual ring.

Almost odorless; almost tasteless.

Under a microscope, a transverse section reveals ray composed of 1 - 2 rows of slender and long cells; the area between rays filled with fiber cells, and large and oblong vessels scattered there; solitary crystals of calcium oxalate in parenchymatous cells of the innermost of xylem.

**Identification** To 0.5 g of pulverized Sappan Wood add 10 mL of dilute ethanol, shake, and filter. To 5 mL of the filtrate add 2 to 3 drops of sodium hydroxide TS: a dark red color develops.

**Purity** Put a small piece of Sappan Wood in calcium hydroxide TS: no purple-blue color develops.

**Loss on drying**

- Not more than 11.5% (6 hours).

**Total ash**

- Not more than 2.0%.

**Extract content**

- Dilute ethanol-soluble extract: not less than 7.0%.

**Containers and storage** Containers—Well-closed containers.

### Saussurea Root

*Saussureae Radix*

モッコウ

Saussurea Root is the root of *Saussurea lappa* Clarke (*Compositae*).

**Description** Nearly cylindrical roots, 5 - 20 cm in length, 1 - 6 cm in diameter; some of them slightly bent, and sometimes longitudinally cut; scar of stem dented on the top of the root with crown; externally yellow-brown to grayish brown, with coarse longitudinal wrinkles and fine reticulate furrows, and also with remains of lateral roots; sometimes root from which periderm has been removed; hard and dense in texture, and difficult to break. A transverse section is yellow-brown to dark brown, and cambium part has a dark color. Under a magnifying glass, medullary rays distinct, here and there, large clefts, and brown oil sacs scattered; in old root, pith existing in the center, and often forming a hollow.

Odor, characteristic; taste, bitter.

**Identification** Warm 0.5 g of pulverized Saussurea Root with 10 mL of ethanol (95) for 1 minute, cool, and filter.
Shake 1 mL of the filtrate with 0.5 mL of hydrochloric acid: a purple color is produced.

**Purity** (1) Arsenic $<1.11$—Prepare the test solution with 0.40 g of pulverized Saussurea Root according to Method 4, and perform the test (not more than 5 ppm).

(2) Foreign matter—Add iodine TS dropwise to a transverse section: no blue-purple color develops.

**Total ash** $<5.01$—Not more than 4.0%.

**Extract content** $<5.01$—Dilute ethanol-soluble extract: not less than 17.0%.

**Containers and storage** Containers—Well-closed containers.

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**Schizonepeta Spike**

**Schizonepetae Spica**

ケイガイ

Schizonepeta Spike is the spike of *Schizonepeta tenuifolia* Briquet (*Labiatae*).

**Description** Oblong spike, 5 – 10 cm in length, 0.5 – 0.8 cm in diameter, purplish green-brown to green-brown in color. Spike, 5 – 10 cm in length, with calyx-tubes containing small labiate flower or often fruits; sometimes leaves under spike; leaf, leaf or small lanceolate; stem, prismatic, purple-brown in color. Under a magnifying glass, it reveals short hairs. It has a characteristic aroma and slightly cool feeling on keeping in the mouth.

**Identification** To 2 g of pulverized Schizonepeta Spike add 20 mL of water, shake well, and distill. To 3 mL of the distillate add 2 or 3 drops of 2,4-dinitrophenylhydrazine-ethanol TS: an orange-red precipitate is formed.

**Total ash** $<5.05$—Not more than 11.0%.

**Acid-insoluble ash** $<5.05$—Not more than 3.0%.

**Extract content** $<5.05$—Dilute ethanol-soluble extract: not less than 8.0%.

**Containers and storage** Containers—Well-closed containers.

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**Scopolia Rhizome**

**Scopoliae Rhizoma**

ロートコン

Scopolia Rhizome is the rhizome with root of *Scopolia japonica* Maximowicz, *Scopolia carnulica* Jacquin or *Scopolia parviflora* Nakai (*Solanaceae*).

When dried, it contains not less than 0.29% of total alkaloids [hyoscyamine ($C_{17}H_{23}NO_3$: 289.37) and scopolamine ($C_{17}H_{21}NO_4$: 303.35)].

**Description** Chiefly irregularly branched, slightly curved rhizome, about 15 cm in length, about 3 cm in diameter, occasionally longitudinally cut; externally grayish brown, with wrinkles; constrictions make the rhizome appear nodular; rarely, stem base at one end; stem scars at upper side of each node; roots or root scars on both sides and lower surface of rhizome; fractured surface granular, grayish white to light brown in color, with lighter colored cortex. Odor characteristic; taste sweet, later slightly bitter.

Under a microscope, xylem reveals groups of vessels arranged stepwise, and accompanied with xylem sieve tubes in medullary rays; parenchyma cells contain starch grains, and sometimes sand crystals of calcium oxalate.

**Identification** (1) To 1 g of pulverized Scopolia Rhizome add 10 mL of diethyl ether and 0.5 mL of ammonia TS, shake for 30 minutes, and filter. Wash the residue with 10
mL of diethyl ether, transfer the filtrate and the washing to a separator, add 20 mL of diluted sulfuric acid (1 in 50), shake well, and drain off the acid extract into another separator. Render the solution slightly alkaline with ammonia TS, add 10 mL of diethyl ether, shake well, transfer the diethyl ether layer to a porcelain dish, and evaporate the diethyl ether on a water bath. To the residue add 5 drops of fuming nitric acid, and evaporate the mixture on a water bath to dryness. Cool, dissolve the residue in 1 mL of \( N, N \)-dimethylformamide, and add 5 to 6 drops of tetracyanomethylenium hydroxide TS: a red-purple to purple color develops.

(2) Place 2.0 g of pulverized Scopolia Rhizome in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate RS and 1 mg of Scopolamine Hydrobromide RS in 1 mL each of ethanol (95), and use these solutions as standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 5 \( \mu L \) each of the sample solution, standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the sample solution, standard solutions (1) and (2) on a plate at 80°C (90:7:3) to a distance of about 10 cm, and dry the plate with a mixture of acetone, water and ammonia water. Cool, dissolve the residue in 1 mL of \( N, N \)-dimethylformamide, and add 5 to 6 drops of tetracyanomethylenium hydroxide TS: a red-purple to purple color develops.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Scopolia Rhizome according to Method 3, and perform the test. Prepare the control solution with 4.5 mL of Standard Lead Solution (not more than 15 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 0.40 g of pulverized Scopolia Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.05> Not more than 7.0%.

Assay Weigh accurately about 0.7 g of pulverized Scopolia Rhizome, previously dried at 60°C for 4 hours, in a glass-stoppered, centrifuge tube, and moisten with 15 mL of ammonia TS. To this add 25 mL of diethyl ether, stopper the centrifuge tube tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the residue using 25-mL portions of diethyl ether. Combine all the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. Filter this solution through a filter of a porosity of not more than 0.8 \( \mu m \), discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate RS (separately determine the loss on drying <2.41> in the same conditions as Atropine Sulfate Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution A. Weigh accurately about 25 mg of Scopolamine Hydrobromide RS (separately determine the loss on drying <2.41> in the same conditions as Scopolamine Hydrobromide Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution B. Pipet 5 mL of standard stock solution A and 1 mL of standard stock solution B, add exactly 3 mL of the internal standard solution, then add 25 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 10 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography <2.05> according to the following conditions. Calculate the ratios, \( Q_{TS} \) and \( Q_{SA} \), of the peak area of hyoscyamine (atropine), and the ratios, \( Q_{TS} \) and \( Q_{SS} \), of the peak area of scopolamine to that of the internal standard in each solution, calculate the amounts of hyoscyamine and scopolamine by the following equation, and designate the total as the amount of total alkaloids.

\[
\text{Amount (mg)} \text{ of hyoscyamine (C}_{17}\text{H}_{23}\text{NO}_{3}) = M_{SA} \times \frac{Q_{TS}}{Q_{SA}} \times \frac{1}{25} \times 0.8551
\]
\[
\text{Amount (mg)} \text{ of scopolamine (C}_{17}\text{H}_{25}\text{NO}_{4}) = M_{SS} \times \frac{Q_{TS}}{Q_{SS}} \times \frac{1}{25} \times 0.7894
\]

\( M_{SA} \): Amount (mg) of Atropine Sulfate RS, calculated on the dried basis

\( M_{SS} \): amount (mg) of Scopolamine Hydrobromide RS, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Operating conditions—
Detector: A ultraviolet absorption spectrometer (wavelength: 210 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadesilcylanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).
Column temperature: A constant temperature of about 20°C.
Mobile phase: Dissolve 6.8 g of potassium dihydrogen-phosphate in 900 mL of water, add 10 mL of triethylamine, adjust with phosphoric acid to pH 3.5, and add water to make 1000 mL. To 9 parts of this solution add 1 part of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.
System suitability—
System performance: When the procedure is run with 10 \( \mu L \) of the standard solution under the above operating conditions, scopolamine, atropine and the internal standard are eluted in this order with the resolution between the peaks of scopolamine and atropine being not less than 11, and with the resolution between the peaks of atropine and the internal standard being not less than 4.

Containers and storage Containers—Well-closed containers.

Scopolia Extract

ロートエキス

Scopolia Extract contains not less than 0.90% and not more than 1.09% of total alkaloids [hyoscyamine...
Scopolia Extract Powder

ロートエキス散

Scopolia Extract Powder contains not less than 0.085% and not more than 0.110% of total alkaloids [hyoscyamine (C₁₇H₂₃NO₃: 289.37) and scopolamine (C₁₇H₂₃NO₄: 303.35)].

**Method of preparation**

Scopolia Extract Powder contains not less than 0.085% and not more than 0.110% of total alkaloids [hyoscyamine (C₁₇H₂₃NO₃: 289.37) and scopolamine (C₁₇H₂₃NO₄: 303.35)].

| Scopolia Extract | 100 g |
| Starch, Lactose Hydrate or their mixture | a sufficient quantity |
| To make | 1000 g |

To Scopolia Extract add 100 mL of Purified Water or Purified Water in Containers, then warm and soften the mixture with stirring. Cool, add 800 g of starch, Lactose Hydrate or their mixture little by little, and mix well. Dry preferably at a low temperature, and dilute with a sufficient additional quantity of starch, Lactose Hydrate or their mixture to make 1000 g of homogeneous powder.

**Description**

Scopolia Extract Powder is a brownish yellow to grayish yellow-brown powder. It has a faint, characteristic odor and a slightly bitter taste.

**Identification (1)**

To 20 g of Scopolia Extract Powder add 15 mL of water and 8 mL of ammonia TS, mix homogeneously, add 100 mL of diethyl ether and 7 g of sodium chloride, stopper tightly, shake for 1 hour, add 5 g of Powdered Tragacanth, and shake vigorously. Allow to stand for 5 minutes, take the clearly separated diethyl ether layer, and filter. Proceed with the filtrate as directed in the Identification (1) under Scopolia Rhizome.

(2) Place 5.0 g of Scopolia Extract Powder in a glass-stoppered centrifuge tube, add 50 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Proceed as directed in Identification (2) under Scopolia Rhizome.

**Assay**

Weigh accurately about 4 g of Scopolia Extract Powder, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 25 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 25 mL each of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. Proceed as directed under Scopolia Rhizome.

Amount (mg) of hyoscyamine (C₁₇H₂₃NO₃)

\[ M_{SA} \times Q_{SA} \times 1/5 \times 0.8551 \]

Amount (mg) of scopolamine (C₁₇H₂₃NO₄)

\[ M_{SS} \times Q_{SS} \times 1/25 \times 0.7894 \]

\[ M_{SA} : \text{Amount (mg) of Atropine Sulfate RS, calculated on the dried basis} \]

\[ M_{SS} : \text{Amount (mg) of Scopolamine Hydrobromide RS, calculated on the dried basis} \]

**Internal standard solution**

A solution of brucine dihydrate in the mobile phase (1 in 2500).

**Containers and storage**

Containers—Tight containers. Storage—Light-resistant, and in a cold place.
Scopolia Extract and Carbon Powder

ロートエキス・カーボン散

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scopolia Extract</td>
<td>5</td>
</tr>
<tr>
<td>Medicinal Carbon</td>
<td>550</td>
</tr>
<tr>
<td>Natural Aluminum Silicate</td>
<td>345</td>
</tr>
<tr>
<td>Starch, Lactose Hydrate or their mixture</td>
<td>a sufficient quantity</td>
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</table>

To make 1000 g

Prepare before use as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

**Description** Scopolia Extract and Carbon Powder is easily dustable and black in color. It is tasteless.

**Containers and storage** Containers—Well-closed containers.

Compound Scopolia Extract and Diastase Powder

複方ロートエキス・ジアスターゼ散

**Method of preparation**

<table>
<thead>
<tr>
<th>Ingredient</th>
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</tr>
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<tr>
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<tr>
<td>Diastase</td>
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<tr>
<td>Precipitate Calcium Carbonate</td>
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<tr>
<td>Sodium Bicarbonate</td>
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<td>Magnesium Oxide</td>
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<tr>
<td>Powdered Gentian</td>
<td>50</td>
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<td>Starch, Lactose Hydrate or their mixture</td>
<td>a sufficient quantity</td>
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</table>

To make 1000 g

Prepare before use as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

**Description** Compound Scopolia Extract and Diastase Powder is light yellow in color. It has a bitter taste.

**Containers and storage** Containers—Well-closed containers.

Scopolia Extract and Ethyl Aminobenzoate Powder

ロートエキス・アネスタミン散

Scopolia Extract and Ethyl Aminobenzoate Powder contains not less than 22.5% and not more than
27.5% of ethyl aminobenzoate (C₉H₁₁NO₂: 165.19).

**Method of preparation**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
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</thead>
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<tr>
<td>Ethyl Aminobenzoate</td>
<td>250 g</td>
</tr>
<tr>
<td>Magnesium Oxide</td>
<td>150 g</td>
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<tr>
<td>Sodium Bicarbonate</td>
<td>500 g</td>
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<tr>
<td>Starch, Lactose Hydrate or</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td>their mixture</td>
<td></td>
</tr>
</tbody>
</table>

**To make 1000 g**

Prepare as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

**Description** Scopolia Extract and Ethyl Aminobenzoate Powder is slightly brownish white in color. It has a slightly bitter taste, leaving a sensation of numbness on the tongue.

**Identification (1)** To 2 g of Scopolia Extract and Ethyl Aminobenzoate Powder add 20 mL of diethyl ether, shake, and filter through a glass filter (G4). Wash the residue with three 10-mL portions of diethyl ether, combine the filtrate and the washings, evaporate to dryness, and perform the following test with the residue (ethyl aminobenzoate).

(i) Dissolve 0.01 g of the residue in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests \(<1.09\) for primary aromatic amines.

(ii) Dissolve 0.1 g of the residue in 5 mL of water with the aid of dilute hydrochloric acid added dropwise, and add iodine TS dropwise: a brown precipitate is produced.

(iii) Warm 0.05 g of the residue with 2 drops of acetic acid (31) and 5 drops of sulfuric acid: the odor of ethyl acetate is perceptible.

(2) To the diethyl ether-insoluble residue obtained in (1) add 30 mL of water, shake gently, and filter: the filtrate responds to the Qualitative Tests \(<1.09\) for sodium salt and for bicarbonate.

(3) To the water-insoluble residue obtained in (2) add 10 mL of dilute hydrochloric acid, shake, and filter: the filtrate responds to the Qualitative Tests \(<1.09\) for magnesium salt.

(4) Place 30 g of Scopolia Extract and Ethyl Aminobenzoate Powder in a glass-stoppered conical flask, add 100 mL of water, shake for 30 minutes, and filter immediately by suction through a glass filter (G3). Transfer the residue in the flask to the same glass filter with the filtrate, and filter the residue by suction while pressing vigorously the residue on the same glass filter. Place 75 mL of the filtrate in a 300-mL beaker, and add cautiously 10 mL of diluted sulfuric acid (1 in 3). Add 0.2 mL of bromocresol green TS to this solution, and add dilute sulfuric acid dropwise while shaking thoroughly, until the color of the solution changes from green to yellow-green. After cooling, place this solution in a separator, wash with two 25-mL portions of a mixture of hexane and diethyl ether (1:1) by shaking well, and place the water layer in another separator. Make slightly alkaline with ammonia TS, add immediately 30 mL of diethyl ether, and shake well. Wash the diethyl ether layer with two 10-mL portions of a saturated solution of sodium chloride, separate the diethyl ether layer, add 3 g of anhydrous sodium sulfate, shake, and filter through a pledget of cotton. Evaporate the filtrate to dryness, dissolve the residue in 0.2 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate RS and 1 mg of Scopolia-, and use these solutions as standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.24>\). Spot 10 \(\mu L\) each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia solution (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragentoff’s TS for spraying on the plate: two principal spots from the sample solution show the same color tone and the same \(R_f\) value with each yellow-red spot from the standard solutions, respectively.

**Assay** Weigh accurately about 0.3 g of Scopolia Extract and Ethyl Aminobenzoate Powder, transfer to a Soxhlet extractor, extract with 100 mL of diethyl ether for 1 hour, and evaporate the diethyl ether on a water bath. Dissolve the residue in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the sample solution. Weigh accurately about 75 mg of Ethyl Aminobenzoate RS, previously dried in a desiccator (silica gel) for 3 hours, dissolve in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, to each add 10 mL of 1 mol/L hydrochloric acid TS, then add 1 mL of a solution of sodium nitrite (1 in 200), prepared before use, and allow to stand for 5 minutes with occasional shaking. Add 5 mL of ammonium amidosulfate TS, shake well, and allow to stand for 10 minutes. Add 2 mL of \(N-N\)-diethyl-1-naphthylethylenediamine oxalate-acetone TS, mix immediately, and add water to make exactly 50 mL. Allow to stand for 2 hours, determine the absorbances, \(A_T\) and \(A_S\), of these solutions at 550 nm, as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\) using a blank prepared in the same manner with 5 mL of water in place of the sample solution.

\[
\text{Amount (mg) of ethyl aminobenzoate (C}_9\text{H}_{11}\text{NO}_2) = M_5 \times \frac{A_T}{A_S}
\]

\(M_5\): Amount (mg) of Ethyl Aminobenzoate RS

**Containers and storage** Containers—Well-closed containers.

**Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder**

Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder contains not less than 10.8% and not more than 13.2% of ethyl aminobenzoate (C₉H₁₁NO₂: 165.19).
Method of preparation

Scopolia Extract 15 g
Papaverine Hydrochloride 15 g
Ethyl Aminobenzoate 120 g
Starch, Lactose Hydrate or their mixture a sufficient quantity

To make 1000 g

Prepare as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

Description Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder is brownish yellow to grayish yellow-brown in color. It has a slightly bitter taste, leaving a sensation of numbness on the tongue.

Identification (1) To 4 g of Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder add 20 mL of diethyl ether, shake, and filter through a glass filter (G4). Wash the residue with three 10-mL portions of diethyl ether, combine the filtrate and the washings, evaporate to dryness, and perform the following test with the residue (ethyl aminobenzoate):

(i) Dissolve 0.01 g of the residue in 1 mL of dilute hydrochloric acid and 4 mL of water; the solution responds to the Qualitative Tests <1.00> for primary aromatic amines.
(ii) Dissolve 0.1 g of the residue in 5 mL of water with the aid of dilute hydrochloric acid added dropwise, and add iodine TS dropwise: a brown precipitate is produced.
(iii) Warm 0.05 g of the residue with 2 drops of acetic acid (31) and 5 drops of sulfuric acid: the odor of ethyl acetate is perceptible.

(2) To the diethyl ether-insoluble residue obtained in (1) add 20 mL of chloroform, shake well, and further wash the residue with 10 mL of chloroform. Combine the filtrate and the washing, transfer this solution to a separator, and add 10 mL of 0.1 mol/L hydrochloric acid TS. After shaking, separate the chloroform layer, add 2 g of anhydrous sodium sulfate, shake, and filter through a pledget of cotton. Evaporate the filtrate to dryness, dry the residue at 105°C for 3 hours, and evaporate the chloroform by shaking.

(i) To 1 mg of the residue add 1 drop of formaldehyde solution-sulfuric acid TS: a colorless or light yellow-green color, changing to red-purple, is produced.
(ii) Dissolve 1 mg of the residue in 3 mL of acetic anhydride and 5 drops of sulfuric acid, heat in a water bath for 1 minute, and view under ultraviolet light: the solution shows a yellow-green fluorescence.

(3) Place 20 g of Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder in a glass-stoppered conical flask, add 80 mL of water, shake for 15 minutes, and filter by suction through a glass filter (G3). Transfer 60 mL of the filtrate to a separator, add 0.5 mL of 1 mol/L hydrochloric acid TS, and extract with three 20-mL portions of chloroform by shaking. Make the aqueous layer slightly alkaline with ammonia TS, add immediately 30 mL of diethyl ether, and shake well. Wash the diethyl ether layer with two 10-mL portions of a saturated solution of sodium chloride, and separate the diethyl ether layer. Add 3 g of anhydrous sodium sulfate, shake, and filter through a pledget of cotton. Evaporate the filtrate to dryness, dissolve the residue in 0.2 mL of ethanol (95), and use the solution as the sample solution. Dissolve 20 mg of atropine sulfate for thin-layer chromatography, 10 mg of scopalone hydrobromide and 20 mg of papaverine hydrochloride in 10 mL each of ethanol (95), and use these solutions as standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm, and dry the plate at 80°C for 20 minutes. After cooling, spray Dragendorff’s TS for spraying upon the plate evenly: three yellow-red principal spots obtained from the sample solution and the corresponding spots from standard solutions (1), (2) and (3) show the same Rf values.

Assay Weigh accurately about 0.6 g of Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder, transfer to a Soxhlet extractor, and extract with 100 mL of diethyl ether for 1 hour, and evaporate the diethyl ether on a water bath. Dissolve the residue in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 75 mg of Ethyl Aminobenzoate RS, previously dried in a desiccator (silica gel) for 3 hours, dissolve in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add 10 mL of 1 mol/L hydrochloric acid TS to each, then add 1 mL of a solution of sodium nitrite (1 in 200) prepared before use, and allow to stand for 5 minutes with occasional shaking. Add 5 mL of ammonium amidosulfate TS, shake well, and allow to stand for 10 minutes. Add 2 mL of N-N-diethyl-N'-1-naphthylethylenediamine oxalate-acetone TS, mix immediately, and add water to make exactly 50 mL. Allow to stand for 2 hours, and determine the absorbances, A1 and A5, of these solutions at 550 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a blank prepared in the same manner with 5 mL of water in place of the sample solution.

Amount (mg) of ethyl aminobenzoate (C9H11NO2)

\[
M_5 = \frac{M_S \times A_1}{A_5}
\]

M5: Amount (mg) of Ethyl Aminobenzoate RS

Containers and storage Containers—Well-closed containers.

Scopolia Extract and Tannic Acid Suppositories

ロートエキス・タンニン坐剤

Method of preparation

Scopolia Extract 0.5 g
Tannic Acid 1 g
Cacao Butter or a suitable base a sufficient quantity
Prepare 10 suppositories as directed under Suppositories, with the above ingredients.

**Description**  Scopolia Extract and Tannic Acid Suppositories are light brown in color.

**Identification** (1) To 2 Scopolia Extract and Tannic Acid Suppositories add 20 mL of diethyl ether, and dissolve the base of suppositories with shaking for 10 minutes. Shake thoroughly the mixture with 15 mL of water, separate the water layer, and filter. To the filtrate add 10 mL of chloroform, shake well, and separate the chloroform layer. Take 5 mL of the chloroform solution, add 5 mL of ammonia TS, shake, and allow to stand: the ammonia layer shows a blue-green fluorescence.

(2) To 1 mL of the aqueous layer obtained in (1) after extraction with diethyl ether, add 2 drops of iron (III) chloride TS: a bluish-black color develops. Allow to stand: a bluish-black precipitate is formed (tannic acid).

**Containers and storage** Containers—Well-closed containers.

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**Scutellaria Root**

*Scutellariae Radix*

オウゴン

Scutellaria Root is the root of *Scutellaria baicalensis* Georgi (*Labiateae*), from which the periderm has been removed.

It contains not less than 10.0% of baicalin (C_{21}H_{18}O_{11}: 446.36), calculated on the basis of dried material.

**Description** Cone-shaped, semitubular or flattened root, 5 – 20 cm in length, 0.5 – 3 cm in diameter; externally yellow-brown, with coarse and marked longitudinal wrinkles, and with scattered scars of lateral root and remains of brown periderm; scars of stem or remains of stem at the crown; xylem rotted in old roots, often forming a hollow; hard in texture and easily broken; fractured surface fibrous and yellow in color.

Almost odorless; taste, slightly bitter.

**Identification** (1) Boil gently 0.5 g of pulverized Scutellaria Root with 20 mL of diethyl ether under a reflux condenser on a water bath for 5 minutes, cool, and filter. Evaporate the filtrate, dissolve the residue in 10 mL of ethanol (95), and to 3 mL of the solution add 1 to 2 drops of dilute iron (III) chloride TS: a grayish green color develops, and it changes to purple-brown.

(2) To 2 g of pulverized Scutellaria Root add 10 mL of methanol, warm on a water bath for 3 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Baicalin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one spot among the spots from the sample solution and a dark green spot from the standard solution show the same color tone and the same Rf value.

**Purity** (1) Heavy metals <1.07> — Proceed with 3.0 g of pulverized Scutellaria Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11> — Prepare the test solution with 0.40 g of pulverized Scutellaria Root according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying** <5.01> Not more than 12.0% (6 hours).

**Total ash** <5.01> Not more than 6.0%.

**Assay** Weigh accurately about 0.5 g of pulverized Scutellaria Root, add 30 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 30 minutes, and cool. Transfer the mixture to a glass-stoppered centrifuge tube, centrifuge, and separate the supernatant liquid. Wash the vessel for the reflux extraction with 30 mL of diluted methanol (7 in 10), transfer the washings to the glass-stoppered centrifuge tube, centrifuge after shaking for 5 minutes, and separate the supernatant liquid. To the residue add 30 mL of diluted methanol (7 in 10), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add diluted methanol (7 in 10) to make exactly 100 mL, then pipet 2 mL of the extract, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water), and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of the solution, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography (<2.03> according to the following conditions. Determine the peak areas, A_P and A_S, of baicalin in each solution.

Amount (mg) of baicalin (C_{21}H_{18}O_{11}) = M_S \times A_P / A_S \times 5 

M_S: Amount (mg) of Baicalin RS, calculated on the anhydrous basis

**Operating conditions**—

- Detector: An ultraviolet absorption photometer (wavelength: 277 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanol silica gel for liquid chromatography (5 µm in particle diameter).
- Column temperature: A constant temperature of about 50°C.
- Mobile phase: A mixture of diluted phosphoric acid (1 in 146) and acetonitrile (18:7).
- Flow rate: Adjust the flow rate so that the retention time of baicalin is about 6 minutes.

**System suitability**—

System performance: Dissolve 1 mg of Baicalin RS and 2 mg of methyl parahydroxybenzoate in methanol to make 100 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, baicalin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times...
with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Scutellaria Root

Scutellariae Radix Pulverata

粉末こんげ

Powdered Scutellaria Root is the powder of Scutellaria Root.

It contains not less than 10.0% of baicalin (C_{21}H_{18}O_{11}: 446.36), calculated on the basis of dried material.

Description Powdered Scutellaria Root occurs as a yellow-brown powder. It is almost odorless, and has a slight, bitter taste.

Under a microscope <5.01>, Powdered Scutellaria Root reveals fragments of parenchyma cells containing small amount of starch grains, fragments of reticulate vessels, tracheids and elongated stone cells; also a few fragments of spiral vessels and xylem fibers are observed.

Identification (1) Boil gently 0.5 g of Powdered Scutellaria Root with 20 mL of diethyl ether under a reflux condenser on a water bath for 5 minutes, cool, and filter. Evaporate the filtrate, dissolve the residue in 10 mL of ethanol (95), and to 3 mL of the solution add 1 to 2 drops of dilute iron (III) chloride TS: a grayish green color develops, and it changes to purple-brown later.

(2) To 2 g of Powdered Scutellaria Root add 10 mL of methanol, warm on a water bath for 3 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Baicalin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one spot among the spots from the sample solution and dark green spot from the standard solution show the same color tone and the same Rf value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Scutellaria Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Scutellaria Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Scutellaria Root does not show crystals of calcium oxalate.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Assay Weigh accurately about 0.5 g of Powdered Scutellaria Root, add 30 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 30 minutes, and cool. Transfer the mixture to a glass-stoppered centrifuge tube, centrifuge, and separate the supernatant liquid. Wash the vessel for the reflux extraction with 30 mL of diluted methanol (7 in 10), transfer the washings to the glass-stoppered centrifuge tube, centrifuge after shaking for 5 minutes, and separate the supernatant liquid. To the residue add 30 mL of diluted methanol (7 in 10), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add diluted methanol (7 in 10) to make exactly 100 mL, then pipet 2 mL of the extract, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water), and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of the solution, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_t and A_s, of baicalin in each solution.

\[
M_t = \frac{A_t}{A_s} \times 5
\]

M_s: Amount (mg) of Baicalin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 146) and acetonitrile (18:7).

Flow rate: Adjust the flow rate so that the retention time of baicalin is about 6 minutes.

System suitability—

System performance: Dissolve 1 mg of Baicalin RS and 2 mg of methyl parahydroxybenzoate in methanol to make 100 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, baicalin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.
Senega

Senegae Radix

セネガ

Senega is the root of *Polygala senega* Linné or *Polygala senega* Linné var. *latisolia* Torrey et Gray (Polygalaceae).

**Description** Slender, conical root often branched, 3 – 10 cm in length; main root 0.5 – 1.5 cm in diameter; externally light grayish brown to grayish brown; with many longitudinal wrinkles and sometimes with twisted protruding lines; tuberously enlarged crown, with remains of stems and red buds; branched rootlets twisted; a transverse section reveals grayish brown cortex and yellowish white xylem; usually round, and sometimes cuneate to semicircular; cortex on the opposite side is thickened.

Odor, characteristic, resembling the aroma of methyl salicylate; taste, sweet at first but leaving an acrid taste.

Under a microscope <5.01>, a transverse section of the main root reveals a cork layer consisting of several rows of light brown cork cells; secondary cortex composed of parenchyma cells and sieve tubes, traversed by medullary rays, 1 to 3 cells wide; medullary rays on xylem not distinct. Its parenchyma cells contain oil droplets, but starch grains and crystals of calcium oxalate are absent.

**Identification** (1) Shake vigorously 0.5 g of pulverized Senega with 30 mL of water: a lasting fine foam is produced. (2) Shake 0.5 g of pulverized Senega with 30 mL of water for 15 minutes, and filter. Take 1 mL of the filtrate, mix with 50 mL of water, and determine the absorption spectrum of the solution according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

**Purity** (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Senega according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Foreign matter <5.07>—Prepare the test solution with 0.40 g of Powdered Senega according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <5.07>—The amount of foreign matter other than the stems is not more than 1.0%.

**Loss on drying** <5.01> Not more than 13.0% (6 hours).

**Total ash** <5.01> Not more than 5.0%.

**Acid-insoluble ash** <5.01> Not more than 2.0%.

**Extract content** <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

**Containers and storage** Containers—Well-closed containers.

Powdered Senega

Senegae Radix Pulverata

セネガ末

Powdered Senega is the powder of Senega.

**Description** Powdered Senega occurs as a light brown powder, and has a characteristic odor resembling the aroma of methyl salicylate; taste, sweet at first, but later acrid.

Under a microscope <5.01>, Powdered Senega reveals fragments of pitted vessels, reticulate vessels and tracheids; fragments of xylem fibers with oblique pits; fragments of xylem parenchyma cells with simple pits; fragments of phloem parenchyma containing oily droplets; fragments of exodermis often composed of cells suberized and divided into daughter cells; oily droplets stained red by sudan III TS. The parenchyma cells of Powdered Senega do not contain starch grains and crystals of calcium oxalate.

**Identification** (1) Shake vigorously 0.5 g of Powdered Senega with 10 mL of water: a lasting fine foam is produced. (2) Shake 0.5 g of Powdered Senega with 30 mL of water for 15 minutes, and filter. Take 1 mL of the filtrate, mix with 50 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum at about 317 nm.

**Purity** (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Senega according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Senega according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.07>, stone cells, starch grains or crystals of calcium oxalate are not observed.

**Loss on drying** <5.01> Not more than 13.0% (6 hours).

**Total ash** <5.01> Not more than 5.0%.

**Acid-insoluble ash** <5.01> Not more than 2.0%.

**Extract content** <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

**Containers and storage** Containers—Well-closed containers.

Senega Syrup

セネガシロップ

**Method of preparation**

<table>
<thead>
<tr>
<th>Senega, in medium cutting</th>
<th>40 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>780 g</td>
</tr>
<tr>
<td>10 vol% Ethanol</td>
<td>a sufficient quantity</td>
</tr>
<tr>
<td>Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL
Add 400 mL of 10 vol% ethanol to Senega, and macerate for one or two days. Filter the extract, wash the residue with a small amount of 10 vol% Ethanol, filter, and combine the filtrate of the extracts and washings until total volume measures about 500 mL. Dissolve Sucrose in the mixture, by warming if necessary, and dilute to 1000 mL with Purified Water or Purified Water in Containers. May be prepared with an appropriate proportion of Ethanol and Purified Water or Purified Water in Containers in place of 10 vol% Ethanol.

**Description** Senega Syrup is a yellow-brown, viscous liquid. It has a characteristic odor resembling methyl salicylate and a sweet taste.

**Identification** Add 5 mL of water to 1 mL of Senega Syrup, and shake: lasting small bubbles are produced.

**Containers and storage** Containers—Tight containers.

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**Senna Leaf**

**Sennae Folium**

センナ

Senna Leaf is the leaflets of *Cassia angustifolia* Vahl or *Cassia acutifolia* Delile (*Leguminosae*).

It contains not less than 1.0% of total sennosides [sennoside A (C_{42}H_{38}O_{20}· 862.74) and sennoside B (C_{42}H_{38}O_{20}· 862.74)], calculated on the basis of dried material.

**Description** Lanceolate to narrow lanceolate leaflets, 1.5 – 5 cm in length, 0.5 – 1.5 cm in width, light grayish yellow to light grayish yellow-green in color; margin entire, apex acute, base asymmetric, petiole short; under a magnifying glass, vein marked, primary lateral veins running toward the apex along the margin and joining the lateral vein above; lower surface having slight hairs.

Odor slight; taste, bitter.

Under a microscope 5.0, a transverse section of Senna Leaf reveals epidermis with thick cuticle, with numerous stomata, and with thick-walled, warty unicellular hairs; epidermal cells are often separated into two loculi by a septum which is in parallel with the surface of the leaf, and contain mucilage in the inner loculus; palisade of a single layer tum which is in parallel with the surface of the leaf, and contain mucilage in the inner loculus; palisade of a single layer (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 by adding 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, shake with 30 mL of tetrahydrofuran for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A RS in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography 2.07 with the sample solution and standard solution. Spot 10 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same RF value.

**Purity** (1) Rachis and fruit—When perform the test of foreign matter 5.0, the amount of rachis and fruits contained in Senna Leaf does not exceed 5.0%.

(2) Foreign matter 5.0—The amount of foreign matter other than rachis and fruits contained in Senna Leaf does not exceed 1.0%.

(3) Total BHC's and total DDT's 5.0—Not more than 0.2 ppm, respectively.

**Loss on drying** 5.0 Not more than 12.0% (6 hours).

**Total ash** 5.0 Not more than 12.0%.

**Acid-insoluble ash** 5.0 Not more than 2.0%.

**Assay** Weigh accurately about 0.5 g of pulverized Senna Leaf in a glass-stoppered centrifuge tube, add 25 mL of diluted methanol (7 in 10), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of diluted methanol (7 in 10), shake for 10 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure once more, combine all the extracts, add diluted methanol (7 in 10) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A RS (separately determine the water), dissolve in a solution of sodium hydroxide carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (1). Weigh accurately about 10 mg of Sennoside B RS (separately determine the water), dissolve in a solution of sodium hydroxide carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (2). Pipet 5 mL of the standard stock solution (1) and 10 mL of the standard stock solution (2), add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.07 according to the following conditions. Determine the peak areas, Sa, Ta, and Sb, of sennoside A, and the peak areas, Sa, Tb, and Sb, of sennoside B in each solution, calculate the amounts of sennoside A and sennoside B by the following equations, and designate the total as the amount of total sennosides.

Amount (mg) of sennoside A (C_{42}H_{38}O_{20})

\[
= M_{SA} \times A_{Tb}/A_{SB} \times 1/2
\]

Amount (mg) of sennoside B (C_{42}H_{38}O_{20})

\[
= M_{SB} \times A_{Ta}/A_{SA} \times 1/4
\]
Operating conditions—
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 50°C.
Mobile phase: Dissolve 2.45 g of tetra-n-heptylammonium bromide in 1000 mL of a mixture of diluted 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0 (1 in 10) and acetonitrile (17:8).
Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 26 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sennoside B and sennoside A are eluted in this order with the resolution between these peaks being not less than 15, and the number of theoretical plates of the peak of sennoside A being not less than 8000.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Senna Leaf

Sennae Folium Pulveratum

センナ末

Powdered Senna Leaf is the powder of Senna Leaf. It contains not less than 1.0% of total sennosides [sennoside A (C_{42}H_{38}O_{20}: 862.74) and sennoside B (C_{42}H_{38}O_{20}: 862.74)], calculated on the basis of dried material.

Description Powdered Senna Leaf occurs as a light yellow to light grayish yellow-green powder. It has a slight odor and a bitter taste.

Under a microscope <5.01>, Powdered Senna Leaf reveals fragments of vessels and vein tissue accompanied with crystal cell rows; fragments of thick-walled, bent, unicellular hairs; fragments of palisade and spongy tissue; clustered and solitary crystals of calcium oxalate, 10 to 20 μm in diameter.

Identification (1) Macerate 0.5 g of Powdered Senna Leaf in 10 mL of diethyl ether for 2 minutes, and filter. Add 5 mL of ammonia TS to the filtrate: a yellow-red color is produced in the water layer. To the residue of maceration add 10 mL of water, and macerate for 2 minutes. Filter, and add 5 mL of ammonia TS: a yellow-red color is produced in the water layer.

(2) To 2 g of Powdered Senna Leaf add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 with 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, shake with 30 mL of tetrahydrofuran for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A RS in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.01> with the sample solution and standard solution. Spot 10 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100:40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same Rf value.

Purity (1) Foreign matter ≤5.01—Under a microscope, stone cells and thick fibers are not observable.
(2) Total BHC's and total DDT's ≤5.01—Not more than 0.2 ppm, respectively.

Loss on drying ≤5.01—not more than 12.0% (6 hours).

Total ash ≤5.01—not more than 12.0%.

Acid-insoluble ash ≤5.01—not more than 2.0%.

Assay Weigh accurately about 0.5 g of Powdered Senna Leaf in a glass-stoppered centrifuge tube, add 25 mL of diluted methanol (7 in 10), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of diluted methanol (7 in 10), shake for 10 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure once more, combine all the extracts, add diluted methanol (7 in 10) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A RS (separately determine the water), dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (1). Weigh accurately about 10 mg of Sennoside B RS (separately determine the water), dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (2). Pipet 5 mL of the standard stock solution (1) and 10 mL of the standard stock solution (2), add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{Sa}, A_{Sb}, and A_{Sb} of sennoside A, and the peak areas, A_{Tb}, and A_{Ab} of sennoside B of each solution, calculate the amounts of sennoside A and sennoside B by the following equations, and designate the total as the amount of total sennoside.

\[
\text{Amount (mg) of sennoside A (C_{42}H_{38}O_{20})} = M_{Sa} \times A_{Sa} / A_{Tb} \times 1/4
\]

\[
\text{Amount (mg) of sennoside B (C_{42}H_{38}O_{20})} = M_{Sb} \times A_{Sb} / A_{Ab} \times 1/2
\]
Sesame / Crude Drugs

M_{Sa}: Amount (mg) of Sennoside A RS, calculated on the anhydrous basis
M_{Sb}: Amount (mg) of Sennoside B RS, calculated on the anhydrous basis

Operating conditions—
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).
Column temperature: A constant temperature of about 50°C.
Mobile phase: Dissolve 2.45 g of tetra-n-heptylammonium bromide in 1000 mL of a mixture of diluted 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0 (1 in 10) and acetonitrile (17:8).
Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 26 minutes.
System suitability—
- System performance: When the procedure is run with 10 \( \mu \)L of the standard solution under the above operating conditions, sennoside B and sennoside A are eluted in this order with the resolution between these peaks being not less than 15, and the number of theoretical plates of the peak of sennoside A being not less than 8000.
- System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Sesame
Sesami Semen
ゴマ

Sesame is the seed of Sesamum indicum Linné (Pedaliaceae).

Description Ovate to spatulate seed, 3 – 4 mm in length, about 2 mm in width, about 1 mm in thickness; externally dark brown to black, rarely light brown to brown. Under a magnifying glass, thin ridges are observed on edges. 100 seeds weigh about 0.2 – 0.3 g.
Odorless; taste, slightly sweet and oily.
Under a microscope, transverse section reveals a seed coat consisting of palisade epidermis and flattened parenchyma; in the interior, endosperm and cotyledon; epidermal cells contain orbicular crystals of calcium oxalate and black pigment; parenchymatous cells of endosperm and cotyledon contain oil drops and aleurone grains.
Identification Grind an amount of Sesame. To 1.0 g of the ground add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of sesamin for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these

Shakuyakukanzoto Extract

Shakuyakukanzoto Extract occurs as a light brown to brown, powder or viscous extract. It has slightly an odor, and a sweet taste.

Identification (1) Shake 0.5 g of dry extract (or 1.5 g of the viscous extract) with 10 mL of water, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Peoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the purple spot from the standard solution (Peony Root).

(2) Shake 0.5 g of dry extract (or 1.5 g of the viscous extract) with 10 mL of water, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Shakuyakukanzoto Extract occurs as a light brown to brown, powder or viscous extract. It has slightly an odor, and a sweet taste.

Method of preparation

<table>
<thead>
<tr>
<th>1) Peony Root</th>
<th>6 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycyrrhiza</td>
<td>6 g</td>
</tr>
<tr>
<td>2) Peony Root</td>
<td>5 g</td>
</tr>
<tr>
<td>Glycyrrhiza</td>
<td>5 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.
solutions as directed under Thin-layer Chromatography <2.09>. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spots among the several spots obtained from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Glycyrrhiza).

**Purity** (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract equivalent to 1.0 g of dried substance) as directed in Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract equivalent to 1.0 g of dried substance) according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying** <2.47>—The dry extract: Not more than 8.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

**Total ash** <5.01>—Not more than 9.0%, calculated on the dried basis.

**Assay** (1) Peoniflorin—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract equivalent to 0.2 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determined to 0.2 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of peoniflorin in each solution.

\[
\text{Amount (mg) of peoniflorin (C₃₉H₄₈O₁₄)} = Mₛ × \frac{A_T}{A_S} × \frac{1}{2}
\]

Mₛ: Amount (mg) of Peoniflorin RS, calculated on the anhydrous basis

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

**Containers and storage**—Containers—Tight containers.

**Shimbuto Extract**

Shimbuto Extract contains not less than 26 mg and not more than 78 mg of peoniflorin (C₃₉H₄₈O₁₄: 480.46), not less than 0.5 mg and not more than 2.0 mg (for preparation prescribed 0.8 g of Ginger) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 1 g of Ginger) or not less than 0.9 mg and not more than 3.6 mg (for preparation prescribed 1.5 g of Ginger) of [6]-gingerol, and not less than 0.7 mg (for preparation prescribed 1 g of Processed
Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride) or not less than 0.2 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) or not less than 0.1 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride), per the extract prepared as directed in the Method of preparation.

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>1)</th>
<th>2)</th>
<th>3)</th>
<th>4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poria Sclerotium</td>
<td>5 g</td>
<td>5 g</td>
<td>5 g</td>
<td>4 g</td>
</tr>
<tr>
<td>Peony Root</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Atractylodes Rhizome</td>
<td>3 g</td>
<td>—</td>
<td>3 g</td>
<td>—</td>
</tr>
<tr>
<td>Atractylodes Lancea Rhizome</td>
<td>—</td>
<td>3 g</td>
<td>—</td>
<td>3 g</td>
</tr>
<tr>
<td>Ginger</td>
<td>1 g</td>
<td>1 g</td>
<td>0.8 g</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Processed Aconite Root (Processed Aconite Root 1)</td>
<td>1 g</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Powdered Processed Aconite Root (Powdered Processed Aconite Root 1)</td>
<td>—</td>
<td>1 g</td>
<td>—</td>
<td>0.5 g</td>
</tr>
</tbody>
</table>

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description

Shimbuto Extract occurs as light yellow-brown to brown powder. It has a characteristic odor and a hot and bitter taste.

Identification

1) To 2.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the blue-white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) (For preparation prescribed Atractylodes Lancea Rhizome) To 2.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an Rf value of about 0.4. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes and allowed to cool (Atractylodes Lancea Rhizome).

4) To 1.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atracylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the blue-white fluorescent spot from the standard solution (Atractylodes Lancea Rhizome).

(5) To 3.0 g of Shimbuto Extract, add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and take the supernatant liquid. Evaporate the supernatant liquid under reduced pressure, add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica
gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and RF value with the yellow-brown spot from the standard solution (Processed Aconite Root or Powdered Processed Aconite Root).

**Purity**

(1) Heavy metals \(<1.07\)—Prepare the test solution with 1.0 g of Shimbuto Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Arsenic \(<1.17\)—Prepare the test solution with 0.67 g of Shimbuto Extract according to Method 3, and perform the test (not more than 3 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately 1.0 g of Shimbuto Extract, add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 \(\mu\)L of each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07\) according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the standard solution.

**System suitability**

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

System performance: When the procedure is run with 20 \(\mu\)L of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

**System repeatability**

When the test is repeated 6 times with 20 \(\mu\)L of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

**Loss on drying** \(<2.47\) Not more than 7.0% (1 g, 105°C, 5 hours).

**Total ash** \(<5.07\) Not more than 10.0%.

**Assay**

(1) Peoniflorin—Weigh accurately about 0.5 g of Shimbuto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin RS (separately determine the water), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu\)L of each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07\) according to the following conditions, and determine the peak areas, \(A_1\) and \(A_5\), of peoniflorin in each solution.

\[
\text{Amount (mg) of peoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) = M_S \times \frac{A_1}{A_5} \times \frac{1}{2}
\]

\(M_S\): Amount (mg) of Peoniflorin RS, calculated on the anhydrous basis.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of peoniflorin is about 9 minutes).

**System suitability**

System performance: Dissolve 1 mg each of Peoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 \(\mu\)L of this solution under the above operating conditions, albiflorin and peoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of peoniflorin is not more than 1.5%.

(2) \([6]-gingerol\)—Weigh accurately about 0.5 g of Shimbuto Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of \([6]-gingerol\) for assay, dissolve in diluted methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu\)L of each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07\) according to the following conditions, and determine the peak areas, \(A_1\) and \(A_5\), of \([6]-gingerol\) in each solution.
Shosaikoto Extract / Crude Drugs

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhypaconine; 254 nm for 14-anisoylaconine).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of water, acetonitrile and phosphoric acid (620:380:1).
Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 15 minutes).
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

Amount (mg) of [6]-gingerol = \( M_A \times A_A \times 1/20 \)

Amount (mg) of [6]-gingerol for assay

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhypaconine; 254 nm for 14-anisoylaconine).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of water, acetonitrile and phosphoric acid (620:380:1).
Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 15 minutes).
System suitability—
System performance: When the procedure is run with 10 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the relative standard deviation of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine is not more than 1.5%.

Amount (mg) of [6]-gingerol = \( M_A \times A_A \times 1/20 \)

Amount (mg) of 14-anisoylaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay

Preparation of syrup—
Shobaikoto Extract, as directed in the Method of preparation, contains not less than 2 mg and not more than 8 mg of saikosaponin b2, not less than 80 mg and not more than 240 mg of baicalin (C\(_{15}\)H\(_{18}\)O\(_{11}\): 446.36), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid (C\(_{22}\)H\(_{22}\)O\(_{16}\): 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Shosaikoto Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b2, not less than 80 mg and not more than 240 mg of baicalin (C\(_{15}\)H\(_{18}\)O\(_{11}\): 446.36), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid (C\(_{22}\)H\(_{22}\)O\(_{16}\): 822.93), per extract prepared with the amount specified in the Method of preparation.

Shosaikoto Extract occurs as a light brown to black-grayish brown, powder or viscous extract. It has a slight odor, and a sweet first then slightly pungent and bitter taste.
Identification (1) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b₂ for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.07. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the red spot from the standard solution (Glycyrrhiza).

(2) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the diethyl ether under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.07. Spot 15 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the blue-green spot from the standard solution (Ginger).

(3) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.07. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100:10:1) to a distance of about 10 cm, and air-dry the plate, and spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Scutellaria Root).

(4) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb₁ RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.07. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the purple spot from the standard solution (Ginseng).

(5) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.07. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution (Glycyrrhiza).

Purity (1) Heavy metals <1.0%—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to about 1.0 g of dried substance) as directed in Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.1%—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.4% The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <5.0% Not more than 10.0%, calculated on the dried basis.

Assay (1) Saikosaponin b₂—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of saikosaponin b₂ for assay, previously dried in a desiccator (silica gel) for more than 24 hours, dissolve in 50 mL of methanol, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.07 according to the following conditions, and determine the peak areas, A₁ and A₅, of saikosaponin b₂ in each solution.

\[
\text{Amount (mg) of saikosaponin b₂} = M₅ \times \frac{A₁}{A₃} \times \frac{1}{20}
\]

Where:
- \(M₅\): Amount (mg) of saikosaponin b₂ for assay

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diame-
Shoseiryuto Extract / Crude Drugs

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).
Flow rate: 1.0 mL per minute (the retention time of saikosaponin b₂ is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b₂ is not more than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b₂ is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water), dissolve in diluted methanol (7 in 10) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.02> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of glycyrrhizic acid in each solution.

Amount (mg) of baicalin (C₂₁H₁₈O₁₁)

\[
M_S = \frac{M_T}{A_T/A_S \times 1/4}
\]

\( M_S \): Amount (mg) of Baicalin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of dilute acetic acid (31) (1 in 15) and acetonitrile (13:7).
Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage—

Containers—Tight containers.

Shoseiryuto Extract

小青竜湯エキス

Shoseiryuto Extract contains not less than 10 mg and not more than 30 mg of the total alkaloids [ephedrine (C₁₀H₁₅NO: 165.23) and pseudoephedrine (C₁₀H₁₅NO: 165.23)], not less than 26 mg and not more than 78 mg of peoniflorin (C₂₃H₂₈O₁₁: 480.46), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid (C₆₂H₉₀O₁₆: 822.93), per extract prepared with the amount specified in the Method of preparation.
Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

**Description** Shoseiryuto Extract occurs as a light brown to blackish brown, powder or viscous extract. It has a characteristic odor and a acid first then pungent taste.

**Identification (1)** Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ephedrine hydrochloride in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and RF value with the blue-green spot from the standard solution (Ephedra Herb).

(2) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Peoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. The plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and RF value with the purple spot from the standard solution (Ephedra Herb).

(3) For preparation prescribed Processed Ginger—Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingeol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Sprinkle 20 μL of the sample solution and 2 μL the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and RF value with the yellow-brown spot from the standard solution (Glycyrrhiza).
among the several spots obtained from the sample solution has the same color tone and $RF$ value with the yellow-orange spot from the standard solution.

(ii) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of hexane and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (E)-2-methoxymercaptoaniline and 0.8 mg of (E)-2-methoxy-3-nitroaniline for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 20 µL of the sample solution and 2 µL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the standard solution has the same color tone and $RF$ value with the bluish white fluorescent spot from the standard solution.

(7) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of asarinin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 20 µL of the sample solution and 5 µL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and $RF$ value with the yellow-brown spot from the standard solution (Asiasarum Root).

(8) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of schisandrin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.03>$. Spot 10 µL of the sample solution and 5 µL of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100:40:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and $RF$ value with the blue-purple spot from the standard solution (Schisandra Fruit).

**Purity (1)** Heavy metals $<1.07>$—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed in Extracts (4), and perform the test (not more than 30 ppm).

**Arsenic** $<1.11>$—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

**Loss on drying** $<2.41>$ The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).
The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

**Total ash** $<5.01>$ Not more than 12.0%, calculated on the dried basis.

**Assay (1)** Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and determine the peak areas, $A_{TP}$ and $A_{TS}$, of ephedrine and pseudoephedrine from the sample solution, and the peak area, $A_{E}$, of ephedrine from the standard solution.

\[
S = \frac{M_S \times (A_{TG} + A_{TR})}{A_S} \times \frac{1}{10} \times 0.819
\]

**Operating conditions**
- **Detector:** An ultraviolet absorption photometer (wavelength: 210 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5µm in particle diameter).
- **Column temperature:** A constant temperature of about 40°C.
- **Mobile phase:** A mixture of a solution of sodium lauryl sulfate (1 in 130), acetonitrile and phosphoric acid (650:350:1).
- **Flow rate:** 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

**System suitability**
- **System performance:** Dissolve 1 mg each of ephedrine hydrochloride for assay and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.
- **System repeatability:** When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

**Peoniflorin**—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin RS, (separately determined the
water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, \( A_1 \) and \( A_6 \), of peoniflorin in each solution.

\[
\text{Amount (mg) of peoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) = M_S \times \frac{A_1}{A_6} \times \frac{1}{2}
\]

\( M_S \): Amount (mg) of Peoniflorin RS, calculated on the anhydrous basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 232 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 20°C.
Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).
Flow rate: 1.0 mL per minute (the retention time of peoniflorin is about 9 minutes).

System suitability—
System performance: Dissolve 1 mg each of Peoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of albiflorin is not more than 1.5%.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of albiflorin is not more than 1.5%.

\( 3 \) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS, (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas, \( A_1 \) and \( A_6 \), of glycyrrhizic acid in each solution.

\[
\text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) = M_S \times \frac{A_1}{A_6} \times \frac{1}{2}
\]

\( M_S \): Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of dilute acetic acid (31) (1 in 15) and acetonitrile (13:7).
Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Sinomenium Stem and Rhizome

\( \text{ポウイ} \)

Sinomenium Stem and Rhizome is the climbing stem and rhizome of Sinomenium acutum Rehder et Wilson (Menispermaceae), usually cut transversely.

Description Round or elliptic sections, 0.2 – 0.4 cm in thickness, 1 – 4.5 cm in diameter; cortex on both fractured surfaces, light brown to dark brown; in xylem, grayish brown vessel portions and dark brown medullary rays lined alternately and radialy; flank, dark gray, with longitudinal wrinkles and warty protrusions. Almost odorless; taste, bitter.

Under a microscope \(<5.01\), a transverse section reveals extremely thick-walled stone cells in primary cortex and pericycle; irregular-sized vessels lined nearly stepwise in the vessel portion; cells of medullary ray mostly not lignified, and extremely thick-walled and large stone cells scattered here and there; primary cortex containing needle crystals of calcium oxalate; medullary rays containing starch gains, simple grain, 3 – 10 μm in diameter, and small needle crystals of calcium oxalate.

Identification To 0.5 g of pulverized Sinomenium Stem and Rhizome add 10 mL of dilute acetic acid, heat for 2 minutes on a water bath with frequent shaking, cool, and filter. To 5 mL of the filtrate add 2 drops of Dragendorff’s TS: an orange-yellow precipitate is immediately produced.

Total ash \(<5.01\) Not more than 7.0%.
Acid-insoluble ash \(<5.01\) Not more than 0.5%.

Containers and storage Containers—Well-closed containers.
Smilax Rhizome

Smilacis Rhizoma

サンキライ

Smilax Rhizome is the rhizome of Smilax glabra Roxburgh (Liliaceae).

Description Flattened and irregular cylindrical tuber, often with node-like branches; usually 5 – 15 cm in length, 2 – 5 cm in diameter; the outer surface grayish yellow-brown to yellow-brown, and the upper surface scattered with knotty remains of stem; transverse section irregular elliptical to obtuse triangular, consisting of extremely thin cortical layer and mostly of stele.

Odor, slight; almost tasteless.

Under a microscope, a transverse section reveals a 2- to 3-cell-wide cork layer, with extremely narrow cortical layer, usually consisting of a 2- to 4-cell-wide, thick-walled parenchyma cells, showing large mucilage cells here and there; mucilage cell containing raphides of calcium oxalate; stele consisting chiefly of parenchyma cells, and scattered with vascular bundles; parenchyma cells containing starch grains composed mostly of simple grains, 12 – 36 μm in diameter, and sometimes mixed with 2- to 4-compound grains.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Smilax Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Smilax Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 5.0%.

Containers and storage Containers—Well-closed containers.

Sodium Bicarbonate and Bitter Tincture Mixture

苦味重曹水

Method of preparation

<table>
<thead>
<tr>
<th>Sodium Bicarbonate</th>
<th>30 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitter Tincture</td>
<td>20 mL</td>
</tr>
<tr>
<td>Water, Purified Water or Purified Water in Containers a sufficient quantity To make</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Prepare before use, with the above ingredients.

Description Sodium Bicarbonate and Bitter Tincture Mixture is a clear, yellowish liquid, having a bitter taste.

Containers and storage Containers—Well-closed containers.

Sophora Root

Sophorae Radix

クジン

Sophora Root is the root of Sophora flavescens Aiton (Leguminoseae) or often such root from which the periderm has been removed.

Description Cylindrical root, 5 – 20 cm in length, 2 – 3 cm in diameter; externally dark brown to yellow-brown, with distinct longitudinal wrinkles, and with laterally extended lenticels; root without periderm, externally yellowish white, with somewhat fibrous surface; the transversely cut surface, light yellow-brown; cortex, 0.1 – 0.2 cm in thickness, slightly tinged with dark color near cambium, forming a crack between xylem.

Odor, slight; taste, extremely bitter and lasting.

Identification To 0.5 g of powdered Sophora Root add 10 mL of dilute acetic acid, heat on a water bath for 3 minutes with occasional shaking, cool, and filter. To 5 mL of the filtrate add 2 drops of Dragendorff’s TS: an orange-yellow precipitate is produced immediately.

Purity (1) Stem—When perform the test of foreign matter <5.01>, the amount of its stems contained in Sophora Root does not exceed 10.0%.
(2) Heavy metals $<1.0\%$—Proceed with 3.0 g of pulverized Sophora Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic $<1.1\%$—Prepare the test solution with 0.40 g of pulverized Sophora Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter $<5.0\%$—The amount of foreign matter other than stems is not more than 1.0%.

**Total ash** $<5.0\%$ Not more than 6.0%.

**Acid-insoluble ash** $<5.0\%$ Not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

### Powdered Sophora Root

**Sophorae Radix Pulverata**

クジン末

Powdered Sophora Root is the powder of Sophora Root.

**Description** Powdered Sophora Root occurs as a light brown powder. It has a slight odor, and an extremely bitter and lasting taste.

Under a microscope $<5.0\%$, Powdered Sophora Root reveals mainly starch grains and fragments of parenchyma cells containing them, fibers, bordered pitted vessels, reticulate vessels; a few fragments of corky tissue and solitary crystals of calcium oxalate. Starch grains usually composed of 2- to 4-compound grains 15 – 20 μm in diameter, and simple grains 2 – 5 μm in diameter.

**Identification** To 0.5 g of Powdered Sophora Root add 10 mL of dilute acetic acid, heat on a water bath for 3 minutes while occasional shaking, cool, and filter. To 5 mL of the filtrate add 2 drops of Dragendorff’s TS: an orange-yellow precipitate is produced immediately.

**Purity (1)** Stem—When perform the test of foreign matter $<5.0\%$, the amount of stems contained in Sweet Hydrangea Leaf does not exceed 3.0%.

(2) Foreign matter $<5.0\%$—The amount of foreign matter other than stems contained in Sweet Hydrangea Leaf does not exceed 1.0%.

**Loss on drying** $<5.0\%$ Not more than 13.0% (6 hours).

**Total ash** $<5.0\%$ Not more than 12.0%.

**Acid-insoluble ash** $<5.0\%$ Not more than 2.5%.

**Containers and storage** Containers—Well-closed containers.

### Sweet Hydrangea Leaf

**Hydrangeae Dulcis Folium**

アマチャ

Sweet Hydrangea Leaf is the leaf with twig of Hydrangea macrophylla Seringe var. thunbergii Makino (Saxifragaceae).

**Description** Usually wrinkled and contracted leaf, dark green to dark yellow-green in color. When soaked in water and smoothed out, it is lanceolate to acuminate ovate, 5 – 15 cm in length, 2 – 10 cm in width; margin serrated, base slightly wedge; coarse hair on both surfaces, especially on the veins; lateral veins not reaching the margin but curving upwards and connecting with each other; petiole short and less than one-fifth of the length of the lamina.

Odor, slight; taste, characteristically sweet.

**Identification** Mix 0.5 g of pulverized Sweet Hydrangea Leaf with 8 mL of a mixture of diethyl ether and petroleum ether (1:1), shake well, filter, and evaporate the filtrate to dryness. Dissolve the residue in 1 mL of dilute ethanol, and add 1 drop of dilute iron (III) chloride TS: a red-purple color develops, which disappears on the addition of 2 to 3 drops of dilute sulfuric acid.

**Purity (1)** Stem—When perform the test of foreign matter $<5.0\%$, the amount of stems contained in Sweet Hydrangea Leaf does not exceed 3.0%.

(2) Foreign matter $<5.0\%$—The amount of foreign matter other than stems contained in Sweet Hydrangea Leaf does not exceed 1.0%.

**Loss on drying** $<5.0\%$ Not more than 13.0% (6 hours).

**Total ash** $<5.0\%$ Not more than 12.0%.

**Acid-insoluble ash** $<5.0\%$ Not more than 2.5%.

**Containers and storage** Containers—Well-closed containers.

### Powdered Sweet Hydrangea Leaf

**Hydrangeae Dulcis Folium Pulveratum**

アマチャ末

Powdered Sweet Hydrangea Leaf is the powder of Sweet Hydrangea Leaf.

**Description** Powdered Sweet Hydrangea Leaf occurs as a dark yellow-green powder, and has a faint odor and a characteristic, sweet taste.

Under a microscope $<5.0\%$, Powdered Sweet Hydrangea Leaf reveals fragments of epidermis with wavy lateral membrane; stomata with two subsidiary cells; unicellular and thin-walled hair with numerous protrusions of the surface, 150 – 300 μm in length; fragments of palisade tissue and spongy tissue; fragments of vascular bundle and mucilage cells containing raphides of calcium oxalate 50 – 70 μm in length.
Identification  Mix 0.5 g of Powdered Sweet Hydrangea Leaf with 8 mL of a mixture of diethyl ether and petroleum ether (1:1), shake well, filter, and evaporate the filtrate to dryness. Dissolve the residue in 1 mL of dilute ethanol, and add 1 drop of dilute iron (III) chloride TS: a red-purple color develops, which disappears on the addition of 2 to 3 drops of dilute sulfuric acid.

Purity  Foreign matter [5.01]—Under a microscope, Powdered Sweet Hydrangea Leaf does not show stone cells, a large quantity of fibers or starch grains. Loss on drying [5.01]  Not more than 12.0% (6 hours).
Total ash [5.01]  Not more than 12.0%.
Acid-insoluble ash [5.01]  Not more than 2.5%.
Containers and storage  Containers—Well-closed containers.

Swertia Herb

Swertia Herb is the whole herb of Swertia japonica Makino (Gentianaceae) collected during the blooming season.

It contains not less than 2.0% of swertiamarin (C_{16}H_{22}O_{10}: 374.34), calculated on the basis of dried material.

Description  Herb, 20 cm in length, having flowers, opposite leaves, stems, and, usually, with short, lignified roots; stems square, about 0.2 cm in diameter, often with branches; the leaves and stems dark green to dark purple or yellow-brown in color; the flowers white to whitish, and the roots yellow-brown. When smoothed by immersing in water, leaves, linear or narrow lanceolate, 1 - 4 cm in length, 0.1 - 0.5 cm in width, entire, and sessile; corolla split deeply as five lobes; the lobes narrow, elongated ellipse shape, and under a magnifying glass, with two elliptical nectaries juxtaposed at the base of the inner surface; the margin of lobe resembles eyelashes; the five stamens grow on the tube of the corolla and stand alternately in a row with corolla-lobes; peduncle distinct. Odor, slight; taste, extremely bitter and persisting.

Identification  To 2 g of pulverized Swertia Herb add 10 mL of ethanol (95), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of Swertiamarin RS in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography [2.03]. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one spot among several spots from the sample solution and a red spot from the standard solution show the same color tone and the same Rf value.

Purity  Foreign matter [5.01]—The amount of straw and other foreign matters contained in Swertia Herb is not more than 1.0%.
Loss on drying [5.01]  Not more than 12.0% (6 hours).
Total ash [5.01]  Not more than 6.5%.
Extract content [5.01]  Dilute ethanol-soluble extract: not less than 20.0%.

Assay  Weigh accurately about 1 g of medium powder of Swertia Herb in a glass-stoppered centrifuge tube, add 40 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 40 mL of methanol, and proceed in the same manner. Combine the extracts, and add methanol to make exactly 100 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Swertiamarin RS (separately determine the water), dissolve in methanol to make exactly 20 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography [2.01] according to the following conditions, and determine the peak areas, A_T and A_S, of swertiamarin in each solution.

\[
\text{Amount (mg) of swertiamarin (C}_{16}\text{H}_{22}\text{O}_{10}) = \frac{M_S \times A_T}{A_S \times 5} \\
M_S: \text{Amount (mg) of Swertiamarin RS, calculated on the anhydrous basis}
\]

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 238 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 50°C.
Mobile phase: A mixture of water and acetonitrile (91:9).
Flow rate: Adjust the flow rate so that the retention time of swertiamarin is about 12 minutes.

System suitability—
System performance: Dissolve 1 mg each of Swertiamarin RS and theophylline in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the theophylline and swertiamarin are eluted in this order with the resolution of these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of swertiamarin is not more than 1.5%.

Containers and storage  Containers—Well-closed containers.
Powdered Swertia Herb

Swertiae Herba Pulverata

Powdered Swertia Herb is the powder of Swertia Herb.

It contains not less than 2.0% of swertiamarin (C_{16}H_{22}O_{10}: 374.34), calculated on the basis of dried material.

**Description** Powdered Swertia Herb occurs as a grayish yellow-green to yellow-brown powder. It has a slight odor, and extremely bitter, persistent taste.

Under a microscope <5.01>, Powdered Swertia Herb reveals xylem tissues with fibers (components of stems and roots); assimilation tissues (components of leaves and calyces); striated epidermis (components of stems and peduncles); tissues of corollas and filaments with spiral vessels; cells of anthers and their inner walls; spherical pollen grains with granular patterns (components of flowers), about 30 μm in diameter; starch grains are simple grain, about 6 μm in diameter, and very few.

**Identification** To 2 g of Powdered Swertia Herb add 10 mL of ethanol (95), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of Swertiamarin RS in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one spot among several spots from the sample solution and a red spot from the standard solution show the same color tone and the same Rf value.

**Purity** Foreign matter—Under a microscope <5.01>, crystals of calcium oxalate, a large quantity of starch grains and groups of stone cells are not observable.

**Loss on drying** <5.01> Not more than 12.0% (6 hours).

**Total ash** <5.01> Not more than 6.5%.

**Acid-insoluble ash** <5.01> Not more than 2.0%.

**Extract content** <5.01> Dilute ethanol-soluble extract: not less than 20.0%.

**Assay** Weigh accurately about 1 g of Powdered Swertia Herb in a glass-stoppered centrifuge tube, add 40 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 40 mL of methanol, and proceed in the same manner. Combine the extracts, and add methanol to make exactly 100 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Swertiamarin RS (separately determine the water), dissolve in methanol to make exactly 20 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_f and A_s, of swertiamarin in each solution.

\[
\text{Amount (mg) of swertiamarin (C}_{16}\text{H}_{22}\text{O}_{10}) = M_s \times A_f / A_s \times 5
\]

M_s: Amount (mg) of Swertiamarin RS, calculated on the basis of anhydrous.

**Operating conditions**—

**Detector**—An ultraviolet absorption photometer (wavelength: 238 nm).

**Column**—A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

**Column temperature**—A constant temperature of about 35°C.

**Mobile phase**—A mixture of water and acetonitrile (91:9).

**Flow rate**—Adjust the flow rate so that the retention time of swertiamarin is about 12 minutes.

**System suitability**—

System performance: Dissolve 1 mg each of Swertiamarin RS and theophylline in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, theophylline and swertiamarin are eluted in this order with the resolution of these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of swertiamarin is not more than 1.5%.

**Containers and storage** Containers—Well-closed containers.

**Swertia and Sodium Bicarbonate Powder**

Prepare as directed under Powders, with the above ingredients.

**Method of preparation**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powdered Swertia Herb</td>
<td>30</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>700</td>
</tr>
<tr>
<td>Starch, Lactose Hydrate</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Powders, with the above ingredients.

**Description** Swertia and Sodium Bicarbonate Powder occurs as a light grayish yellow powder, having a bitter taste.

**Identification** (1) To 10 g of Swertia and Sodium Bicarbonate Powder add 10 mL of ethanol (95), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Swertiamarin RS in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-
layer Chromatography <2.05>. Spot 30 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Identification under Powdered Swertia Herb.

(2) To 0.5 g of Swertia and Sodium Bicarbonate Powder add 10 mL of water. After stirring, centrifuge the mixture with 500 revolutions per minute. Use 5 mL of acetone, shake for 10 minutes, filter, and use this solution as the standard solution. Perform the test with 10 mL of the internal standard solution, add methanol to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg, about 20 mg and about 20 mg of bufalin for assay, cinobufagin for assay and resibufogenin for assay, respectively, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 25 mL, and use this solution as the sample solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.05> according to the following conditions. Calculate the ratios, \( Q_{TR} \) and \( Q_{SR} \), of the peak area of bufalin, \( Q_{TC} \) and \( Q_{SC} \), of the peak area of cinobufagin, and \( Q_{TR} \) and \( Q_{SR} \), of the peak area of resibufogenin, respectively, to that of the internal standard, and designate the total amount as an amount of bufoteroid.

\[
\begin{align*}
\text{Amount (mg) of bufalin} &= M_{SB} \times \frac{Q_{TR}}{Q_{SB}} \\
\text{Amount (mg) of cinobufagin} &= M_{SC} \times \frac{Q_{TC}}{Q_{SC}} \\
\text{Amount (mg) of resibufogenin} &= M_{SR} \times \frac{Q_{TR}}{Q_{SR}} \\
M_{SB}: \text{Amount (mg) of bufalin for assay} \\
M_{SC}: \text{Amount (mg) of cinobufagin for assay} \\
M_{SR}: \text{Amount (mg) of resibufogenin for assay}
\end{align*}
\]

Internal standard solution—A solution of indomethacin in methanol (1 in 4000).

Operating conditions—
Detector: An ultraviolet spectrophotometer (wavelength: 300 nm).
Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (11:9).
Flow rate: Adjust the flow rate so that the retention time of the internal standard is 16 to 19 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column giving elution of bufalin, cinobufagin, resibufogenin and the internal standard in this order, and clearly dividing each peak.

Containers and storage Containers—Well-closed containers.

**Toad Venom**

*Bufo venenum* |

センソ

Toad Venom is the venomous secretion of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider (*Bufoidae*).

When dried, it contains not less than 5.8% of bufotox.

**Description** A round disk with slightly dented bottom and protuberant surface, about 8 cm in diameter, about 1.5 cm in thickness, the mass of one disk being about 80 to 90 g; or a round disk with almost flattened surfaces on both sides, about 3 cm in diameter, and about 0.5 cm in thickness, the mass of one disk being about 8 g; externally red-brown to blackish brown, somewhat lustrous, approximately uniform and horny, hard in texture, and difficult to break; fractured surface nearly flat, and edges of broken pieces red-brown and translucent.

Odorless; taste, bitter and irritating, followed a little later by a lasting sensation of numbness.

**Identification** To 1 g of pulverized Toad Venom add 10 mL of acetone, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of resibufogenin for thin-layer chromatography in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 mL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of cyclohexane and acetone (3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of several spots obtained from the sample solution has the same color tone and the same RF value with the blue-green spot obtained from the standard solution.

**Total ash** <5.01> Not more than 5.0%.

**Acid-insoluble ash** <5.01> Not more than 2.0%.

**Assay** Weigh accurately about 0.5 g of pulverized Toad Venom, previously dried in a desiccator (silica gel) for 24 hours, add 50 mL of methanol, heat under a reflux condenser on a water bath for 1 hour, cool, and filter. Wash the residue with 30 mL of methanol, and combine the washing and filtrate. To this solution add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg, about 20 mg and about 20 mg of bufalin for assay, cinobufagin for assay and resibufogenin for assay, respectively, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, proceed in the same manner as the sample solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.05> according to the following conditions. Calculate the ratios, \( Q_{TR} \) and \( Q_{SR} \), of the peak area of bufalin, \( Q_{TC} \) and \( Q_{SC} \), of the peak area of cinobufagin, and \( Q_{TR} \) and \( Q_{SR} \), of the peak area of resibufogenin, respectively, to that of the internal standard, and designate the total amount as an amount of bufoteroid.
Containers and storage

Containers—Well-closed containers.

Identification (1) To 1 g of Powdered Tragacanth add 50 mL of water: a nearly uniform, slightly turbid mucilage is formed.

(2) To pulverized Tragacanth add dilute iodine TS, and examine the mixture microscopically <5.01>: a few blue-colored starch grains are observable.

Purity Karaya gum—Boil 1 g of Powdered Tragacanth with 20 mL of water until a mucilage is formed, add 5 mL of hydrochloric acid, and again boil the mixture for 5 minutes: no light red color develops.

Total ash <5.01> Not more than 4.0%.

Containers and storage Containers—Tight containers.

**Tragacanth**

*Tragacantha*

トラガント

Tragacanth is the exudation obtained from the trunks of *Astragalus gummifer* Labillardiére or other species of the same genus (*Leguminosae*).

Description Tragacanth occurs as curved, flattened or lamellate fragments, 0.5 – 3 mm in thickness. It is white to light yellow in color, translucent, and horny in texture. It is easily broken, and swells in water.

Odorless; tasteless and mucilaginous.

Identification (1) To 1 g of powdered Tragacanth add 50 mL of water: a nearly uniform, slightly turbid mucilage is formed.

(2) To pulverized Tragacanth add dilute iodine TS, and examine the mixture microscopically <5.01>: a few blue-colored starch grains are observable.

Purity Karaya gum—Boil 1 g of Powdered Tragacanth with 20 mL of water until a mucilage is formed, add 5 mL of hydrochloric acid, and again boil the mixture for 5 minutes: no light red color develops.

Total ash <5.01> Not more than 4.0%.

Containers and storage Containers—Well-closed containers.

**Powdered Tragacanth**

*Tragacantha Pulverata*

トラガント末

Powdered Tragacanth is the powder of Tragacanth.

Description Powdered Tragacanth occurs as a white to yellowish white powder. It is odorless, tasteless and mucilaginous.

Under a microscope <5.01>, it, immersed in olive oil or liquid paraffin, reveals numerous angular fragments with a small amount of the circular or irregular lamellae or of starch grains. Starch grains are spherical to elliptical, mostly simple and occasionally 2- to 4-compound grains, simple starch grains. Starch grains are spherical to elliptical, mostly simple and occasionally 2- to 4-compound grains, simple starch grains.

Loss on drying <5.01> Not more than 11.0% (6 hours).

Total ash <5.01> Not more than 13.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 8.5%.

Containers and storage Containers—Well-closed containers.

**Tribulus Fruit**

*Tribułu Fructus*

シツリシ

Tribulus Fruit is the fruit of *Tribulus terrestris* Linné (*Zygophyllaceae*).

Description Pentagonal star shaped fruit, composed of five mericarps, 7 – 12 mm in diameter, often each mericarp separated; externally grayish green to grayish brown; a pair of longer and shorter spines on surface of each mericarp, the longer spine 3 – 7 mm in length, the shorter one 2 – 5 mm in length, numerous small processes on midrib; pericarp hard in texture, cut surface light yellow; each mericarp contains 1 – 3 seeds.

Almost odorless; taste, mild at first, followed by bitterness.

Under a microscope <5.01>, a transverse section reveals epicarp composed of a single-layered epidermis; mesocarp composed of parenchyma and sclerenchyma layer; endocarp composed of several-layered fiber cells; a single-layer of cell between mesocarp and endocarp contain solitary crystals of calcium oxalate; cotyledons of seed contain oil drops and aleurone grains, and occasionally starch grains.

Identification To 2 g of pulverized Tribulus Fruit add 5 mL of methanol, shake for 10 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.01>. Spot 10 µL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and water (40:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): a bluish white fluorescent spot appears at an Rf value of about 0.4.

Purity (1) Peduncle—When perform the test of foreign matter <5.01>, the amount of peduncle contained in Tribulus Fruit does not exceed 4.0%.

(2) Foreign matters <5.01>—Not more than 1.0% of foreign matters other than peduncle.

Loss on drying <5.01> Not more than 11.0% (6 hours).

Total ash <5.01> Not more than 13.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 8.5%.

Containers and storage Containers—Well-closed containers.
Trichosanthes Root

**Trichosanthis Radix**

カロコン

Trichosanthes Root is the root of *Trichosanthes kirilowii* Maximowicz, *Trichosanthes kirilowii* Maximowicz var. *Japonicum* Kitamura or *Trichosanthes bracteata* Voigt (*Cucurbitaceae*), from which the cortical layer has been removed.

**Description** Irregular cylindrical root 5–10 cm in length, 3–5 cm in diameter, often cut lengthwise; externally light yellowish white, and with irregular pattern of vascular bundles appearing as brownish yellow lines; fractured surface somewhat fibrous and light yellow in color; under a magnifying glass, the transverse section reveals wide medullary rays and brownish yellow spots or small holes formed by vessels.

Odorless; taste, slightly bitter.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Trichosanthes Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Trichosanthes Root according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 4.0%.

**Containers and storage** Containers—Well-closed containers.

Turmeric

**Curcumae Rhizoma**

ウコン

Turmeric is the rhizome of *Curcuma longa* Linné (*Zingiberaceae*) with or without cork layers, usually with the application of blanching.

It contains not less than 1.0% and not more than 5.0% of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), calculated on the basis of dried material.

**Description** Turmeric is a main rhizome or a lateral rhizome; main rhizome, nearly ovoid, about 3 cm in diameter, about 4 cm in length; lateral rhizome, cylindrical, with round tips, curved, about 1 cm in diameter, 2–6 cm in length; both main and lateral rhizomes with cyclic nodes; rhizome with cork layer, yellow-brown, lustrous; rhizome without cork layer, dark yellow-red, with yellow-red powders on surface; hard in texture, not easily broken; transversely cut surface yellow-brown to red-brown, lustrous like wax.

Odor, characteristic; taste, slightly bitter and stimulant, it colors a saliva yellow on chewing.

Under a microscope <5.01>, a transverse section reveals the outermost layer to be composed of a cork layer 4–10 cells thick; sometimes a cork layer partly remains; cortex and stele, divided by a single-layered endodermis, composed of parenchyma, vascular bundles scattered; oil cells scattered in parenchyma; parenchymatous cells contain yellow substances, sandy and solitary crystals of calcium oxalate, and gelatinized starch.

**Identification (1)** To 0.5 g of pulverized Turmeric, add 20 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.01>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100:70:30:1) to a distance about 10 cm, and air-dry the plate: a yellow spot appears at *Rf* value of about 0.4.

(2) To 0.2 g of pulverized Turmeric, add 25 mL of a mixture of methanol and acetic acid (100:99:1), centrifuge after shaking for 20 minutes. Perform the test with the supernatant liquid as directed in the Assay, and determine the peak areas of curcumin, demethoxycurcumin and bisdemethoxycurcumin: the peak area of curcumin is larger than the peak area of demethoxycurcumin and is larger than 0.69 times the peak area of bisdemethoxycurcumin.

**Purity (1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Turmeric according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Turmeric according to Method 4, and perform the test (not more than 5 ppm).

**Loss on drying** <5.01> Not more than 17.0% (6 hours).

Total ash <5.01> Not more than 7.5%.

**Acid-insoluble ash** <5.01> Not more than 1.0%.

**Extract content** <5.01> Not less than 9.0% (dilute ethanol-soluble extract).

**Assay** Weigh accurately about 0.2 g of pulverized Turmeric, add 25 mL of a mixture of methanol and acetic acid (100:99:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 25 mL of a mixture of methanol and acetic acid (100:99:1), and proceed in the same manner as described above. Combine all the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of curcumin for assay, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as described under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, *A*<sub>TC</sub>, *A*<sub>TDP</sub> and *A*<sub>TD</sub> of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the sample solution as well as the peak area *A*<sub>5</sub> of curcumin in the standard solution.

\[
\text{Amount (mg) of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin)} = M_S \times \left( A_{TC} + A_{TDP} + A_{TD} \times 0.69 \right)/A_S \times 1/5
\]

*M*<sub>S</sub>: Amount (mg) of curcumin for assay
Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (56:43:1).

Flow rate: 1.0 mL per minute (the retention time of curcumin is about 11 minutes).

System suitability—

System performance: Dissolve 1 mg each of curcumin for assay, demethoxycurcumin and bisdemethoxycurcumin in methanol to make 5 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, bisdemethoxycurcumin, demethoxycurcumin and curcumin are eluted in this order with the resolution among these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of curcumin is not more than 1.5%.

Containers and storage—Containers—Well-closed containers.

Powdered Turmeric

Curcumae Rhizoma Purveratum

ウコン末

Powdered Turmeric is the powder of Turmeric.

It contains not less than 1.0% and not more than 5.0% of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), calculated on the basis of dried material.

Description—Powdered Turmeric occurs as a yellow-brown to dark yellow-brown powder. It has a characteristic odor and a bitter, stimulant taste, and colors the saliva yellow on chewing.

Under a microscope <5.01>, all elements are yellow in color; it reveals parenchymatous cells containing mainly masses of gelatinized starch or yellow substances, also fragments of scalariform vessels; fragments of cork layers, epidermis, thick-walled xylem parenchymatous cells, and non-glandular hairs are occasionally observed.

Identification—(1) To 0.5 g of Powdered Turmeric, add 20 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (70:30:1) to a distance of about 10 cm, and air-dry the plate: a yellow spot appears at Rf value of about 0.4.

(2) To 0.2 g of Powdered Turmeric, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), centrifuge after shaking for 20 minutes. Perform the test with the supernatant liquid as directed in the Assay, and determine the peak areas of curcumin, demethoxycurcumin and bisdemethoxycurcumin: the peak area of curcumin is larger than the peak area of demethoxycurcumin and is larger than 0.69 times the peak area of bisdemethoxycurcumin.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Turmeric according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 0.40 g of Powdered Turmeric according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <5.01>—Not more than 17.0% (6 hours).

Total ash <5.01>—Not more than 7.5%.

Acid-insoluble ash <5.01>—Not more than 1.0%.

Extract content <5.01>—Dilute ethanol-soluble extract: not less than 9.0%.

Assay—Weigh accurately about 0.2 g of Powdered Turmeric, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), and proceed in the same manner as described above. Combine all the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of curcumin for assay, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as described under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A1C, A1D, A1B of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the sample solution as well as the peak area A5 of curcumin in the standard solution.

Amount (mg) of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) = M5 × (A1C + A1D + A1B × 0.69)/A5 × 1/5

M5: Amount (mg) of curcumin for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (56:43:1).

Flow rate: 1.0 mL per minute (the retention time of curcumin is about 11 minutes).

System suitability—

System performance: Dissolve 1 mg each of curcumin for assay, demethoxycurcumin and bisdemethoxycurcumin in methanol to make 5 mL. When the procedure is run with 10 μL of this solution under the above operating conditions,
Uncaria Hook

Uncariae Uncis Cum Ramulus

Odorless and practically tasteless.

Uncaria Hook is, hook or the hook-bearing stem, of Uncaria rhynchophylla Miquel, Uncaria sinensis Haviland or Uncaria macrophylla Wallich (Rubiacae).

Uncaria Hook contains not less than 0.03% of total alkaloids (rhynchophylline and hirsutine), calculated on the dried basis.

Description Uncaria Hook is uncinate hook or short stem with opposite or single hook; the hook, 1 - 4 cm in length, curved and acuminate; externally red-brown to dark brown or yellow-brown, some one with hairs, the transverse section oblong to elliptical, light brown; stem thin and prismatic square to cylindrical, 2 - 5 mm in diameter, externally, red-brown to dark brown or yellow-brown; the transverse section, square to elliptical; the pith light brown, square to elliptical; hard in texture.

Odorless and practically tasteless.

Under a microscope <5.01>, a transverse section of the hook reveals vascular bundles in the cortex, unevenly distributed and arranged in a ring. Parenchyma cells in the secondary cortex containing sand crystals of calcium oxalate.

Identification To 1 g of pulverized Uncaria Hook add 20 mL of methanol, boil under a reflux condenser on a water bath for 5 minutes, and filter. Evaporate the filtrate to dryness, add 5 mL of dilute acetic acid to the residue, warm the mixture on a water bath for 1 minute, and filter after cooling. Spot 1 drop of the filtrate on a filter paper, air-dry, spray Dragendorff’s TS for spraying on it, and allow to stand: a yellow-red color develops.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 4.0%.

Extract content <5.01> Dilute ethanol-souble extract: not less than 7.5%.

Assay Weigh accurately about 0.2 g of medium powder of Uncaria Hook, transfer into a glass-stoppered centrifuge tube, add 30 mL of a mixture of methanol and dilute acetic acid (7:3), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add two 10-mL portions of a mixture of methanol and dilute acetic acid (7:3), proceed in the same manner, and combine all of the supernatant liquid. To the combined liquid add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this as the sample solution. Separately, weigh accurately about 5 mg of rhynchophylline for assay, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL. Pipet 1 mL of this solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 10 mL, and use this solution as the standard solution (1). Separately, dissolve 1 mg of hirsutine in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{Tb}$ and $A_{Ta}$, of rhynchophylline and hirsutine obtained from the sample solution, and the peak area, $A_{S}$, of rhynchophylline from the standard solution (1).

System suitability—

System performance: Dissolve 5 mg of rhynchophylline for assay in 100 mL of a mixture of methanol and dilute acetic acid (7:3). To 5 mL of this solution add 1 mL of ammonium solution (28), and reflux for 10 minutes or warm at about 50°C for 2 hours. After cooling, to 1 mL of the solution so obtained add a mixture of methanol and dilute acetic acid (7:3) to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the peak of rhynchophylline is about 17 minutes.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak areas of rhynchophylline is not less than 1.5%.
Zanthoxyli Fructus

Sanchoi

Zanthoxyllum Fruit is the pericarps of the ripe fruit of Zanthoxylum piperitum De Candolle (Rutaceae), from which the seeds separated from the pericarps have been mostly removed.

**Description** Capsules of 2 or 3 flattened spheroidal mericarps, which are dehiscent in 2 pieces about 5 mm in diameter; the outer surface of pericarp, dark yellow-red to dark red-brown, with numerous dented spots originated from oil sacs; the inner surface, light yellowish white.

Odor, characteristically aromatic; taste, acrid, which gives numbing sensation to the tongue.

Under a microscope less than 5.01, transverse section of Zanthoxyllum Fruit reveals the external epidermis and the adjoined unicellular layer containing red-brown tannin; the pericarp holds oil sacs being up to approximately 500 μm in diameter and sporadically vascular bundles consisting mainly of spiral vessels; the endocarp consists of stone cell layers; inner epidermal cells very small.

**Identification** To 0.5 g of pulverized Zanthoxyllum Fruit add 100 mL of diluted ethanol (7 in 10), stopper the vessel tightly, shake for 30 minutes, filter, and use this filtrate as the sample solution.

Perform the test with the sample solution as directed under Thin-layer Chromatography to 0.5 g. Spot 10 μL of the sample solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum): one spot showing a grayish red to red color at an Rf value of about 0.7 appears.

**Purity**

1. Seed—When perform the test of foreign matter less than 5.01, the amount of the seeds contained in Zanthoxyllum Fruit does not exceed 20.0%.

2. Peduncle and twig—The amount of the peduncles and twigs contained in Zanthoxyllum Fruit does not exceed 5.0%.

3. Foreign matter less than 5.01—The amount of foreign matter other than peduncles and twigs contained in Zanthoxyllum Fruit does not exceed 1.0%.

**Total ash** less than 5.01 Not more than 8.0%.

**Acid-insoluble ash** less than 5.01 Not more than 1.5%.

**Essential oil content** less than 5.01 Perform the test with 30.0 g of pulverized Zanthoxyllum Fruit: the volume of essential oil is not less than 1.0 mL.

**Containers and storage** Containers—Well-closed containers.

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**Powdered Zanthoxyllum Fruit**

Sanchoi末

Powdered Zanthoxyllum Fruit is the powder of Zanthoxyllum Fruit.

**Description** Powdered Zanthoxyllum Fruit occurs as a dark yellow-brown powder. It has a strong, characteristic aroma and an acrid taste leaving a sensation of numbness on the tongue.

Under a microscope less than 5.01, Powdered Zanthoxyllum Fruit reveals fragments of inner tissue of pericarp consisting of stone cells with membranes about 2.5 μm in thickness; fragments of spiral and annular vessels 10 to 15 μm in diameter; fragments of oil sacs containing essential oil or resin; fragments of epidermal cells, polygonal in surface view, containing tannin; numerous oil drops; masses of tannin, colored red by adding vanillin-hydrochloric acid TS.

**Identification** To 0.5 g of Powdered Zanthoxyllum Fruit add 100 mL of diluted ethanol (7 in 10), stopper the vessel tightly, shake for 30 minutes, filter, and perform the test with the filtrate as the sample solution as directed under Thin-layer Chromatography less than 2.03. Spot 10 μL of the sample solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one spot showing a grayish red to red color at the Rf value of about 0.7 appears.

**Total ash** less than 5.01 Not more than 8.0%.

**Acid-insoluble ash** less than 5.01 Not more than 1.5%.

**Essential oil content** less than 5.01 Perform the test with 30.0 g of Powdered Zanthoxyllum Fruit: the volume of essential oil is not less than 0.8 mL.

**Containers and storage** Containers—Tight containers.

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**Zedoary**

Zedoariae Rhizoma

ガジュツ

Zedoary is the rhizome of Curcuma zedoaria Roscoe (Zingiberaceae), usually after being passed through hot water.

**Description** Nearly ovoid rhizome, 4 – 6 cm in length, 2.5 – 4 cm in diameter; externally grayish yellow-brown to grayish brown; nodes protruded as rings; internode of 0.5 – 0.8 cm, with thin, longitudinal wrinkles, scars of removed roots, and small protrusions of branched rhizomes; under a magnifying glass, external surface covered with coarse hairs; horny in texture and difficult to cut; transverse section grayish brown in color; cortex 2 – 5 mm in thickness, stele thick, a light
grayish brown ring separating them.

Odor, characteristic; taste, pungent, bitter and cooling.

**Purity** (1) Heavy metals $\leq 1.07$—Proceed with 1.0 g of pulverized Zedoary according to Method 3, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic $\leq 1.11$—Prepare the test solution with 0.40 g of pulverized Zedoary according to Method 4, and perform the test (not more than 5 ppm).

**Total ash** $\leq 5.01$—Not more than 7.0%.

**Essential oil content** $\leq 5.01$—Perform the test with 50.0 g of pulverized Zedoary, provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.5 mL.

**Containers and storage** Containers—Well-closed containers.
The infrared reference spectra presented here were obtained by the use of Fourier-transform infrared spectrophotometers under the conditions specified in the individual monographs. The horizontal axis indicates the wave numbers (cm$^{-1}$) and the vertical axis indicates the transmittance ($\%$). A spectrum of polystyrene obtained in the same manner is also presented for reference.
Acetohexamide

Acetylcholine Chloride for Injection

Acetylcysteine
Alminoprofen

Alprenolol Hydrochloride

Alprostadil
**JP XVI**

Infrared Reference Spectra

**Amantadine Hydrochloride**

**Ambenonium Chloride**

**Amidotrizoic Acid**
Amikacin Sulfate

Amiodarone Hydrochloride

Amlexanox
Amoxicillin Hydrate

Anhydrous Ampicillin

Ampicillin Hydrate
Infrared Reference Spectra

Benidipine Hydrochloride

Benserazide Hydrochloride

Benzbromarone
Beraprost Sodium

Berberine Chloride Hydrate

Berberine Tannate
Bufetolol Hydrochloride

Buformin Hydrochloride

Bumetanide
Carbazochrome Sodium Sulfonate Hydrate

Carbidopa Hydrate

l-Carbocisteine
Cefadroxil

Cefalexin

Cefalotin Sodium
Cefotetan

Cefotiam Hydrochloride

Cefpodoxime Proxetil


Clindamycin Hydrochloride

Clindamycin Phosphate

Clinofibrate
JP XVI

Infrared Reference Spectra

Cloxacillin Sodium Hydrate

Cocaine Hydrochloride

Codeine Phosphate Hydrate
Deferoxamine Mesilate

Demethylchlortetracycline Hydrochloride

Dexamethasone
Dextromethorphan Hydrobromide Hydrate

Diazepam

Dibucaine Hydrochloride
Dihydroergotamine Mesilate

Dihydroergotoxine Mesilate

Dilazep Hydrochloride Hydrate
Dinoprost

Diphenhydramine Hydrochloride

Dipyridamole
Dobutamine Hydrochloride

Domperidone

Donepezil Hydrochloride
JP XVI

Infrared Reference Spectra

Enalapril Maleate

Enflurane

Enoxacin Hydrate
JP XVI

Anhydrous Ethanol

Ethenzamide

Ethionamide
Ethyl L-Cysteine Hydrochloride

Ethyl Icosapentate

Ethylmorphine Hydrochloride Hydrate
Ethyl Parahydroxybenzoate

Etidronate Disodium

Etilefrine Hydrochloride
Flopropione

Fluconazole

Fludiazepam
Flurazepam Hydrochloride

Flurbiprofen

Flutamide
1864

Infrared Reference Spectra

L-Glutamic Acid

L-Glutamine

Glutathione
Halothane

Haloxazolam

L-Histidine
1-Histidine Hydrochloride Hydrate

Homochlorcyclizine Hydrochloride

Hydralazine Hydrochloride
JP XVI

Infrared Reference Spectra

1871

Hydrocortisone Sodium Succinate

Hydrocortisone Succinate

Hydrocotarnine Hydrochloride Hydrate
Hymecromone

Hypromellose Phthalate (200731)

Hypromellose Phthalate (220824)
Imidapril Hydrochloride

Imipenem Hydrate

Indapamide
Isosorbide

Isoxsuprine Hydrochloride

Itraconazole
Ketamine Hydrochloride

Ketoconazole

Ketoprofen
JP XVI

Latamoxef Sodium

Lenampicillin Hydrochloride

L-Leucine
Losartan Potassium

Loxoprofen Sodium Hydrate

l-Lysine Acetate
JP XVI

Infrared Reference Spectra

1-Lysine Hydrochloride

Manidipine Hydrochloride

p-Mannitol
Mosapride Citrate Hydrate

Nabumetone

Nafamostat Mesilate
Nalidixic Acid

Naloxone Hydrochloride

Naproxen
Nicorandil

Nifedipine

Nilvadipine
Noscapine

Ofloxacin

Omeprazole
Pindolol

Pioglitazone Hydrochloride

Pipemidic Acid Hydrate
Piperacillin Hydrate

Piperacillin Sodium

Piperazine Adipate
JP XVI
Infrared Reference Spectra

Prednisolone Succinate

Probucol

Procainamide Hydrochloride
JP XVI

Infrared Reference Spectra

**Progesterone**

**Proglumide**

1-Proline
Infrared Reference Spectra

Promethazine Hydrochloride

Propafenone Hydrochloride

Propiverine Hydrochloride
Quinine Ethyl Carbonate

Quinine Sulfate Hydrate

Rabeprazole Sodium
Infrared Reference Spectra

**Rifampicin**

**Risperidone**

**Ritodrine Hydrochloride**
Siccanin

Simvastatin

Sodium Fusidate
Sodium Picosulfate Hydrate

Sodium Polystyrene Sulfonate

Sodium Prasterone Sulfate Hydrate
Sodium Starch Glycolate, Type B

Sodium Valproate

Spiramycin Acetate
Sulfamonomethoxine Hydrate

Sulindac

Sulpiride
Testosterone Propionate

Tetracycline Hydrochloride

Theophylline
Tocopherol Nicotinate

Todralazine Hydrochloride Hydrate

Tofisopam
Tolazamide

Tolnaftate

Tosufloxacin Tosilate Hydrate
JP XVI
Infrared Reference Spectra

Vinblastine Sulfate

Vincristine Sulfate

Voglibose
ULTRAVIOLET-VISIBLE REFERENCE SPECTRA

The ultraviolet-visible reference spectra presented here were obtained by the use of double-beam spectrophotometers with sample solutions prepared as specified in the individual monographs. The horizontal axis indicates the wavelength (nm) and the vertical axis indicates the absorbance.
Acetohexamide 2

Aciclovir

Aclarubicin Hydrochloride
Afloqualone

Alimemazine Tartrate

Allopurinol
Arotinolol Hydrochloride

Aspoxicillin Hydrate

Atenolol
Atorvastatin Calcium Hydrate

Azathioprine

Azelastine Hydrochloride
JP XVI

Ultraviolet-visible Reference Spectra

1979

Benzylpenicillin Benzathine Hydrate

Benzylpenicillin Potassium

Beraprost Sodium
Bleomycin Hydrochloride

Bleomycin Sulfate

Bromazepam
Bromhexine Hydrochloride

Bromocriptine Mesilate

Bucumolol Hydrochloride
Bufetolol Hydrochloride

Buformin Hydrochloride

Bumetanide
Bupranolol Hydrochloride

Buprenorphine Hydrochloride

Butenafine Hydrochloride
Butropium Bromide 1

Butropium Bromide 2

Cadralazine
Carbidopa Hydrate

Carmofur

Carteolol Hydrochloride
Cefadroxil

Cefalexin

Cefalotin Sodium
Ultraviolet-visible Reference Spectra

Cefatrizine Propylene Glycolate

Cefazolin Sodium

Cefazolin Sodium Hydrate
Ultraviolet-visible Reference Spectra

Cefotaxime Sodium

Cefotetan

Cefotiam Hexetil Hydrochloride
JP XVI

Ultraviolet-visible Reference Spectra 2001

Ceferam Pivoxil

Ceftibuten Hydrate

Ceftizoxime Sodium
Ceftriaxone Sodium Hydrate

Cefuroxime Axetil

Cetirizine Hydrochloride
Cibenzoline Succinate

Cilostazol

Cinoxacin
Clocapramine Hydrochloride Hydrate

Clofedanol Hydrochloride

Clofibrate 1
Clofibrate 2

Clomifene Citrate

Clomipramine Hydrochloride
Colchicine

Cortisone Acetate

Croconazole Hydrochloride
Demethylchortetracycline Hydrochloride

Dexamethasone

Dextromethorphan Hydrobromide Hydrate
Diazepam

Dibucaine Hydrochloride

Diclofenamide
JP XVI

Ultraviolet-visible Reference Spectra 2021

Dimemorfan Phosphate

Dimorpholamine

Dinoprost
Diphenhydramine Hydrochloride

Dipyridamole

Disopyramide
**Elcatonin**

**Emorfazone**

**Enoxacin Hydrate**
Ethionamide

Ethosuximide

Ethyl Icosapentate
Felbinac

Fenbufen

Fentanyl Citrate
Fexofenadine Hydrochloride

Flavoxate Hydrochloride

Flecainide Acetate
Fludrocortisone Acetate

Flunitrazepam

Fluocinonide
Fluorometholone

Fluorouracil

Fluoxymesterone
Fluphenazine Enanthate

Flurazepam 1

Flurazepam 2
Formoterol Fumarate Hydrate

Furosemide

Gabexate Mesilate
JP XVI

Ultraviolet-visible Reference Spectra

$\beta$-Galactosidase (Aspergillus)

Glibenclamide

Gliclazide
Hydroxocobalamin Acetate

Hydroxyzine Hydrochloride

Hydroxyzine Pamoate
Indenolol Hydrochloride 1

Indenolol Hydrochloride 2

Indigocarmine
JP XVI

Ultraviolet-visible Reference Spectra

Ketoprofen

Ketotifen Fumarate

Kitasamycin
JP XVI

Ultraviolet-visible Reference Spectra

Limaprost Alfadex

Liothyronine Sodium

Lisinopril Hydrate
Lorazepam

Losartan Potassium

Loxoprofen Sodium Hydrate
Lysozyme Hydrochloride

Manidipine Hydrochloride

Maprotiline Hydrochloride
JP XVI

Ultraviolet-visible Reference Spectra

Methoxsalen

Methyldopa Hydrate

dl-Methylephedrine Hydrochloride
JP XVI

Ultraviolet-visible Reference Spectra

Methyltestosterone

Meticrane

Metildigoxin
Ultraviolet-visible Reference Spectra

**Metoclopramide**

**Metoprolol Tartrate**

**Metronidazole**
JP XVI

Ultraviolet-visible Reference Spectra

Metyrapone

Mexiletine Hydrochloride

Miconazole
JP XVI

Ultraviolet-visible Reference Spectra

Nabumetone

Nadolol

Nafamostat Mesilate
Nalidixic Acid

Naloxone Hydrochloride

Naproxen
Nateglinide

Neostigmine Methylsulfate

Nicardipine Hydrochloride
Nitrendipine

Nizatidine

Noradrenaline
Norfloxacin

Nortriptyline Hydrochloride

Noscapine
Phenethicillin Potassium

Phenobarbital

Phenolsulfonphthalein
Ultraviolet-visible Reference Spectra

Pimaricin

Pimozide

Pindolol
Pioglitazone Hydrochloride

Pipemidic Acid Hydrate

Pirarubicin
JP XVI

Ultraviolet-visible Reference Spectra

Procaterol Hydrochloride Hydrate

Progesterone

Promethazine Hydrochloride
Propafenone Hydrochloride

Propiverine Hydrochloride

Propranolol Hydrochloride
Pyridoxine Hydrochloride

Pyrrolnitrin

Quinapril Hydrochloride
Ranitidine Hydrochloride

Rebamipide

Reserpine
Riboflavin

Riboflavin Butyrate

Riboflavin Sodium Phosphate
Rokitamycin

Roxatidine Acetate Hydrochloride

Salazosulfapyridine
Salbutamol Sulfate

Salicylic Acid

Santonin
Simvastatin

Sodium Cromoglicate

Sodium Picosulfate Hydrate
Ultraviolet-visible Reference Spectra

Sulindac

Sulpiride

Sultiame
Theophylline

Thiamine Chloride Hydrochloride

Thiamylal Sodium
Triamterene

Trichlormethiazide

Trimebutine Maleate
JP XVI

Ultraviolet-visible Reference Spectra

Trimetazidine Hydrochloride

Trimetoquinol Hydrochloride Hydrate

Trozipide
Verapamil Hydrochloride

Vinblastine Sulfate

Vincristine Sulfate
Warfarin Potassium

Zaltoprofen

Zolpidem Tartrate
GENERAL INFORMATION

G1 Physics and Chemistry

Guideline for Residual Solvents and Models for the Residual Solvents Test

1. Guideline for Residual Solvents
   Since the acceptable limits of residual solvents recommended in the Guideline were estimated to keep the safety of patients, the levels of residual solvents in pharmaceuticals must not exceed the limits, except for in a special case. Pharmaceutical manufacturers should assure the quality of their products by establishing their own specification limits or manufacturing process control limits for residual solvents present in their products in consideration of the limits recommended in the Guideline and the observed values in their products, and by performing the test with the products according to the Residual Solvents Test.

2. Residual Solvents Test
   Generally, the test is performed by using gas chromatography <2.02> as directed in the Residual Solvents Test <2.46>. If only the class 3 solvents with low toxic potential to man are present in the products, Loss on Drying Test <2.41> can be applied in place of gas chromatography, in which case the limit value of residual solvents is not more than 0.5%.
   The test may also be performed according to the EP method (2.4.24 Identification and control of residual solvents) or the USP method (<467> Residual Solvents). Even in this case, description should be in the JP style and the system suitability test should be performed according to the JP rule.

3. Models for the operating conditions and system suitability of gas chromatography for residual solvents test
   The following are typical examples of the operating conditions of gas chromatography for residual solvents test, described in the EP and the USP, but these do not necessarily imply that other suitable operating conditions can not be used. In the operating conditions, generally, items required for the test such as detector, column, column temperature, injection port temperature, detector temperature, carrier gas, flow rate, and time span of measurement should be specified, and in the system suitability, items such as test for required detectability, system performance, and system repeatability should be specified. The following are several models for the operating conditions and the system suitability:

3.1. Models for operating conditions for a head-space sample injection device (Models described in the EP and the USP)
   (i) Operating conditions (1) for the head-space sample injection device—
   Equilibration temperature—A constant temperature of about 80°C for inside vial
   Equilibration time for inside vial—60 minutes
   Transfer-line temperature—A constant temperature of about 85°C
   Carrier gas—Nitrogen
   Pressurisation time—30 seconds
   Injection volume of sample—1.0 mL
   (ii) Operating conditions (2) for the head-space sample injection device—
   Equilibration temperature—A constant temperature of about 105°C for inside vial
   Equilibration time for inside vial—45 minutes
   Transfer-line temperature—A constant temperature of about 110°C
   Carrier gas—Nitrogen
   Pressurisation time—30 seconds
   Injection volume of sample—1.0 mL
   (iii) Operating conditions (3) for the head-space sample injection device—
   Equilibration temperature—A constant temperature of about 80°C for inside vial
   Equilibration time for inside vial—45 minutes
   Transfer-line temperature—A constant temperature of about 105°C
   Carrier gas—Nitrogen
   Pressurisation time—30 seconds
   Injection volume of sample—1.0 mL

3.2. Models for operating conditions and system suitability of gas chromatography
   (I) Test conditions (I) (A model described under Procedure A in the EP and the USP)
   Operating conditions—
   Detector: Hydrogen flame-ionization detector.
   Column: Coat the inside wall of a fused silica tube, 0.32 mm (or 0.53 mm) in inside diameter and 30 m in length, to 1.8 μm (or 3 μm) thickness with 6% cyanopropylphenylmethyl silicon polymer for gas chromatography. Use a guard column if necessary.
   Column temperature: Maintain at 40°C for 20 minutes, then increase to 240°C at 10°C per minute if necessary, and keep at 240°C for 20 minutes.
   Injection port temperature: A constant temperature of about 140°C.
   Detector temperature: A constant temperature of about 250°C.
   Carrier gas: Helium.
   Flow rate: 35 cm/second.
   Split ratio: 1:5.

System suitability—
System performance: When the procedure is run with the standard solution under the above operating conditions, the
resolution between the peaks is not less than 1.0. (Note: In the case that the number of substances to be tested is two or more.) System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of the substance to be tested is not more than 15%.

(ii) Test conditions (2) (A model described under Procedure B in the EP and the USP)

**Operating conditions—**

- **Detector:** Hydrogen flame-ionization detector.
- **Column:** Coat the inside wall of a fused silica tube, 0.32 mm (or 0.53 mm) in inside diameter and 30 m in length, to 0.25 μm thickness with polyethylene glycol 20M for gas chromatography. Use a guard column if necessary.
- **Column temperature:** Maintain at 50°C for 20 minutes, then increase to 165°C at 6°C per minute if necessary, and keep at 165°C for 20 minutes.
- **Injection port temperature:** A constant temperature of about 140°C.
- **Detector temperature:** A constant temperature of about 250°C.
- **Carrier gas:** Helium.
- **Flow rate:** 35 cm/second.
- **Split ratio:** 1:5.

**System suitability—**

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the peaks is not less than 1.0. (Note: In the case that the number of substances to be tested is two or more.) System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of the substance to be tested is not more than 15%.

### Inductively Coupled Plasma Atomic Emission Spectrometry

Inductively coupled plasma (ICP) atomic emission spectrometry is a method for qualitative and quantitative analysis for the element in the sample specimen, which is nebulized into argon plasma induced by radio frequency power, by measuring the wavelength and the intensity of the emission spectra generated by the target element, atomized and excited in the plasma. Argon gas plasma used in this method is characteristic of higher temperature of 6000 – 8000 K and electronic density of 10^13 cm⁻³.

When the high energy is externally given to an atom, the orbit transition of the most exterior electron is occurred and attained to the excited state. When the excited state of atom returns to the basic state, the energy obtained by the excitation is radiated as a light. The emitted light has an intrinsic wave number ν and wave length λ, attributed to the individual element. Assuming the Planck constant h and the light velocity c, the emitted light energy ΔE is expressed by the following formula.

\[ ΔE = hν = hc/λ \]

Since there are many of combinations of the orbit transition energy level of the most exterior electron and the emitted light energy, usually, there are plenty of spectral lines emitted from an element, summing up every strong and weak emitted lights. However, the number of emitted lights of being in the ultra-violet and visible region and having suitable detection sensitivity for qualitative and quantitative analysis are limited to a certain number. Since the atomic emission spectra give intrinsic wave number or wave length for the respective element, many of elements contained in a sample specimen can be identified by the spectroscopic analysis of the emitted lights. Furthermore, the quantitative analysis for an element can also be done by measuring the spectral intensity of the emitted light.

The characteristics of ICP emission spectrometry are summarized as follows;

1. (i) Microanalysis for plenty of elements is possible.
2. (ii) Analytical precision is high, since the stable plasma generation can be kept.
3. (iii) Linearity of the calibration curve is ensured in the wide range of 4 ~ 5 digit.
4. (iv) Multi-element simultaneous analysis is possible.
5. (v) Chemical interferences are almost negligible.

The most serious problem in the ICP emission spectrometry is that the spectral interferences are inevitable to this analysis, and disturb the analysis for the target element. It results from many of emitted spectral lines due to coexisting elements, because the atomization and excitation of elements are done in very high temperature of plasma. Thus it is the key technique to obtain an accurate analytical result how to reduce the spectral interferences and how to correct the in-
The present method is superior to analyze inorganic impurities and/or coexisting elements in drug substances and preparations specifically, and also to analyze metal residues in crude drugs and preparations. The qualitative and quantitative analysis of alkali, alkali earth, and heavy metals are possible, and also of many other elements which are required for suitable control for the safety assurance of drug medicines. Furthermore, since the simultaneous analysis for lots of elements are possible, this method can be used for the profile analysis of inorganic impurities such as metal elements for the quality control of drug substances.

In this General Information, a spectroscopic detection method of atomic emission spectra (AES) due to metal elements introduced into inductively coupled plasma (ICP), is described as ICP-AES. Separately, since ICP is not only a good excitation source, but also a good ionizing source, inductively coupled plasma-mass spectrometry (ICP-MS) is also possible by using ICP as an ionizing source.

1. Apparatus

1.1. Composition of the apparatus

The apparatus is composed of the excitation source unit, sample introduction unit, the emission unit, the spectroscopy unit, the detection unit, and the data processing unit.

The excitation source unit is composed of the electric source circuit, the control circuit to supply and to control electric energy, and the gas supplying part. The sample introduction unit is the part of introducing the sample into the emission unit and composed of a nebulizer and a spray chamber, etc.

The emission unit is a part where the target element in the sample is excited and emitted, and it is composed of the torch and the radio frequency induction coil. The torch usually consists of a threefold tube, in which the sample is introduced by the central tube. Argon is used for the gas to form plasma and also to carry the sample. There are two types of observation way of the light emitted, one is the radial viewing, and the other the axial viewing.

The spectroscopy unit is a part separating the emitted light into the individual spectral line and composed of convergent lens system and optical elements such as the diffraction grating, etc. There are two types of spectrometers, one is the wavelength scanning type of monochrometer, the other is the fixed wavelength type of polychrometer. For measuring spectral lines in the vacuum UV region, in which the wavelength is not more than 190 nm, the evacuation of the interior of the spectroscope or the substitution of air for argon or nitrogen gas are necessary.

Detection unit is a part converting the incident light intensity to the corresponding strength of the electric signal and composed of the detector and the signal processing system. Regarding the detector, either the photomultiplier or the charge transfer device (CTD) such as the charge coupled device, the charge injection device, and the photo diode array etc., is used.

Data processing unit is a part of processing the detected signal and the measurement result and of giving the calibration curve etc., which are given by the displaying apparatus or the printer, etc.

2. Pretreatment of sample

2.1. Dilution and/or dissolution method

Take a specified amount of sample in the respective monograph, then dilute by water or an appropriate solvent, or dissolve in water or a specified solvent to prepare the sample solution.

2.2. Dry ashing method

Applying the “Residue on Ignition Test”, take a specified amount of sample in the crucible, moisten it with a small amount of sulfuric acid, then heat gently to carbonize it thoroughly. After cooling, moisten the charred residue with a small amount of sulfuric acid, heat gently again, then ignite the residue to ash in the range of 400 ~ 600°C. Dissolve the ash by adding a small amount of nitric acid or hydrochloric acid to prepare the sample solution.

Separately, applying the 3rd Method in the “Heavy Metals Limit Test”, ignite the sample to ash without moistening it with sulfuric acid. In this case, since it is digested and incinerated in the open system, be careful to the vaporization loss of low boiling point elements such as mercury.

2.3. Acid digestion method

Take an amount of sample into the beaker or the flask as specified in the monograph, add nitric acid, sulfuric acid, or the mixture of these acids, then digest by heating. If it is necessary, an auxiliary oxidant such as hydrogen peroxide may be available. After the digestion is completed, add a small amount of nitric acid or hydrochloric acid to the residues, dissolve them by heating to prepare the sample solution.

Acid digestion can be done either in the open or in the closed system. Regarding the closed system, the heat digestion at high temperature under high pressure conditions might be applicable by using the pressure tolerant digestion vessel of polytetrafluoroethylene fitted with the outer tube of stainless steel. Where the heat digestion method is used in the
closed system, be careful enough to accidents such as the expansion and the liquid leak.

2.4. Microwave digestion method

Take an amount of sample into the pressure tolerable closed vessel as specified in the monograph, add an appropriate amount of nitric acid, then digest under heating conditions by using the microwave digestion apparatus. Since it is a digestion procedure under high temperature/high pressure conditions, the temperature and the pressure in the closed vessel should be appropriately controlled, in consideration of amounts of the acid and the sample.

The pretreatment method should be selected appropriately, corresponding to the attribute of the sample and the target element. Where the ignition or the digestion is done in the open system, be careful to the vaporization loss of the target element or the contamination from the operating environment. Furthermore, where the residues obtained by the ignition or the digestion are dissolved in acid solution and prepared to the sample solution, if necessary, the filtration with membrane filter (pore size: $1 \mu m$) may be useful.

3. Procedure

3.1. Performance test of the spectroscope

3.1.1. Wavelength calibration

The wavelength calibration should be suitably performed according to the indicated methods and procedures of the apparatus, since there is specific calibration method of the individual instrument. For example, some of calibration methods are available, such as a method of using standard solution containing several elements, spectral lines of mercury lamp, and emission lines of argon gas, etc.

3.1.2. Wavelength resolution

The wavelength resolution is usually specified by the half wavelength (nm) of the spectral lines for some representative elements. During the lower and the higher wavelength, following spectral lines of As (193.696 nm), Mn (257.610 nm), Cu (324.754 nm), and Ba (455.403 nm) are usually selected. The specification of the half wavelength for the individual element is responsible to the user, because the characteristics of the instrument and the spectroscope are intrinsic and different each other.

However, in the case of the simultaneous analysis type of instrument fitted with semiconductor detector, this specification is not always required.

3.2. Preparation of sample solution and standard solution

Sample of drug substances, preparations, and crude drugs are pretreated by using anyone of the method described in the above section (2. Pretreatment of sample) and prepared to the sample solution. Usually, the sample solution is prepared to as a dilute nitric acid solution, while hydrochloric acid is also available in place of nitric acid.

Where the reference standard solutions are specified in the Japanese Pharmacopoeia (JP) and/or the Japanese Industrial Standard (JIS), the standard solutions are prepared by diluting those official standards to the definite concentrations with the water for ICP analysis etc. When those reference standard solutions are not specified both in JP and JIS, the reference substances certified and provided by the official organization or the academic association etc., are used for the preparation of the standard solutions. Where the reference standard solutions or the reference substances are not given by the above mentioned official or semi-official organizations, either the pure metals or the compounds containing the target element of high purity (not less than 99.99%) can be used for preparing the calibration standards, in which one or more target elements are included. In case of preparing multi-component standard solution, the reagent solutions and the element combinations are required not to form precipitates, and not to give spectral interferences with the analytical line of the target element.

3.3. Optimization of the operating conditions

The operating conditions are usually as follows.

After the plasma is attained to the stable generation by the warm-up driving for 15 ~ 30 minutes, the optimum operating conditions are adjusted for the intended use. Radio frequency power output of 0.8 ~ 1.4 kW, argon gas flow rate for the cooling gas of $10 \sim 18 L/min$, for auxiliary gas of $0 \sim 2 L/min$, and for carrier gas of $0.5 \sim 2 L/min$, sample introduction of $0.5 \sim 2 mL/min$ are usual operating conditions. Where the radial viewing method is applied, the observation height is adjusted to $10 \sim 25 mm$ above the induction coil, and the axial viewing is applied, the optical axis is adjusted to obtain the greatest emission intensity. The integration time is set appropriately between 1 and several decades of second, in consideration of the stability of the emission intensity.

As the analytical line of the individual target element, the representative emission lines are shown in Table 1. However, where the concentration of the target element is markedly high, it is recommended either to dilute the sample solution or to select an appropriate another spectral line, based on the expected concentration of the element. Furthermore, when the spectral interferences due to coexisting elements are occurred, another spectral line should be selected so as to be free from the interference.

Where this test method is specified in the respective monograph, the necessary operating conditions such as the analytical line (nm), the radio frequency power unit (kW), argon gas flow rate (L/min) etc., should be specified for the intended use. However, all the specified conditions except for the analytical line are the referring ones, and the optimization of those conditions are expected to be found according to the individual instrument and the observation way etc.

3.4. Water and reagents

Water and reagents for this test are as follows.

(i) As for water, use water for ICP analysis described below.

Water for ICP analysis: The electric conductivity should be below $1 \mu S \cdot cm^{-1}$ (25°C). Further it should be confirmed that the contaminated impurities do not interfere with the emission of the target element.

(ii) The quality of reagents should be suitable, as it does not contain the interfering substances of the test.

(iii) As for argon gas, use that specified below;

Argon gas: The purity should be not less than 99.99 vol%, as specified in JIS K 1105. Either the liquefied argon or pressurized argon gas can be used.

3.5. Procedure

After confirmation of the normal action of the instrumental parts usually switched on electrically, the electric source of the instrument body and the surrounding equipments should be switched on. After setting argon gas flow rate to the decided value, switch on the radio frequency source, and generate argon plasma. After confirming the stable plasma is given, the sample solution and the standard solution prepared as specified in the individual monograph, are intro-
standard solution is 10

Assuming that the concentration of the target element in the solution, the standard solution of the target element can be prepared, as mentioned in the section 3.2. Next, diluting this standard solution to 1/10 times of the concentration, this is produced into the plasma, and the emission intensity is measured at the indicated analytical line. Where the qualitative test for the confirmation or the identification is performed, the measurement wavelength range of the emission spectra should be sufficiently wide, in which all the specified analytical lines are included.

Where the emission lines in vacuum UV region are measured by using the vacuum type spectroscope, the air around the optical axis between the emission part and the spectroscope part should be substituted for argon or nitrogen gas.

4. System suitability

When this test is applied to the limit test or the quantitative test of metal elements etc., it is required to confirm previously whether the performance ability of the apparatus is adequate or not by the system suitability test specified below. However, in the assay test, the test for 4.1 Confirmation of detection and evaluation of linearity, is not required.

4.1. Confirmation of detection and evaluation of linearity

In the limit test of metal elements etc., where the pretreatment method of the sample specimen is specified in the respective monograph, it can be speculated what is the concentration of the specified limit in the sample solution (μg/mL). Assuming that the concentration of the target element in the standard solution is 10 ~ 20 times that of the sample solution, the standard solution of the target element can be prepared, as mentioned in the section 3.2. Next, diluting this standard solution to 1/10 times of the concentration, this is the system suitability test solution.

Under the optimum conditions for an individual instrument, ICP emission spectra of the standard solution and the system suitability test solution of the target element are measured and the followings should be confirmed. Regarding the system suitability test solution, it should be confirmed that the spectra of the target element are clearly observed at the specified wavelength, and the emission intensity is within a specified range (for example, 80 ~ 120%) compared to the theoretical emission intensity, which is calculated by multiplying the concentration of the standard solution by the dilution ratio.

4.2. System repeatability

As for the standard solution of the target element described in the section 4.1, when the emission intensity measurement is repeated 6 times under the optimum conditions for an individual instrument, the relative standard deviation of the emission intensity for the target element should be below a certain specified value (for example, not more than 3.0%).

In case of the assay test, one of the standard solutions for the calibration curve, which are specified in the monograph, can be selected as a test solution of the system repeatability.

5. Interferences and their reduction or correction

Interferences accompanied to ICP-AES are a general expression of the negative effects caused by the coexisting substances or the matrix containing the target element. Various interferences are classified to the spectral interference and the non-spectral one, and in the latter there are interferences such as the physical interference and the ionization interference etc. Those interferences can be removed or reduced by the application of a suitable reduction or correction methods.

5.1. Physical interference

When there are differences of physical properties such as the viscosity, the density, and the surface tension etc. between the sample solution and the standard solution, the analytical result might be affected by the differences, due to the induction of the difference in the sample nebulization efficiency. It is called the physical interference. In order to remove or reduce this kind of interference, dilute the sample solution until the interference cannot occur, or conform the liquid characteristics of the sample solution to those of the standard solution (Matrix matching method). Separately, it is a useful correction method to adopt the internal standard method (Intensity ratio method) or the standard addition method as an assay method.

5.2. Ionization interference

The ionization interference indicates the effect that the marked increase of electronic density in plasma may affect the ionization ratio of the target element, where the coexisting element highly contained in the sample solution are easily ionized and release a great number of electrons in the plasma. The reduction or the correction method against the ionization interference effect is essentially the same as that described in the section 5.1 Physical interference. Furthermore, the ionization interference is moderately reduced by the selection of the observation way and the adjustments of the observation height, the radio frequency power output, and the flow rate of carrier gas, etc.

5.3. Spectral interference

The spectral interference indicates that analytical results are affected by the overlapping of various emission lines or continuous spectra to the analytical line of the target element. The causes and the correction methods to this interference are shown below.

5.3.1. Interference by the coexisting elements

This interference occurs when the emission line of coexisting element in the sample solution come close to the analytical line of the target element. The degree of the interference depends on the resolution ability of the spectroscope, the wavelength difference between two emission lines, and the intensity ratio. To avoid this interference another analytical line can also be available. Where an appropriate analytical line cannot be found, it is necessary to do the spectral interference correction.

The inter-element correction is one of the correction methods for the spectral interference. If the effect of coexisting element on the analytical line of the target element are measured in advance as a function of the emission intensity or the concentration by using the known concentration of two-component or multi-component standard solution, the
degree of interference on the emission intensity of the analytical line or the concentration will be speculated by measuring the emission intensity of the coexisting element together with measurement of the target element. Separately, the matrix matching method or the mathematical spectral separation technique, by which the spectra of coexisting element superimposed to the analytical line are separated, can also be used for the correction.

5.3.2. Background interference

The background interference indicates the following effect that analytical results are affected by the increase of background due to the emission lines caused by the element highly contained in the sample. In this case, this effect will be removed by applying the following correction method. Based on the background behavior before and after the analytical line, estimate the background intensity at the position of the analytical line, and obtain an intrinsic emission intensity of the target element by subtraction of this background intensity from the apparent emission intensity.

Furthermore where the pretreatment of organic sample is not complete, the molecular band spectra (NO, OH, NH, CH, etc.) generated by elements of N, O, H, C in the sample solution may happen to affect the intensity of the analytical line. This kind of interference is moderately reduced by the selection of the observation way and the adjustments of the observation height, the radio frequency power output, and the flow rate of carrier gas, etc.

6. Qualitative and quantitative analysis

6.1. Qualitative analysis

6.1.1. Identification of inorganic impurities such as metal elements

In JP, the confirmation test for drug substances is usually specified by applying the method of spectral analysis, by which the structural features of the target molecule can be totally found. Drug substances frequently contain specific elements such as N, S, P, halogens, and metals etc. in their molecule, which are required for their existence in the individual specification. Where the existence is not confirmed by such as spectral analyses, it is required for their confirmation by using chemical reactions. Thus the present method can be applied to their confirmation test, except for nitrogen (N).

When a few of wavelengths and their relative intensity of the target element in the sample solution coincide with those of the standard solution, the existence of the target element is confirmed. In place of the standard solution, the spectra library attached to the individual instrument or the wavelength table provided by the academic society, can also be used for the confirmation.

6.1.2. Profile analysis

If the target elements such as metal catalysts, inorganic elements, and other high toxicity elements such as Pb and As, which should be ordinarily controlled in view of the drug safety, are decided to be controlled, the profile analysis of those inorganic impurities can be performed by the present method as a part of the good manufacturing practice of drug substances.

Although the analytical line of the individual element can be selected referring to Table 1, an appropriate another emission line can also be available, when the spectral interferences are supposed to affect the analytical results. The standard solution of the individual element is adjusted to a suitable concentration, in consideration of a permitted limit of the respective element, specified separately. However, when the multi-component standard solution is prepared, it should be confirmed beforehand that any trouble such as coprecipitation does not occur.

The confirmation test of the target element should be done according to the section 6.1.1, and the impurity content of each element in the sample specimen is roughly estimated by the ratio of emission intensity of the sample solution and the standard solution at the analytical line (1 point calibration method).

6.2. Quantitative analysis

The quantitative evaluation of inorganic impurities in the sample specimen is usually done by measuring the emission intensity during a certain time of integration.

6.2.1. Calibration curve method

Regarding to the target element, not less than 4 kinds of the calibration standard solutions with different concentrations are prepared. By using these calibration standards, plot the emission intensity at the analytical line versus the concentration to obtain the calibration curve. After measuring the emission intensity of the sample solution, the concentration of the target element is determined by the calibration curve.

6.2.2. Internal standard method

Regarding to the target element, not less than 4 kinds of calibration standard solutions with different concentrations, in which a definite concentration of internal standard is contained, are prepared. Usually, yttrium (Y) is used as an internal standard. By using these calibration standards, plot the ratio of emission intensity of the target element and the internal standard versus the concentration to obtain the calibration curve. In the preparation of the sample solution, the internal standard element is added to give the same concentration as the calibration standards. After measuring the ratio of the emission intensity of the target element and the internal standard, the concentration of the target element is determined by the calibration curve.

Applying this method to inorganic impurity analysis, it should be confirmed that the internal standard element is not contained in the sample. As for the internal standard element, it is expected that changes of the emission intensity due to the operational conditions and the solution characteristics, are similar to those of the target element, and the selection of emission line is necessary so as not to give the spectral interference to the analytical line.

6.2.3. Standard addition method

Take not less than 4 sample solutions of equivalent volume, add the target element to these solutions to prepare the calibration standard solutions with different concentrations containing zero addition. After measuring the emission intensity of these calibration standards, plot the emission intensity at the analytical line versus the concentration. The concentration of the target element is determined to be the concentration, at which the regression line and the abscissa cross each other.

This method can be applied only to the case of analysis with no spectral interference, or where the background and the spectral interference are exactly corrected, and the relation between the emission intensity and the concentration keeps good linearity.

References

1) Japanese Industrial Standard: General Rules for Atomic
Near Infrared Spectrometry

Near infrared spectrometry (NIR) is one of spectroscopic methods used to qualitatively and quantitatively evaluate substances from analysis of data obtained by determining their absorption spectrum of light in the near-infrared range.

The near-infrared range lies between the visible light and infrared light, typically of wavelengths (wave numbers) between 750 and 2500 nm (13333 – 4000 cm⁻¹). The absorption of near-infrared light occurs due to harmonic overtones from normal vibration or combination tones in the infrared range (4000 to 400 cm⁻¹), primarily absorption of O–H, N–H, C–H and S–H that involve hydrogen atoms, in particular. For instance, the asymmetrical stretching vibration of N–H, C–H and S–H occurs in the vicinity of 3400 cm⁻¹, but the absorption due to the first harmonic overtone occurs in the vicinity of 6600 cm⁻¹ (wavelength 1515 nm), which is near double 3400 cm⁻¹.

Absorption in the near-infrared range is far weaker than absorption due to normal vibration that occurs in the infrared range. Furthermore, in comparison with visible light, near-infrared light has longer wavelength, which makes it possible for the light to penetrate to a depth of several mm into solid specimens including fine particles. This method is often utilized as a nondestructive analysis, as changes occurring with absorbed light spectrum (transmitted light or reflected light) in this process provide physical and chemical information pertaining to specimens.

Conventional spectrometry, such as calibration curve method, is used as a method for analyzing near-infrared absorption spectrum whenever applicable. Ordinarily, however, chemometrics methods are used for analysis. Chemometrics ordinarily involve quantification of chemical data, as well as numerical and statistical procedures for computerization of information. Chemometrics for near-infrared spectrometry includes various types of multivariate analysis such as multiple regression analysis, to perform qualitative or quantitative evaluation of active substances.

Near-infrared spectrometry is used as a rapid and nondestructive method of analysis that replaces conventional and established analysis methods for water determinations or substance verifications. It is necessary to perform a comparison test to evaluate this method against an existing analysis method, to verify that this method is equivalent to such existing analysis method, before using this analysis method as a quality evaluation test method in routine tests.

Applications of near-infrared spectrometry in the pharmaceutical field include qualitative or quantitative evaluation of ingredients, additives or water contents of active substances or preparations. Furthermore, near-infrared spectrometry can also be used for evaluation of physical conditions of substances, such as crystal forms, crystallinity, particle diameters. It is also possible to perform spectrometry on samples that are located in a remote location away from equipment main units, without sampling, by using optical fibers. It can therefore be used as an effective means to perform pharmaceutical manufacturing process control online.

1. Equipment

Near-infrared spectrophotometers can either be a distributed near-infrared spectrophotometer or a Fourier transform near-infrared spectrophotometer. Interference filter-type near-infrared spectrophotometers that use interference filter in the spectrometry section are also available, however, this type of equipment is hardly used in the field of pharmaceutical quality control.

1.1. Distributed near-infrared spectrophotometer

This equipment is comprised of light source section, sample section, spectrometry section, photometry section, signal processing section, data processing section, display-record-output section. Halogen lamps, tungsten lamps, light emitting diodes and other such devices that can emit high intensity near-infrared light in a stable manner are used in the light source section. The sample section is comprised of a sample cell and a sample holder. Equipment that have an optical fiber section that is comprised of optical fibers and a collimator are equipped with a function for transmitting light to sample section, which is remotely located away from the spectrophotometer main unit. Quartz is ordinarily used as material for optical fibers.

The spectrometry section is intended to extract light of required wavelength, using dispersive devices and is comprised of slits, mirrors and dispersive devices. Potential dispersive devices include prisms, diffraction grating, acousto-optical tunable filters (AOTF), or liquid crystal tunable filters (LCTF).

The photometry section is comprised of detectors and amplifiers. Sensors include semiconductor detectors (silicon, lead sulfide, indium-gallium-arsenic, indium-antimony), as well as photomultiplier tubes. Detecting methods that use semiconductor detectors generally perform detections with single elements, but there are also occasions where array-type detectors that use multiple elements are used. Such detectors are capable of simultaneously detecting multiple wavelengths (wave numbers). The signal processing section separates signals required for measurements from output signals fed by amplifiers and then outputs such isolated signals. The data processing section performs data conversions and spectral analysis, etc. The display-record-output section outputs data, analysis results and data processing results to a printer.

1.2. Fourier transform near-infrared spectrophotometer

The configuration of the equipment is fundamentally same as that of the distributed-type equipment described in Section 1.1, except for the spectrometry section and the signal processing section.

The spectrometry section is comprised of interferometers, sampling signal generators, detectors, amplifiers, A/D conversion devices, etc. Interferometers include Michelson inter-
ferometers, transect interferometers and polarization interferometers. The signal processing section is equipped with functions that are required for spectrometer, as well as a function for translating acquired interference waveform (interferogram) into absorption spectrum by Fourier transformation.

2. Determination

There are three types of measurement methods that are used with near-infrared spectrometry: transmittance method, diffuse reflectance method and transmittance reflectance method. The selection of measurement methods relies on the shape of samples and applications. The transmittance method or diffuse reflectance method is used for solid samples, including fine particles. The transmittance method or transmittance reflectance method is used for liquid samples.

2.1. Transmittance method

The degree of decay for incident light intensity as the light from a light source passes through a sample, is represented as transmittance rate \( T(\%) \) or absorbance \( A \) with the transmittance method. A sample is placed in the light path between a light source and a detector, the arrangement of which is ordinarily same as that of the spectroscopic method.

\[
T = 100r \\
T = \frac{I}{I_0} = 10^{-\alpha c} \\
I_0: \text{Incident light intensity} \\
I: \text{Transmitted light intensity} \\
\alpha: \text{Absorptivity} \\
c: \text{Solution concentration} \\
l: \text{Layer length (sample thickness)} \\
T = \log \left(\frac{I}{I_0}\right) = \log \left(\frac{I}{I_0}\right) = \alpha cl \\
A = -\log t = \frac{\log (I/I_0)}{\alpha c l} \\
A = \log \left(\frac{I_0}{I}\right)
\]

This method is applied for taking measurements of samples that are liquids and solutions. Quartz glass cells and flow cells are used, with the layer length of 1 - 5 mm along. Furthermore, this method can also be applied for taking measurements of samples that are solids, including fine particles. It is also known as diffuse transmittance method. Selecting appropriate layer length is critical for this method, since the transmitted light intensity varies depending on grain sizes and surface conditions of samples.

2.2. Diffuse reflectance method

The ratio of the reflection light intensity \( I_r \) emitted from the sample in a wide reflectance range and a control reflection light intensity \( I_r \) emitted from surface of a substance, is expressed as reflectance \( R(\%) \) with the diffuse reflectance method. The near-infrared light penetrates to a depth of several mm into solid samples, including fine particles. In that process, transmission, refraction, reflection and dispersion are repeated, and diffusion takes place, but a portion of the diffused light is emitted again from the surface of the sample and captured by a detector. The spectrum for the diffuse reflectance absorbance \( A_r \) can ordinarily be obtained by plotting logarithm of inverse numbers for reflectance \( 1/R \) against wavelengths (wave numbers).

\[
R = 100r \\
r = \frac{I}{I_0} \\
I: \text{Reflection light intensity of light, diffuse reflected off the sample} \\
I_0: \text{Control reflection light intensity of light emitted from surface of reference substance} \\
A_r = \log \left(\frac{I}{I}\right) = \log \left(\frac{I_0}{I}\right)
\]

The intensity of diffuse reflectance spectrum can also be expressed with the Kubelka-Munk (K-M) function. The K-M function is derived, based on the existence of a sample with sufficient thickness, and expressed in terms of light scattering coefficient, which is determined by absorptivity, grain size, shape and fill condition (compression).

This method is applied to solid samples, including fine particles, and requires a diffuse reflector.

2.3. Transmittance reflectance method

The transmittance reflectance method is a combination of the transmittance method and reflectance method. A mirror is used to re-reflect a light that has passed through a sample in order to take a measurement of transmittance reflectance rate, \( T^* (\%) \). Light path must be twice the thickness of the sample. On the other hand, the light reflected off a mirror and enters into a detector is used as the control light. When this method is applied to suspended samples, however, a metal plate or a ceramic reflector with rough surface that causes diffuse reflectance is used instead of a mirror.

Transmittance reflectance absorbance \( A^* \) is obtained by the following formula with this method:

\[
T^* = 100r^* \\
t^* = \frac{I}{I_0} \\
I: \text{Intensity of transmitted and reflected light, in cases where a sample is placed} \\
l: \text{Intensity of reflected light, in cases where is no sample} \\
A^* = \log \left(\frac{I}{I^*}\right)
\]

This is a method that is applied to solid samples, including fine particles, as well as liquids and suspended samples. The thickness of a sample must be adjusted when applying this method to a solid sample. Ordinarily adjustment is made by setting absorbance to 0.1 - 2 (transmittance of 79 - 1%), which provides the best linearity and S/N ratio of detector. A cell with appropriate layer length, according to the grain size of the fine particle, must be selected when applying the method to a fine particle sample.

3. Factors that affect spectrum

Following items must be considered as factors that can affect spectrum when applying near-infrared spectrometry, particularly when conducting quantitative analysis.

(i) Sample temperature: A significant change (wavelength shift, for example) can occur when the temperature varies by a several degree (°C). Care must be taken, particularly when the sample is a solution or contains water.

(ii) Water or residual solvent: Water or residual solvent contents of a sample, as well as water (humidity) in the environment wherein measurements are taken, can potentially significantly affect absorption band of the near-infrared range.

(iii) Sample thickness: The thickness of a sample is a factor for spectral changes and therefore needs to be controlled at a certain thickness. A sample may be considered to be of adequate thickness for the diffuse reflectance method, however, if the thickness is less than a certain amount, for example, the sample may have to be placed on a support plate with high reflectance to take measurements by the transmittance reflectance method.

(iv) Fill condition of sample: The condition of sample fill can potentially affect spectrum, when taking measure-
ments of samples that are solids or fine particles. Care must be taken when filling samples in a cell, to ensure that a certain amount is filled through a specific procedure.

(v) Optical characteristics of samples: When a sample is physically, chemically or optically uneven, relatively large beam size must be used, multiple samples must be used, measurements must be taken at multiple points on the same sample, or a sample must be pulverized to ensure averaging of the sample. Grain size, fill condition, as well as roughness of surface can also affect fine particle samples.

(vi) Crystal forms: Variations in crystal structures (crystal forms) can also affect spectrum. In cases where multiple crystal forms exist, it is necessary to have consideration for characteristics of samples to be considered and care must be taken to ensure that even standard samples for calibration curve method have diversified distributions similar to that of samples that are subject to analysis.

(vii) Temporal changes in characteristics of samples: Samples can potentially undergo chemical, physical or optical property changes, due to passing of time or storage after sampling, and such changes affect spectrum in a subtle manner. For instance even with identical samples, if elapsed times differ, then their characteristics of near-infrared spectrum can vary significantly. In creating calibration curves, therefore, measurements must be taken offline in a laboratory or online in manufacturing process (or inline) and samples for calibration curves must be prepared with adequate considerations for the passing of time before measurements are taken.

4. Control of equipment performance2,3)

4.1. Accuracy of wavelengths (wave numbers)

The accuracy of wavelengths (wave numbers) of an equipment is derived from the deviation of substances for which peak absorption wavelengths (wave numbers) have been defined, such as polystyrene, mixture of rare earth oxides (dysprosium, holmium and erbium; 1:1:1) or steam, from the figures indicated on the equipment. Tolerance figures in the vicinity of 3 peaks are ordinarily set in the following manner:

\[
1200 \pm 1 \text{ nm} (8300 \pm 8 \text{ cm}^{-1})
\]
\[
1600 \pm 1 \text{ nm} (6250 \pm 4 \text{ cm}^{-1})
\]
\[
2000 \pm 1.5 \text{ nm} (5000 \pm 4 \text{ cm}^{-1})
\]

Since the location of absorption peaks vary, depending on the substance used as reference, absorption peaks of wavelengths (wave numbers) that are closest to the above 3 peaks are selected for suitability evaluations. A mixture of rare earth oxides, for instance, would indicate characteristic absorption peaks at 1261 nm, 1681 nm and 1971 nm.

Absorption peaks at 1155 nm, 1417 nm, 1649 nm, 2352 nm (layer length: 1.0 mm) can be used, when taking measurements with transmittance method that involve the use of dichloromethane as reference. The absorption peak of steam measurements with transmittance method that involve the use of dichloromethane as reference. The absorption peak of steam

\[
1261 \text{ nm}, 1681 \text{ nm} \text{ and } 1971 \text{ nm}
\]

2000 nm against absorbance \( (A_{\text{REF}}) \) assigned to each standard plate, verifications must be made to ensure that the gradient of linearity obtained are ordinarily within the range 1.0 \( \pm \) 0.05 for each of these wavelengths and 0 \( \pm \) 0.05 for ordinate intercept.

4.3. Spectrophotometric noise

The spectrophotometric noise of the equipment can be checked using appropriate reflectance standard plates, such as white-colored reflecting ceramic tiles or reflective thermoplastic resin (such as polytetrafluoroethylene).

4.3.1. High flux noise

Spectrophotometric noise is evaluated by using standard plates with high reflectance, such as reflectance of 99%. Standard plates are used to take measurements for both samples and control samples. Generally, the average value obtained from calculation of mean square root (RMS) of noise for each 100 nm segments in the wavelength range of 1200 – 2200 nm ordinarily must not be more than 0.3 \( \times \) \( 10^{-3} \) and individual values must not exceed 0.8 \( \times \) \( 10^{-3} \).

\[
RMS = \left( \frac{1}{N} \sum_{i=1}^{N} (A_{\text{OBS}} - A_{\text{REF}})^2 \right)^{1/2}
\]

\(N\): Number of measurement points per segment

\(A_{i}\): Absorbance at each measurement point of segment

\(A_{\text{avg}}\): Average absorbance for segment

4.3.2. Low flux noise

Spectrophotometric noise is evaluated by using standard plates with low reflectance, such as reflectance of 10%, when the amount of light is low. In such cases, light source, optical system, detector and electronic circuit systems all have some impact on noise. Similar to the cases of high flux noise, generally, the average value obtained from calculation of RMS for each 100 nm segments in the wavelength range of 1200 – 2200 nm ordinarily must not be more than 1.0 \( \times \) \( 10^{-3} \) and individual values must not exceed 2.0 \( \times \) \( 10^{-3} \).

5. Application to qualitative or quantitative analysis

Unlike in the infrared range, mainly harmonic overtones and combinations manifest as spectrum in the near-infrared range. Such absorbance spectrums are often observed as overlay of absorption bands of functional groups and atomic groups. The near-infrared spectrometry, therefore, differs from conventional analysis methods and it is usually necessary to establish analysis methods that correspond to each application, by preparing model analysis methods using methodologies of chemometrics, such as multivariate analysis.

Characteristics of near-infrared absorption spectrum must be emphasized and effects of complexities of spectrums, as well as overlay of absorption bands must be reduced by performing mathematical preprocessing, such as primary or secondary spectral differentiation processes or normalizations, which becomes one of vital procedures in establishing analysis methods that use methodologies of chemometrics.

While there are many chemometrics methodologies and mathematical preprocessing methods for data, appropriate combinations must be selected that suit the purposes of
Chemometrics methodologies for obtaining quantitative models include multiple regression analysis method, main ingredient regression analysis method and PLS (partial least squares) regression analysis method.

In cases where the composition of a sample is simple, concentrations of ingredients in the sample that are subject to analysis can be calculated, by plotting a calibration curve using the absorbance of a specific wavelength (wave number) or the correlating relationship between the parameters and concentration, using samples for preparation of calibration curves with known concentrations (calibration curve method).

6. Reference

2) Near-infrared Spectrophotometry, 2.2.40, European Pharmacopoeia 5.0 (2005)

pH Test for Gastrointestinal Medicine

In this test, medicine for the stomach and bowels, which is said to control stomach acid, is stirred in a fixed amount of the 0.1 mol/L hydrochloric acid for a fixed duration, and the pH value of this solution is obtained. The pH value of a stomach medicine will be based on the dose and the dosage of the medicine (when the dosage varies, a minimum dosage is used) and expressed in the pH value obtained from the test performed by the following procedure.

1. Preparation of Sample

Solid medicine which conforms to the general regulations for medicine (the powdered medicine section) can be used as a sample. When the medicine is in separate packages, the content of 20 or more packages is accurately weighed to calculate the average mass for one dose and mixed evenly to make a sample. For granules and similar types in separate packages, among the solid medicine which does not conform to the general regulations for medicine (the powdered medicine section), the content of 20 or more packages is accurately weighed to calculate the average mass for one dose and is then powdered to make sample. For granules and similar types not in separate packages, among solid medicine which does not conform to the general regulations for medicine (the powdered medicine section), 20 doses or more are powdered to make a sample. For capsules and tablets, 20 doses or more are weighed accurately to calculate the average mass for one dose or average mass and then powdered to make a sample.

Liquid medicine is generously mixed to make a sample.

2. Procedure

Put 50 mL of the 0.1 mol/L hydrochloric acid with the molarity coefficient adjusted to 1.000, or equivalent 0.1 mol/L hydrochloric acid with its volume accurately measured in a 100-mL beaker. Stir this solution with a magnetic stirrer and a magnetic stirrer rotator (35 mm length, 8 mm diameter) at the speed of about 300 revolutions per minute.
While stirring, add the accurately weighed one-dose sample. After 10 minutes, measure the pH value of the solution using the pH Determination. The solution temperature should be maintained at \(37 \pm 2{}^\circ\text{C}\) throughout this operation.

## System Suitability

In order to ensure the reliability on the results of drug analyses, it is essential to verify that the test method to be applied to the test, including the method prescribed in the Japanese Pharmacopoeia (JP), can give the results adequate for its intended use using the analytical system in the laboratory in which the test is to be performed, then to carry out system suitability testing for confirming that the analytical system maintains the state suitable for the quality test.

### 1. Definition and role of system suitability

“System Suitability” is the concept for ensuring that the performance of the analytical system is as suitable for the analysis of the drug as was at the time when the verification of the test method was performed using the system.

Usually, system suitability testing should be carried out at every series of drug analysis. The test procedures and acceptance criteria of system suitability testing must be prescribed in the test methods of drugs. The results of drug analyses are not acceptable unless the requirements of system suitability have been met.

System suitability testing is an integral part of test methods using analytical instruments, and based on the concept that the equipments, electronic data processing systems, analytical operations, samples to be analyzed and operators constitute an integral system that can be evaluated, when the test procedures and acceptance criteria of system suitability testing are prescribed in the test methods.

### 2. Points to consider in setting system suitability

Parameters of system suitability testing to be prescribed in the test method depend on the intended use and type of analytical method. Since system suitability testing is to be carried out in a routine manner, it is preferable to select the parameters necessary for ensuring that the analytical system maintains the state suitable for the analysis of the drug and to prescribe its test procedure able to carry out easily and rapidly.

For example, in the case of quantitative purity tests using liquid chromatography or gas chromatography, the evaluation of parameters such as “System performance” (to confirm the ability to analyze target substance specifically), “System repeatability” (to confirm that the degree of variation in the analytical results of target substance in replicate injections is within the allowable limit) and “Test for required detectability” (to confirm the linearity of chromatographic response around the specification limit) are usually required.

The followings are supplements to the section of system suitability prescribed in “Liquid Chromatography”.

### 2.1. System repeatability of HPLC and GC

#### 2.1.1. Allowable limit of system repeatability

It is described in the section of system suitability in “Liquid Chromatography” that “In principle, total number of replicate injections should be 6”, and “The allowable limit of “System repeatability” should be set at an appropriate level based on the data when suitability of the method for the evaluation of quality of the drug was verified, and the precision necessary for the quality test”.

Based on the above description, an allowable limit of system repeatability for 6 replicate injections should be set in consideration with the following descriptions. However, in the case that the test method prescribed in the JP monograph is used for the test, the allowable limit of system repeatability prescribed in the monograph should be applied.

(i) Assay for drug substance (for drug substance with the content nearby 100%): An adequate allowable limit should be set at the level that the chromatographic system is able to give the precision suitable for the evaluation of variation in the content of active ingredient within and among the batches of drug substance. For example, the allowable limit of “not more than 1.0%” is usually recommended for the drug substances whose width of content specification are not more than 5%, as is in the case of content specification of 98.0 – 102.0% which is often observed in the assay using liquid chromatography.

(ii) Assay for drug products: An adequate allowable limit should be set considering the width of content specification of the drug product and the allowable limit prescribed in the assay of drug substance (when the drug product is analyzed by a method with the same chromatographic conditions as those used for the analysis of drug substance).

(iii) Purity test for related substances: An adequate allowable limit should be set considering the concentration of active ingredients in the solution used for the system suitability testing. In the case that a solution with active ingredient concentration of 0.5 – 1.0% is used for the test of system repeatability, an allowable limit of “not more than 2.0%” is usually recommended.

Recommendations for allowable limits described above should not be applicable to gas chromatography.

### 2.1.2. Method for decreasing the number of replicate injections without losing the quality of system repeatability testing

It is described in the section of system suitability in “Liquid Chromatography” that “In principle, total number of replicate injections should be 6. However, in the case that a long time is necessary for one analysis, such as the analysis using the gradient method, or the analysis of samples containing late eluting components, it may be acceptable to decrease the number of replicate injections by adopting new allowable limit of “System repeatability” which can guarantee a level of “System repeatability” equivalent to that at 6 replicate injections.”

In consideration of the above description, a method for decreasing the number of replicate injections without losing the quality of system repeatability testing is adopted. One can set the test for system repeatability with reduced number of replicate injections by utilizing this method, if necessary, and can also apply it as an alternative for the method prescribed in a monograph.

The following table shows the allowable limits to be attained in the test at 3 – 5 replicate injections \((n = 3 – 5)\) to keep the quality test equivalent to that of test at \(n = 6\).

However, it should be kept in mind that since decrease in the number of replicate injections results in increase in the weight of each injection, it becomes more important to perform the test by the experienced operator, and to maintain the equipment in a suitable state.
3. Points to consider at the change of analytical system (Change control of analytical system)

When the test method and analytical system verified is continuously used for the quality test without any change, it is sufficient to confirm the compliance to the requirements of system suitability at every series of drug analysis. However, when the test is performed for a long period, a situation in which some changes in the analytical system are inevitable, may occur. These changes don’t affect the quality of the product itself, but they affect the scale in the evaluation of product quality. If the change in the analytical system may induce a significant deviation of the scale, it may lead to the acceptance of products with inadequate quality and/or the rejection of products with adequate quality. Thus, at the time of change in the analytical system, it is necessary to check whether the change is appropriate or not, to avoid the deviation of the scale in the evaluation of product quality.

In the case of the change of test method, it is required to perform an adequate validation depending on the extent of the change.

On the other hand, in the case of the change of analytical system in a laboratory, such as renewal of apparatus or column of liquid chromatography, and the change of operator, it is necessary to perform at least system suitability testing using the system after change, and to confirm that the equivalency of the results before and after change.

In the case that equivalent results would not be obtained after change, for example, when a renewal of column of liquid chromatography may induce a significant change of elution pattern, such as the reversal of elution order between target ingredient of the test and substance for checking resolution, it is required to perform a revalidation of the analytical system for the test using new column, since it is uncertain whether the specificity and/or other validation characteristics necessary for estimating target ingredient is kept or not.

### Test for Trace Amounts of Aluminum in Trans Parenteral Nutrition (TPN) Solutions

Trans parenteral nutrition solutions (TPNs) are nutrient preparations for intravenous injection. Since toxic effects to the central nervous system, bone, etc. due to trace amounts of aluminum have recently been reported in several countries, testing methods for trace amounts of aluminum contaminating TPNs are required for the official standard. The following three analytical methods are available: High-Performance Liquid Chromatography using a fluorescence photometric detector (HPLC with fluorescence detection), Inductivity Coupled Plasma-Atomic Emission Spectrometry (ICP-AES method), Inductivity Coupled Plasma-Mass Spectrometry (ICP-MS method). Detection sensitivity by HPLC with fluorescence detection is about 1 µg/L (ppb), while ICP-AES fitted with special apparatus and ICP-MS have higher sensitivity.

Since TPNs are nutrient preparations, they contain many nutrients such as sugars, amino acids, electrolytes, etc., in various compositions. Thus, care is needed in the selection of a suitable analytical method, because these coexisting components may affect the measurement of trace amounts of aluminum.

In view of the general availability of HPLC apparatus, the present general information describes procedures for the determination of trace levels of aluminum in TPNs by means of HPLC with a fluorescence photometric detector, using two kinds of fluorescent chelating agents, i.e., Quinolinol complexing method, Lumogallion complexing method.

#### 1. Quinolinol complexing method

After forming a complex of aluminum ion in the sample solution with quinolinol, the assay for aluminum by HPLC fitted with a fluorescence photometer is performed.

##### 1.1. Preparation of sample solution

Pipet 1 mL of the sample (TPNs) exactly, and after adding 10 µL of water for aluminum test, make up the sample solution to 10 mL exactly by adding the mobile phase.

##### 1.2. Preparation of a series of standard solutions for calibration curve

Pipet 1 mL of water for aluminum test exactly, and after adding 10 µL each of standard solutions of aluminum (1)–(5), make up the standard solutions for calibration curve to 10 mL (Aluminum concentration: 0, 1.25, 2.5, 5.0, and 10.0 ppb).

##### 1.3. Standard testing method

Pipet 0.1 mL each of the sample solution and standard solutions, and perform the test by HPLC under the following conditions. Calculate the aluminum content in the sample solution using a calibration curve method.

**Operating conditions—**
Detector: Fluorescence photometer(excitation wavelength: 380 nm, emission wavelength: 520 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 8-quinolinol in acetonitrile (3 in 100) and diluted 0.5 mol/L ammonium acetate TS (2 in 5) (1:1).

Flow rate: Adjust the flow rate so that the retention time of aluminum/8-quinolinol complex is about 9 minutes.

System suitability—

The correlation coefficient of the calibration curve, which is prepared using a series of standard solutions, is not less than 0.99.

Furthermore there is an alternative method, in which the chelating agent 8-quinolinol is not included in the mobile phase. In this method also, aluminum is detected as a complex with 8-quinolinol in the sample solution by using HPLC fitted with fluorescence photometer. But it is necessary to form a more stable aluminum/8-quinolinol complex in the sample solution, because the chelating agent is not included in the mobile phase. Further, since the analytical wavelength for the fluorescence detection is different from that in the standard method, excitation WL: 370 nm, emission WL: 504 nm, the detection sensitivity is different. Thus, it is appropriate to obtain the calibration curve between 0 – 25 ppb of aluminum. Other than the above-mentioned differences, the size of column, column temperature, and the mobile phase are also different from those used in the standard method, so suitable analytical conditions should be established for performing precise and reproducible examinations of trace amounts of aluminum in the sample specimen.

2. Lumogallion complexing method

After forming a complex of aluminum ion in the sample specimen with the fluorescent reagent of lumogallion, the solution is examined by HPLC fitted with a fluorescence photometer.

2.1. Preparation of sample solution

Pipet 70 μL of the sample specimen (TPN) exactly, add 0.15 mL of lumogallion hydrochloric acid TS and 0.6 mL of buffer solution for aluminum test, pH 7.2 exactly, then mix the solution. After this solution has been allowed to stand for 4 hours at 40°C, it can be used for the measurement as a sample solution.

2.2. Preparation of a series of standard solutions for calibration curve

Pipet 1 mL each of standard aluminum solutions (1) – (5) exactly, and add diluted nitric acid for aluminum test (1 in 100) to make exactly 100 mL. Pipet 70 μL each of these solutions exactly, and add exactly 0.15 mL of lumogallion hydrochloric acid TS and exactly 0.6 mL of buffer solution for aluminum test, pH 7.2 then allow to stand for 4 hours at 40°C to make a series of standard solutions for obtaining the calibration curve (Aluminum: 0, 1.07, 2.13, 4.27, and 8.54 ppb).

2.3. Standard examination method

Take 0.1 mL each of the sample solution and standard aluminum solutions for the calibration curve, and perform HPLC analysis under the following conditions. Calculate the aluminum content in the sample solution by using a calibration curve method.

Operating conditions—

Detector: Fluorescence photometer(excitation wavelength 505 nm, emission wavelength 574 nm)

Column: A stainless steel column 6.0 mm in inside diameter and 10 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Take 100 mL of 2-propanol, and add a diluted 1 mol/L acetic acid-sodium acetate buffer solution of pH 5.0 (1 in 10) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of aluminum/lumogallion complex is about 5 minutes.

System suitability—

The correlation coefficient of the calibration curve, which is prepared using a series of standard solutions, is not less than 0.99.

3. Notes

(i) Regarding water, solvents, reagents, vessels and other tools used for the examination, select those not contaminated with aluminum. Further, keep the testing environment clean and free from dust in the testing room.

(ii) Before the measurement, it is necessary to confirm that the characteristic properties of the sample do not affect the formation of the complex.

(iii) Reference substances of river water for analysis of trace elements, distributed by the Japan Society for Analytical Chemistry, contain certified amounts of aluminum: JSAC 0301-1 and JSAC 0302 (a known amount of aluminum is artificially added to JSAC 0301-1).

4. Standard Solutions, Reagents and Test Solutions

Other than the standard solutions, reagents and test solutions specified in the Japanese Pharmacopoeia, those described below can be used in this test.

(i) \(N, N\)-Bis(2-hydroxyethyl)-2-aminoethane sulfonic acid \(C_6H_{15}NO_5S\) White crystals or powder.

(ii) Hydrochloric acid for aluminum test Same as the reagent Hydrochloric acid. Further, it contains not more than 1 ppb of aluminum.

(iii) Lumogallion [5-Chloro-2-hydroxy-3(2,4-dihydroxyphenylazo)benzenesulfonic acid] \(C_9H_7ClNO_6S\) Red-brown to dark brown powder. Further, it contains not more than 1 ppm of aluminum.

(iv) Lumogallion hydrochloric acid TS Dissolve 0.86 g of lumogallion in 300 mL of 2-propanol, and add 350 mL of diluted Hydrochloric acid for aluminum test (9 in 50) and Water for aluminum test to make 1000 mL exactly.

(v) Nitric acid for aluminum test Same as the reagent Nitric acid. Further, it contains not more than 1 ppb of aluminum.

(vi) pH buffer solution for aluminum test, pH 7.2 Dissolve 106.6 g of \(N, N\)-bis(2-hydroxyethyl)-2-aminoethane sulfonic acid in 800 mL of Water for aluminum test, adjust the pH 7.2 by using Tetramethylammonium hydroxide aqueous solution, and add Water for aluminum test to make 1000 mL exactly.

(vii) Standard aluminum solution Pipet a constant volume each of Water for aluminum test or the Standard aluminum stock solution, dilute and adjust the aluminum concentration to 0, 1.25, 2.5, 5.0, and 10 ppm by using diluted Nitric acid for aluminum test (1 in 100), to make...
Standard aluminum solutions (1) – (5).

(viii) Tetramethylammonium hydroxide TS (CH₃)₄NOH
It is a 25% aqueous solution, prepared for aluminum test. Further, it contains not more than 1 ppb of aluminum.

(ix) Water for aluminum test It contains not more than 1 ppb of aluminum.

**Validation of Analytical Procedures**

The validation of an analytical procedure is the process of confirming that the analytical procedure employed for a test of pharmaceutics is suitable for its intended use. In other word, the validation of an analytical procedure requires us to demonstrate scientifically that risks in decision by testing caused by errors from analytical steps are acceptably small.

The performance of an analytical procedure is established by various kinds of validation characteristics. The validity of a proposed analytical procedure can be shown by demonstrating experimentally that the validation characteristics of the analytical procedure satisfy the standards set up according to the acceptable limits of testing.

When an analytical procedure is to be newly carried in the Japanese Pharmacopoeia, when a test carried in the Japanese Pharmacopoeia is to be revised, and when the test carried in the Japanese Pharmacopoeia is to be replaced with a new test according to regulations in general notices, analytical procedures employed for these tests should be validated according to this document.

1. **Required data for analytical procedures to be carried in the Japanese Pharmacopoeia**

   1.1. Outline

   This section should provide a brief explanation of the principle of a proposed analytical procedure, identify the necessity of the analytical procedure and its advantage compared with other procedures, and summarize the validation.

   When an analytical procedure is revised, the limitation of the current analytical procedure and the advantage offered by the new analytical procedure should be described.

1.2. Analytical procedure

   This section should contain a complete description of the analytical procedure to enable skilled persons to evaluate correctly the analytical procedure and replicate it if necessary. Analytical procedures include all important operating procedures for performing analyses, the preparation of standard samples, reagents and test solutions, precautions, procedures to verify system suitability (e.g., the verification of the separating performance of a chromatographic system), formulas to obtain results, the number of replications and so forth. Any instruments and apparatus that are not stated in the Japanese Pharmacopoeia should be described in detail. The physical, chemical or biological characteristics of any new reference standards should be clarified and their testing methods should be established.

1.3. Data showing the validity of analytical procedures

   This section should provide complete data showing the validity of the analytical procedures. This includes the experimental designs to determine the validation characteristics, experimental data, calculation results and results of hypothesis tests.

2. **Validation characteristics**

   The definition of typical validation characteristics to be assessed in validation of analytical procedures and examples of assessing procedures are given below.

   The terminology and definitions of the validation characteristics may possibly vary depending upon the fields to which analytical procedures are applied. The terminology and definitions shown in this document are established for the purpose of the Japanese Pharmacopoeia. Typical methods for assessing the validation characteristics are shown in the item of assessment. Various kinds of methods to determine the validation characteristics have been proposed and any methods that are widely accepted will be accepted for the present purpose. However, since values of the validation characteristics may possibly depend upon methods of determination, it is required to present the methods of determining the validation characteristics, the data and calculation methods in sufficient detail.

   Although robustness is not listed as a validation characteristic, it should be considered during the development of analytical procedures. Studying the robustness may help to improve analytical procedures and to establish appropriate analytical conditions including precautions.

2.1. **Accuracy/Trueness**

2.1.1. Definition

   The accuracy is a measure of the bias of observed values obtained by an analytical procedure. The accuracy is expressed as the difference between the average value obtained from a large series of observed values and the true value.

2.1.2. Assessment

   The estimate of accuracy of an analytical method is expressed as the difference between the total mean of observed values obtained during investigation of the reproducibility and the true value. The theoretical value is used as the true value (e.g., in the case of titration methods, etc.). When there is no theoretical value or it is difficult to obtain a theoretical value even though it exists, a certified value or a consensus value may be used as the true value. When an analytical procedure for a drug product is considered, the observed value of the standard solution of the drug substance may be used as the consensus value.

   It may be inferred from specificity data that an analytical procedure is unbiased.

   The estimate of accuracy and a 95% confidence interval of the accuracy should be calculated using the standard error based on the reproducibility (intermediate precision). It should be confirmed that the confidence interval includes zero or that the upper or lower confidence limits are within the range of the accuracy required of the analytical procedure.

2.2. **Precision**

2.2.1. Definition

   The precision is a measure of the closeness of agreement between observed values obtained independently from multiple samplings of a homogenous sample and is expressed as the variance, standard deviation or relative standard deviation (coefficient of variation) of observed values.

   The precision should be considered at three levels with different repetition conditions; repeatability, intermediate precision and reproducibility.

   (i) Repeatability/Intra-assay precision

   The repeatability expresses the precision of observed values obtained from multiple samplings of a homogenous
sample over a short time interval within a laboratory, by the same analyst, using the same apparatus and instruments, lots of reagents and so forth (repeatability conditions).

(ii) Intermediate precision

The intermediate precision expresses the precision of observed values obtained from multiple samplings of a homogenous sample by changing a part of or all of the operating conditions including analysts, experimental dates, apparatus and instruments and lots of reagents within a laboratory (intermediate precision condition).

(iii) Reproducibility

The reproducibility expresses the precision of observed values obtained from multiple samplings of a homogenous sample in different laboratories (reproducibility condition).

2.2.2. Assessment

A sufficient volume of a homogenous sample should be prepared before studying the precision. The solution is assumed to be homogenous. When it is difficult to obtain a homogenous sample, the following samples may be used as homogenous samples; e.g., a large amount of drug products or mixture of drug substance and vehicles that are crushed and mixed well until they can be assumed to be homogenous.

Suitable experimental designs such as one-way layout may be employed when more than one level of precision is to be investigated simultaneously. A sufficient number of repetitions, levels of operating conditions and laboratories should be employed. Sources of variations affecting analytical results should be evaluated as thoroughly as possible through the validation.

It is required to show the variance, standard deviation and relative standard deviation (coefficient of variation) of each level of precision. The 90% confidence interval of the variance and corresponding intervals of the standard deviation and relative standard deviation should also be established. The validity of the proposed analytical procedure for its intended use may be confirmed by comparing obtained values with the required values of the analytical procedure. Whether the proposed analytical procedure is acceptable may normally be decided based on the reproducibility.

2.3. Specificity

2.3.1. Definition

The specificity is the ability of an analytical procedure to measure accurately an analyte in the presence of components that may be expected to be present in the sample matrix. The specificity is a measure of discriminating ability. Lack of specificity of an analytical procedure may be compensated by other supporting analytical procedures.

2.3.2. Assessment

It should be confirmed that the proposed analytical procedure can identify an analyte or that it can accurately measure the amount or concentration of an analyte in a sample. The method to confirm the specificity depends very much upon the purpose of the analytical procedure. For example, the specificity may be assessed by comparing analytical results obtained from a sample containing the analyte only with results obtained from samples containing excipients, related substances or degradation products, and including or excluding the analyte. If reference standards of impurities are unavailable, samples that are expected to contain impurities or degradation products may be used (e.g. samples after accelerated or stress tests).

2.4. Detection limit

2.4.1. Definition

The detection limit is the lowest amount or concentration of the analyte in a sample that is detectable, but not necessarily quantifiable.

2.4.2. Assessment

The detection limit should be normally determined so that producer’s and consumer’s risks are less than 5%. The detection limit may be calculated using the standard deviation of responses of blank samples or samples containing an analyte close to the detection limit and the slope of the calibration curve close to the detection limit. The following equation is an example to determine the detection limit using the standard deviation of responses of blank samples and the slope of the calibration curve.

$$ DL = 3.3\sigma/slope $$

$DL$: detection limit  
$\sigma$: the standard deviation of responses of blank samples  
$slope$: slope of the calibration curve

The noise level may be used as the standard deviation of responses of blank samples in chromatographic methods. It should be ensured that the detection limit of the analytical procedure is lower than the specified limit for testing.

2.5. Quantitation limit

2.5.1. Definition

The quantitation limit is the lowest amount or concentration of the analyte in a sample that can be determined. The precision expressed as the relative standard deviation of samples containing an analyte at the quantitation limit is usually 10%.

2.5.2. Assessment

The quantitation limit may be calculated using the standard deviation of responses of blank samples or samples containing an analyte close to the quantitation limit and the slope of the calibration curve close to the quantitation limit. The following equation is an example to determine the quantitation limit using the standard deviation of responses of blank samples and the slope of the calibration curve.

$$ QL = 10\sigma/slope $$

$QL$: quantitation limit  
$\sigma$: the standard deviation of responses of blank samples  
$slope$: slope of the calibration curve

The noise level may be used as the standard deviation of responses of blank samples in chromatographic methods. It should be ensured that the quantitation limit of the analytical procedure is lower than the specified limit for testing.

2.6. Linearity

2.6.1. Definition

The linearity is the ability of an analytical procedure to elicit responses linearly related to the amount or concentration of an analyte in samples. A well-defined mathematical transformation may sometimes be necessary to obtain a linear relationship.

2.6.2. Assessment

Responses are obtained after analyzing samples with various amounts or concentrations of an analyte according to described operating procedures. The linearity may be evaluated in terms of the correlation coefficient, and the slope and y-intercept of the regression line. It may be also helpful for evaluating the linearity to plot residual errors from the
impurities in pharmaceuticals.

Components in pharmaceuticals according to their characteristics and their intended use.

The range for the validation of analytical procedures is the interval between the lower and upper limits of the amount or concentration of an analyte providing sufficient accuracy and precision. The range for the validation of analytical procedures for an analytical procedure with linearity is the interval between the lower and upper limits providing sufficient accuracy, precision and linearity.

2.7.2. Assessment

When the range for the validation of analytical procedures is investigated, 80 to 120% of specified limits of testing should be usually considered. The accuracy, precision and linearity should be evaluated using samples containing the lower and upper limits and in the middle of the range.

3. Categories of tests employing analytical procedures

Tests covered with this document are roughly classified into three categories shown below according to their purposes. The table lists the normally required validation characteristics to be evaluated in the validation of analytical procedures used in these tests. This list should be considered to represent typical validation characteristics. A different approach to validating analytical procedures should be considered depending upon the characteristics of analytical procedures and their intended use.

(i) Type I Identification. Tests for identifying major components in pharmaceuticals according to their characteristics.

(ii) Type II Impurity tests. Tests for determination of impurities in pharmaceuticals.

(iii) Type III Tests for assaying drug substances, active ingredients, and major components in pharmaceuticals.

4. Terminology used in the validation of analytical procedures

(i) Analytical procedure: This document covers analytical procedures applied to identification, and ones that provides responses depending upon the amount or concentration of analytes in samples.

(ii) Laboratory: The laboratory means an experimental room or facility where tests are performed. In this document different laboratories are expected to perform an analytical procedure using different analysts, different experimental apparatus and instruments, different lots of reagents and so forth.

(iii) Number of replications: The number of replications is one that is described in analytical procedures. An observed value is often obtained by more than one measurement in order to achieve good precision of analytical procedures. Analytical procedures including the number of replications should be validated. This is different from repetition in the validation of analytical procedures to obtain accuracy or precision.

(iv) Observed value: The value of a characteristic obtained as the result of performing an analytical procedure.

(v) Consumer’s risk: This is the probability that products out of the specification of tests are decided to be accepted after testing. It is usually expressed as β, and is called the probability of type II error or the probability of false negative in impurity tests.

(vi) Producer’s risk: This is the probability that products satisfying the specification of tests are decided to be rejected after testing. It is usually expressed as α, and is called the probability of type I error or the probability of false positive in impurity tests.

(vii) Robustness: The robustness is a measure of the capacity to remain unaffected by small but deliberate variations in analytical conditions. The stability of observed values may be studied by changing various analytical conditions within suitable ranges including pH values of solutions, reaction temperature, reaction time or amount of reagents added. When observed values are unstable, the analytical procedure should be improved. Results of studying robustness may be reflected in the developed analytical procedure as precautions or significant digits describing analytical conditions.

(viii) Test: Tests mean various tests described in general tests and official monographs in the Japanese Pharmacopoeia such as impurity tests and assay. They includes sampling methods, specification limits and analytical procedures.

<table>
<thead>
<tr>
<th>Validation characteristics</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy/Trueness Precision</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Repeatability</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Intermediate precision</td>
<td>−</td>
<td>−*</td>
<td>−</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>−</td>
<td>+*</td>
<td>−*</td>
</tr>
<tr>
<td>Specificity**</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Detection limit</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Quantitation limit</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Linearity</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Range</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- Usually need not to be evaluated.
+ Usually need to be evaluated.
* Either intermediate precision or reproducibility should be evaluated depending upon circumstances in which analytical procedures or tests are performed. The latter should be normally evaluated in the validation of analytical procedures proposed to be included in the Japanese Pharmacopoeia.

** The lack of the specificity of an analytical procedure may be compensated by other relevant analytical procedures.
G2 Solid-state Properties

Laser Diffraction Measurement of Particle Size

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

The laser light diffraction technique used for the determination of particle-size distribution is based on the analysis of the diffraction pattern produced when particles are exposed to a beam of monochromatic light. Historically, the early laser diffraction instruments only used scattering at small angles. However, the technique has since been broadened to include laser light scattering in a wider range and application of the Mie theory, in addition to the Fraunhofer approximation and anomalous diffraction.

The technique cannot distinguish between scattering by single particles and scattering by clusters of primary particles, i.e. by agglomerates or aggregates. As most particulate samples contain agglomerates or aggregates and as the focus of interest is generally on the size distribution of primary particles, the clusters are usually dispersed into primary particles before measurement.

For non-spherical particles, an equivalent sphere-size distribution is obtained because the technique assumes spherical particles in its optical model. The resulting particle-size distribution may differ from those obtained by methods based on other physical principles (e.g. sedimentation, sieving).

This chapter provides guidance for the measurement of size distributions of particles in different dispersed systems, for example, powders, sprays, aerosols, suspensions, emulsions, and gas bubbles in liquids, through analysis of their angular light-scattering patterns. It does not address specific requirements of particle-size measurement of specific products.

1. Principle

A representative sample, dispersed at an adequate concentration in a suitable liquid or gas, is passed through a beam of monochromatic light, usually a laser. The light scattered by the particles at various angles is measured by a multi-element detector. Numerical values representing the scattering pattern are then recorded for subsequent analysis. These scattering pattern values are then transformed, using an appropriate optical model and mathematical procedure, to yield the proportion of total volume to a discrete number of size classes, forming a volumetric particle-size distribution.

2. Instrument

The instrument is located in an environment where it is not affected by electrical noise, mechanical vibrations, temperature fluctuations, humidity or direct bright light. An example of a set-up of a laser light diffraction instrument is given in Fig. 1. Other equipment may be used.

The instrument comprises a laser light source, beam processing optics, a sample measurement region (or cell), a Fourier lens, and a multi-element detector for measuring the scattered light pattern. A data system is also required for deconvolution of the scattering data into a volumetric size distribution and associated data analysis and reporting.

The particles can enter the laser beam in 2 positions. In the conventional case the particles enter the parallel beam before the collecting lens and within its working distance. In so-called reversed Fourier optics the particles enter behind the collecting lens and thus, in a converging beam. The advantage of the conventional set-up is that a reasonable path length for the sample is allowed within the working distance of the lens. The second set-up allows only small path lengths but enables measurement of scattered light at larger angles, which is useful when submicron particles are present.

The interaction of the incident light beam and the ensemble of dispersed particles results in a scattering pattern with different light intensities at various angles. The total angular intensity distribution, consisting of both direct and scattered light, is then focused onto a multi-element detector by a lens or a series of lenses. These lenses create a scattering pattern that, within limits, does not depend on the location of the particles in the light beam. Hence, the continuous angular intensity distribution is converted into a discrete spatial intensity distribution on a set of detector elements.

It is assumed that the measured scattering pattern of the particle ensemble is identical to the sum of the patterns from all individual single scattering particles presented in random relative positions. Note that only a limited angular range of scattered light is collected by the lens(es) and, therefore, by the detector.

3. Development of the method

The measurement of particle size by laser diffraction can give reproducible data, even in the sub-micron region, provided the instrument used and the sample tested are carefully controlled to limit variability of the test conditions (e.g. dispersion medium, method of preparation of the sample dispersion).

Traditionally, the measurement of particle size using laser diffraction has been limited to particles in the range of approximately 0.1 \( \mu \text{m} \) to 3 mm. Because of recent advances in lens and equipment design, newer instruments are capable of exceeding this range routinely. With the validation report the user demonstrates the applicability of the method for its intended use.

3.1. Sampling

The sampling technique must be adequate to obtain a representative sample of a suitable volume for the particle-size measurement. Sample splitting techniques such as rotating riffler or the cone and quartering method may be applied.

3.2. Evaluation of the dispersion procedure

Inspect the sample to be analyzed, visually or with the aid of a microscope, to estimate its size range and particle shape. The dispersion procedure must be adjusted to the purpose of the measurement. The purpose may be such that it is preferable to deagglomerate clusters into primary particles as far as possible, or it may be desirable to retain clusters as intact as possible. In this sense, the particles of interest may be either primary particles or clusters.

For the development of a method it is highly advisable to check that comminution of the particles does not occur, and conversely, that dispersion of particles or clusters is satisfactory. This can usually be done by changing the dispersing energy and monitoring the change of the particle-size distribution. The measured size distribution must not change sig-
significantly when the sample is well dispersed and the particles are neither fragile nor soluble. Moreover, if the manufacturing process (e.g., crystallization, milling) of the material has changed, the applicability of the method must be verified (e.g., by microscopic comparison).

Sprays, aerosols and gas bubbles in a liquid should be measured directly, provided that their concentration is adequate, because sampling or dilution generally alters the particle-size distribution.

In other cases (such as emulsions, pastes and powders), representative samples may be dispersed in suitable liquids. Dispersing aids (wetting agents, stabilizers) and/or mechanical forces (e.g., agitation, sonication) are often applied for deagglomeration or deaggregation of clusters and stabilization of the dispersion. For these liquid dispersions, a recirculating system is most commonly used, consisting of an optical measuring cell, a dispersion bath usually equipped with stirrer and ultrasonic elements, a pump, and tubing. Non-recirculating, stirred cells are useful when only small amounts of a sample are available or when special dispersion liquids are used.

Dry powders can also be converted into aerosols through the use of suitable dry powder dispersers, which apply mechanical force for deagglomeration or deaggregation. Generally, the dispersers use the energy of compressed gas or the differential pressure of a vacuum to disperse the particles to an aerosol, which is blown through the measuring zone, usually into the inlet of a vacuum unit that collects the particles. However, for free flowing, coarser particles or granules the effect of gravity may be sufficient to disperse the particles adequately.

If the maximum particle size of the sample exceeds the measuring range of the instrument, the material that is too coarse can be removed by sieving and the mass and percentage of removed material are reported. However, after pre-sieving, note that the sample is no longer representative, unless otherwise proven.

3.3 Optimization of the liquid dispersion

Liquids, surfactants, and dispersing aids used to disperse powders must:
(i) be transparent at the laser wavelength and practically free from air bubbles or particles;
(ii) have a refractive index that differs from that of the test material;
(iii) be non-solvent of the test material (pure liquid or pre-filtered, saturated solution);
(iv) not alter the size of the test materials (e.g., by solubility, solubility enhancement, or recrystallization effects);
(v) favor easily formation and stability of the dispersion;
(vi) be compatible with the materials used in the instrument (such as O-rings, gaskets, tubing, etc.);
(vii) possess a suitable viscosity to facilitate recirculation, stirring and filtration.

Surfactants and/or dispersing aids are often used to wet the particles and to stabilize the dispersion. For weak acids and weak bases, buffering of the dispersing medium at low or high pH respectively can assist in identifying a suitable dispersant.

A preliminary check of the dispersion quality can be performed by visual or microscopic inspection. It is also possible to take fractional samples out of a well-mixed stock dispersion. Such stock dispersions are formed by adding a liquid to the sample while mixing it with, for example, a glass rod, a spatula or a vortex mixer. Care must be taken to ensure the transfer of a representative sample and that settling of larger particles does not occur. Therefore a sample paste is prepared or sampling is carried out quickly from a suspension maintained under agitation.

3.4 Optimization of the gas dispersion

For sprays and dry powder dispersions, a compressed gas free from oil, water and particles may be used. To remove such materials from the compressed gas, a dryer with a filter can be used. Any vacuum unit should be located away from the measurement zone, so that its output does not disturb the measurement.

3.5 Determination of the concentration range

In order to produce an acceptable signal-to-noise ratio in the detector, the particle concentration in the dispersion must exceed a minimum level. Likewise, it must be below a maximum level in order to avoid multiple scattering. The concentration range is influenced by the width of the laser beam, the path length of the measurement zone, the optical properties of the particles, and the sensitivity of the detector elements.

In view of the above, measurements must be performed at different particle concentrations to determine the appropri-
ate concentration range for any typical sample of material. 
(Note: in different instruments, particle concentrations are usually represented by differently scaled and differently named numbers, e.g. obscuration, optical concentration, proportional number of total mass).

3.6. Determination of the measuring time

The time of measurement, the reading time of the detector and the acquisition frequency is determined experimentally in accordance with the required precision. Generally, the time for measurement permits a large number of detector scans or sweeps at short time intervals.

3.7. Selection of an appropriate optical model

Most instruments use either the Fraunhofer or the Mie theory, though other approximation theories are sometimes applied for calculation of the scattering matrix. The choice of the theoretical model depends on the intended application and the different assumptions (size, absorbance, refractive index, roughness, crystal orientation, mixture, etc.) made for the test material. If the refractive index values (real and imaginary parts for the used wavelength) are not exactly known, then the Fraunhofer approximation or the Mie theory with a realistic estimate of the refractive index can be used. The former has the advantages that it is simple and it does not need refractive index values; the latter usually provides less-biased particle-size distributions for small particles. For instance, if the Fraunhofer model is used for samples containing an appreciable amount of small, transparent particles, a significantly large amount of small particles may be calculated. In order to obtain traceable results, it is essential to document the refractive index values used, since small differences in the values assumed for the real and imaginary part of the complex refractive index may cause significant differences in the resulting particle-size distributions. Small values of the imaginary part of the refractive index (about 0.01 – 0.1) are often applied to allow the correction of the absorbance for the surface roughness of the particles. It should be noted, in general, that the optical properties of the substance to be tested, as well as the structure (e.g. shape, surface roughness and porosity) bear upon the final result.

3.8. Validation

Typically, the validity of a procedure may be assessed by the evaluation of its specificity, linearity, range, accuracy, precision and robustness. In particle-size analysis by laser light diffraction, specificity as defined by ICH is not applicable as it is not possible to discriminate different components into a sample, as is neither possible to discriminate between agglomerates from dispersed particles unless properly complemented by microscopic techniques. Exploring a liner relationship between concentration and response, or a mathematical model for interpolation, is not applicable to this procedure. Rather than evaluating linearity, this method requires the definition of a concentration range within which the result of the measurements does not vary significantly. Concentrations below that range produce an error due to a poor signal to noise ratio, while concentrations above that range produce an error due to multiple scattering. The range depends mostly in the instrument hardware. Accuracy should be confirmed through an appropriate instrument qualification and comparison with microscopy, while precision may be assessed by means of a repeatability determination. The attainable repeatability of the method mainly depends on the characteristics of the material (milled/not milled, robust/fragile, width of its size distribution, etc.), whereas the required repeatability depends on the purpose of the measurement. Mandatory limits cannot be specified in this chapter, as repeatabilities (different sample preparations) may vary appreciably from one substance to another. However, it is good practice to aim at acceptance criteria for repeatability such as RSD (%$\leq 10\%$ [$n = 6$] for any central value of the distribution (e.g. for $x_0$). Values at the sides of the distribution (e.g. $x_{50}$ and $x_{100}$) are oriented towards less stringent acceptance criteria such as RSD $\leq 15\%$ [$n = 6$]. Below $10\mu m$, these values must be doubled. Robustness may be tested during the selection and optimization of the dispersion media and forces. The change of the dispersing energy may be monitored by the change in the particle-size distribution.

4. Measurement

4.1. Precautions

(i) never look into the direct path of the laser beam or its reflections;
(ii) earth all instrument components to prevent ignition of solvents or dust explosions;
(iii) check the instrument set-up (e.g. warm-up, required measuring range and lens, appropriate working distance, position of the detector, no direct bright daylight);
(iv) in the case of wet dispersions, avoid air bubbles, evaporation of liquid, schlieren or other inhomogeneities in the dispersion; similarly, avoid improper mass-flow from the disperser or turbulent air-flow in the case of dry dispersions; such effects can cause erroneous particle-size distributions.

4.2. Measurement of the light scattering of dispersed sample(s)

After proper alignment of the optical part of the instrument, a blank measurement of the particle-free dispersion medium must be performed using the same method as that used for the measurement of the sample. The background signal must be below an appropriate threshold. The detector data are saved in order to subtract them later from the data obtained with the sample. The sample dispersion is measured according to the developed method.

For each detector element, an average signal is calculated, sometimes together with its standard deviation. The magnitude of the signal from each detector element depends upon the detection area, the light intensity and the quantum efficiency. The co-ordinates (size and position) of the detector elements together with the focal distance of the lens determine the range of scattering angles for each element. Most instruments also measure the intensity of the central (unscattered) laser beam. The ratio of the intensity of a dispersed sample to that in its absence (the blank measurement) indicates the proportion of scattered light and hence the particle concentration.

4.3. Conversion of scattering pattern into particle-size distribution

This deconvolution step is the inverse of the calculation of a scattering pattern for a given particle-size distribution. The assumption of spherical particle shape is particularly important as most algorithms use the mathematical solution for scattering from spherical particles. Furthermore, the measured data always contain some random and systematic errors, which may vitiate the size distributions. Several mathematical procedures have been developed for use in the available instruments. They contain some weighting of devi-
ations between measured and calculated scattering patterns (e.g. least squares), some constraints (e.g. non-negativity for amounts of particles), and/or some smoothing of the size distribution curve.

The algorithms used are specific to each make and model of equipment, and are proprietary. The differences in the algorithms between different instruments may give rise to differences in the calculated particle-size distributions.

4.4. Replicates

The number of replicate measurements (with individual sample preparations) to be performed, depends on the required measurement precision. It is recommended to set this number in a substance-specific method.

5. Reporting of results

The particle-size distribution data are usually reported as cumulative undersize distribution and/or as density distribution by volume. The symbol \( x \) is used to denote the particle size, which in turn is defined as the diameter of a volume-equivalent sphere. \( Q_3(x) \) denotes the volume fraction undersize at the particle size \( x \). In a graphical representation, \( x \) is plotted on the abscissa and the dependent variable \( Q_3 \) on the ordinate. Most common characteristic values are calculated from the particle-size distribution by interpolation. The particle sizes at the undersize values of 10\%, 50\%, and 90\% (denoted as \( x_{10}, x_{50}, \) and \( x_{90} \) respectively) are frequently used. \( x_{50} \) is also known as the median particle size. It is recognized that the symbol \( d \) is also widely used to designate the particle size, thus the symbol \( x \) may be replaced by \( d \).

Moreover, sufficient information must be documented about the sample, the sample preparation, the dispersion conditions, and the cell type. As the results depend on the particular instrument, data analysis program, and optical model used, these details must also be documented.

6. Control of the instrument performance

Use the instrument according to the manufacturer’s instructions and carry out the prescribed qualifications at an appropriate frequency, according to the use of the instrument and substances to be tested.

6.1. Calibration

Laser diffraction systems, although assuming idealized properties of the particles, are based on first principles of laser light scattering. Thus, calibration in the strict sense is not required. However, it is still necessary to confirm that the instrument is operating correctly. This can be undertaken using any certified reference material that is acceptable in industrial practice. The entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, measurement, and the deconvolution procedure. It is essential that the total operational procedure is fully described.

The preferred certified reference materials consist of spherical particles of a known distribution. They must be certified as to the mass-percentage size distribution by an absolute technique, if available, and used in conjunction with an agreed, detailed operation procedure. It is essential that the real and imaginary parts of the complex refractive index of the material are indicated if the Mie theory is applied in data analysis. The representation of the particle-size distribution by volume will equal that of the distribution by mass, provided that the density of the particles is the same for all size fractions.

The response of a laser diffraction instrument is considered to meet the requirements if the mean value of \( x_{50} \) from at least 3 independent measurements does not deviate by more than 3\% from the certified range of values of the certified reference material. The mean values for \( x_{10} \) and \( x_{90} \) must not deviate by more than 5\% from the certified range of values. Below 10 \( \mu m \), these values must be doubled.

Although the use of materials consisting of spherical particles is preferable, non-spherical particles may also be employed. Preferably, these particles have certified or typical values from laser diffraction analysis performed according to an agreed, detailed operating procedure. The use of reference values from methods other than laser diffraction may cause a significant bias. The reason for this bias is that the different principles inherent in the various methods may lead to different sphere-equivalent diameters for the same non-spherical particle.

Although the use of certified reference materials is preferred, other well-defined reference materials may also be employed. They consist of substances of typical composition and particle-size distribution for a specified class of substances. Their particle-size distribution has proven to be stable over time. The results must comply with previously determined data, with the same precision and bias as for the certified reference material.

6.2. Qualification of the system

In addition to the calibration, the performance of the instrument must be qualified at regular time intervals or as frequently as appropriate. This can be undertaken using any suitable reference material as mentioned in the previous paragraph.

The qualification of the system is based on the concept that the equipment, electronics, software and analytical operations constitute an integral system, which can be evaluated as an entity. Thus the entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, and the measurement and deconvolution procedure. It is essential that the total operational procedure is fully described.

In general, unless otherwise specified in the individual monograph, the response of a laser diffraction instrument is considered to meet the requirements if the \( x_{50} \) value does not deviate by more than 10\% from the range of values of the reference material. If optionally the values at the sides of the distribution are evaluated (e.g. \( x_{10} \) and \( x_{90} \)), then these values must not deviate by more than 15\% from the certified range of values. Below 10 \( \mu m \), these values must be doubled.

Note 1: For calibration of the instrument stricter requirements are laid down in 6.1. Calibration.


**Powder Fineness**

This classification is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

A simple descriptive classification of powder fineness is provided in this chapter. Sieving is most suitable where a majority of the particles are larger than about 75 \( \mu m \), although it can be used for some powders having smaller particle sizes where the method can be validated. Light
diffraction is also a widely used technique for measuring the size of a wide range of particles. Where the cumulative distribution has been determined by analytical sieving or by application of other methods, particle size may be characterized in the following manner:

- \( x_{90} \): particle size corresponding to 90% of the cumulative undersize distribution
- \( x_{50} \): median particle size (i.e., 50% of the particles are smaller and 50% of the particles are larger)
- \( x_{10} \): particle size corresponding to 10% of the cumulative undersize distribution

It is recognized that the symbol \( d \) is also widely used to designate these values. Therefore, the symbols \( d_{90} \), \( d_{50} \), \( d_{10} \) may be used.

The following parameters may be defined based on the cumulative distribution.

- \( Q_r(x) \): cumulative distribution of particles with a dimension less than or equal to \( x \) where the subscript \( r \) reflects the distribution type

<table>
<thead>
<tr>
<th>( r )</th>
<th>Distribution type</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Number</td>
</tr>
<tr>
<td>1</td>
<td>Length</td>
</tr>
<tr>
<td>2</td>
<td>Area</td>
</tr>
<tr>
<td>3</td>
<td>Volume</td>
</tr>
</tbody>
</table>

Therefore, by definition:

- \( Q_3(x) = 0.90 \) when \( x = x_{90} \)
- \( Q_3(x) = 0.50 \) when \( x = x_{50} \)
- \( Q_3(x) = 0.10 \) when \( x = x_{10} \)

An alternative but less informative method of classifying powder fineness is by use of the descriptive terms in the following table.

<table>
<thead>
<tr>
<th>Classification of powders by fineness</th>
<th>( x_{50} ) (μm)</th>
<th>Cumulative distribution by volume basis, ( Q_3(x) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse</td>
<td>&gt; 355</td>
<td>( Q_3(355) &lt; 0.50 )</td>
</tr>
<tr>
<td>Moderately fine</td>
<td>180–355</td>
<td>( Q_3(180) &lt; 0.50 ) and ( Q_3(355) \geq 0.50 )</td>
</tr>
<tr>
<td>Fine</td>
<td>125–180</td>
<td>( Q_3(125) \leq 0.50 ) and ( Q_3(180) \geq 0.50 )</td>
</tr>
<tr>
<td>Very fine</td>
<td>( \leq 125 )</td>
<td>( Q_3(125) \geq 0.50 )</td>
</tr>
</tbody>
</table>

**Powder Flow**

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The widespread use of powders in the pharmaceutical industry has generated a variety of methods for characterizing powder flow. Not surprisingly, scores of references appear in the pharmaceutical literature, attempting to correlate the various measures of powder flow to manufacturing properties. The development of such a variety of test methods was inevitable; powder behavior is multifaceted and thus complicates the effort to characterize powder flow. The purpose of this chapter is to review the methods for characterizing powder flow that have appeared most frequently in the pharmaceutical literature. In addition, while it is clear that no single and simple test method can adequately characterize the flow properties of pharmaceutical powders, this chapter proposes the standardization of test methods that may be valuable during pharmaceutical development.

Four commonly reported methods for testing powder flow are (1) angle of repose, (2) compressibility index or Hausner ratio, (3) flow rate through an orifice, and (4) shear cell. In addition, numerous variations of each of these basic methods are available. Given the number of test methods and variations, standardizing the test methodology, where possible, would be advantageous.

With this goal in mind, the most frequently used methods are discussed below. Important experimental considerations are identified and recommendations are made regarding standardization of the methods. In general, any method of measuring powder flow should be practical, useful, reproducible, sensitive, and yield meaningful results. It bears repeating that no one simple powder flow method will adequately or completely characterize the wide range of flow properties experienced in the pharmaceutical industry. An appropriate strategy may well be the use of multiple standardized test methods to characterize the various aspects of powder flow as needed by the pharmaceutical scientist.

1. **Angle of repose**

The angle of repose has been used in several branches of science to characterize the flow properties of solids. Angle of repose is a characteristic related to interparticulate friction, or resistance to movement between particles. Angle of repose test results are reported to be very dependent upon the method used. Experimental difficulties arise due to segregation of material and consolidation or aeration of the powder as the cone is formed. Despite its difficulties, the method continues to be used in the pharmaceutical industry, and a number of examples demonstrating its value in predicting manufacturing problems appear in the literature.

The angle of repose is the constant, three-dimensional angle (relative to the horizontal base) assumed by a cone-like pile of material formed by any of several different methods (described briefly below).

1.1. **Basic methods for angle of repose**

A variety of angle of repose test methods are reported in the literature. The most common methods for determining the static angle of repose can be classified based on two important experimental variables:

(i) The height of the “funnel” through which the powder passes may be fixed relative to the base, or the height may be varied as the pile forms.

(ii) The base upon which the pile forms may be of fixed diameter or the diameter of the powder cone may be allowed to vary as the pile forms.

1.2. **Variations in angle of repose methods**

In addition to the above methods, variations of them have been used to some extent.

(i) Drained angle of repose: This is determined by
allowing an excess quantity of material positioned above a fixed diameter base to "drain" from the container. Formation of a cone of powder on the fixed diameter base allows determination of the drained angle of repose.

(ii) Dynamic angle of repose: This is determined by forming a cone of powder on a fixed base with a retaining lip to retain a layer of powder on the base. The base should be free of vibration. Vary the height of the funnel to care- fully build up a symmetrical cone of powder. Care should be taken to prevent vibration as the funnel is moved. The angle of repose is defined by the plane separating those particles sliding down the top layer of the powder and those particles that are rotating with the drum (with roughened surface).

1.3. Angle of repose general scale of flowability

While there is some variation in the qualitative description of powder flow using the angle of repose, much of the pharmaceutical literature appears to be consistent with the classification by Carr\(^1\), which is shown in Table 1. There are examples of formulations with an angle of repose in the range of 40 to 50 degrees that manufactured satisfactorily. When the angle of repose exceeds 50 degrees, the flow is rarely acceptable for manufacturing purposes.

| Table 1 Flow properties and corresponding angles of repose\(^1\) |
|----------------|-----------------|
| Flow property | Angle of repose (degrees) |
| Excellent     | 25 – 30          |
| Good          | 31 – 35          |
| Fair          | 36 – 40          |
| Passable      | 41 – 45          |
| Poor          | 46 – 55          |
| Very poor     | 56 – 65          |
| Very, very poor | > 66          |

1.4. Experimental considerations for angle of repose

Angle of repose is not an intrinsic property of the powder, that is to say, it is very much dependent upon the method used to form the cone of powder. On this subject, the existing literature raises these important considerations:

(i) The peak of the cone of powder can be distorted by the impact of powder from above. By carefully building the powder cone, the distortion caused by impact can be minimized.

(ii) The nature of the base upon which the powder cone is formed influences the angle of repose. It is recommended that the powder cone be formed on a "common base", which can be achieved by forming the cone of powder on a layer of powder. This can be done by using a base of fixed diameter with a protruding outer edge to retain a layer of powder upon which the cone is formed.

1.5. Recommended procedure for angle of repose

Form the angle of repose on a fixed base with a retaining lip to retain a layer of powder on the base. The base should be free of vibration. Vary the height of the funnel to carefully build up a symmetrical cone of powder. Care should be taken to prevent vibration as the funnel is moved. The funnel height should be maintained approximately 2 – 4 cm from the top of the powder pile as it is being formed in order to minimize the impact of falling powder on the tip of the cone. If a symmetrical cone of powder cannot be successful-

ly or reproducibly prepared, this method is not appropriate. Determine the angle of repose by measuring the height of the cone of powder and calculating the angle of repose, \(\alpha\), from the following equation:

\[
\tan \alpha = \frac{\text{height}}{(\text{base} \times 0.5)}
\]

2. Compressibility index and Hausner ratio

In recent years the compressibility index and the closely related Hausner ratio have become the simple, fast and popular methods of predicting powder flow characteristics. The compressibility index has been proposed as an indirect measure of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials because all of these can influence the observed compressibility index. The compressibility index and the Hausner ratio are determined by measuring both the bulk volume and tapped volume of a powder.

2.1. Basic methods for compressibility index and Hausner ratio

While there are some variations in the method of determining the compressibility index and Hausner ratio, the basic procedure is to measure (1) the unsettled apparent volume, \(V_o\), and (2) the final tapped volume, \(V_t\), of the powder after tapping the material until no further volume changes occur. The compressibility index and the Hausner ratio are calculated as follows:

\[
\text{Compressibility Index} = \frac{V_o - V_t}{V_o} \times 100
\]

\[
\text{Hausner Ratio} = \frac{V_o}{V_t}
\]

Alternatively, the compressibility index and Hausner ratio may be calculated using measured values for bulk density \((\rho_{\text{bulk}})\) and tapped density \((\rho_{\text{tapped}})\) as follows:

\[
\text{Compressibility Index} = \frac{\rho_{\text{bulk}} - \rho_{\text{tapped}}}{\rho_{\text{tapped}}} \times 100
\]

\[
\text{Hausner Ratio} = \frac{\rho_{\text{tapped}}}{\rho_{\text{bulk}}}
\]

In a variation of these methods, the rate of consolidation is sometimes measured rather than, or in addition to, the change in volume that occurs on tapping. For the compressibility index and the Hausner ratio, the generally accepted scale of flowability is given in Table 2.

| Table 2 Scale of flowability\(^1\) |
|----------------|-----------------|
| Compressibility index (%) | Flow character | Hausner ratio |
| \(\geq 10\) | Excellent | 1.00 - 1.11 |
| 11 – 15 | Good | 1.12 – 1.18 |
| 16 – 20 | Fair | 1.19 – 1.25 |
| 21 – 25 | Passable | 1.26 – 1.34 |
| 26 – 31 | Poor | 1.35 – 1.45 |
| 32 – 37 | Very poor | 1.46 – 1.59 |
| > 38 | Very, very poor | > 1.60 |

2.2. Experimental considerations for the compressibility index and Hausner ratio

Compressibility index and Hausner ratio are not intrinsic properties of the powder, that is to say, they are dependent upon the methodology used. The existing literature points out several important considerations affecting the determination of the (1) unsettled apparent volume, \(V_o\), (2) the final tapped volume, \(V_t\), (3) the bulk density, \(\rho_{\text{bulk}}\), and (4) the tapped density, \(\rho_{\text{tapped}}\):

...
Flow rate through an orifice can be used only with free-flowing materials. Provided that the height of the powder bed (the ‘head’ of powder) is much greater than the diameter of the orifice, the flow rate is virtually independent of the powder head. Use a cylinder as the container because the cylinder material should have little effect on flow. This configuration results in flow rate being determined by the movement of powder over powder rather than powder along the wall of the container. Powder flow rate often increases when the height of the powder column is less than two times the diameter of the column. The orifice should be circular and the cylinder should be free of vibration. General guidelines for dimensions of the cylinder are as follows:

1. Diameter of opening > 6 times the diameter of the particles
2. Diameter of the cylinder > 2 times the diameter of the opening

Use of a hopper as the container may be appropriate and representative of flow in a production situation. It is not advisable to use a funnel, particularly one with a stem, because flow rate will be determined by the size and length of the stem as well as the friction between the stem and the powder. A truncated cone may be appropriate, but flow will be influenced by the powder—wall friction coefficient, thus, selection of an appropriate construction material is important.

For the opening in the cylinder, use a flat-faced bottom plate with the option to vary orifice diameter to provide maximum flexibility and better ensure a powder-over-powder flow pattern. Rate measurement can be either discrete or continuous. Continuous measurement using an electronic balance can more effectively detect momentary flow rate variations.

4. Shear cell methods

In an effort to put powder flow studies and hopper design on a more fundamental basis, a variety of powder shear testers and methods that permit more thorough and precisely defined assessment of powder flow properties have been developed. Shear cell methodology has been used extensively in the study of pharmaceutical materials. From these methods, a wide variety of parameters can be obtained, including the yield loci representing the shear stress-shear strain relationship, the angle of internal friction, the unconfined yield strength, the tensile strength, and a variety of derived parameters such as the flow factor and other flowability indices. Because of the ability to more precisely control experimental parameters, flow properties can also be determined as a function of consolidation load, time, and other environmental conditions. The methods have been successfully used to determine critical hopper and bin parameters.
4.1. Basic methods for shear cell

One type of shear cell is the cylindrical shear cell which is split horizontally, forming a shear plane between the lower stationary base and the upper movable portion of the shear cell ring. After powder bed consolidation in the shear cell (using a well-defined procedure), the force necessary to shear the powder bed by moving the upper ring is determined. Annular shear cell designs offer some advantages over the cylindrical shear cell design, including the need for less material. A disadvantage, however, is that because of its design, the powder bed is not sheared as uniformly because material on the outside of the annulus is sheared more than material in the inner region. A third type of shear cell (plate-type) consists of a thin sandwich of powder between a lower stationary rough surface and an upper rough surface that is moveable.

All of the shear cell methods have their advantages and disadvantages, but a detailed review is beyond the scope of this chapter. As with the other methods for characterizing powder flow, many variations are described in the literature. A significant advantage of shear cell methodology in general is a greater degree of experimental control. The methodology generally is rather time-consuming and requires significant amounts of material and a well-trained operator.

4.2. Recommendations for shear cell

The many existing shear cell configurations and test methods provide a wealth of data and can be used very effectively to characterize powder flow. They are also helpful in the design of equipment such as hoppers and bins.

Because of the diversity of available equipment and experimental procedures, no specific recommendations regarding methodology are presented in this chapter. It is recommended that the results of powder flow characterization using shear cell methodology include a complete description of equipment and methodology used.

References

Solid and Particle Densities

Density of a solid or a powder as a state of aggregation has different definitions depending on the way of including of the interparticulate and intraparticulate voids that exist between the particles or inside the powder. Different figures are obtained in each case, and there are different practical meanings. Generally, there are three levels of definitions of the solid or powder density.

1) Crystal density: It is assumed that the system is homogeneous with no intraparticulate void. Crystal density is also called true density.
2) Particle density: The sealed pores or the experimentally non-accessible open pores is also included as a part of the volumes of the solid or the powder.
3) Bulk density: The interparticulate void formed in the powder bed is also included as a part of the volumes of the solid or the powder. Bulk density is also called apparent density. Generally, the powder densities at loose packing and at tapping are defined as the bulk density and the tapped density, respectively.

Generally, the densities of liquid or gas are affected only by temperature and pressure, but the solid or powder density is affected by the state of aggregation of the molecules or the particles. Therefore, the solid or powder densities naturally vary depending on crystal structure or crystallinity of the substance concerned, and also varies depending on the method of preparation or handling if the sample is amorphous form or partially amorphous. Consequently, even in a case that two solids or powders are chemically identical, it may be possible that the different figures of density are obtained if their crystal structures are different. As the solid or powder particle densities are important physical properties for the powdered pharmaceutical drugs or the powdered raw materials of drugs, the Japanese Pharmacopoeia specifies each density determination as “Powder Particle Density Determination” for the particle density and as “Determination of Bulk and Tapped Densities” for the bulk density.

The solid or powder densities are expressed in mass per unit volume (kg/m³), and generally expressed in g/cm³ (1 g/cm³ = 1000 kg/m³).

Crystal Density

The crystal density of a substance is the average mass per unit volume, exclusive of all voids that are not a fundamental part of the molecular packing arrangement. It is an intrinsic property concerning the specific crystal structure of the substance, and is not affected by the method of determination. The crystal density can be determined either by calculation or by simple measurement.

A. The calculated crystal density is obtained using:
1) For example, the crystallographic data (volume and composition of the unit cell) obtained by indexing the perfect crystal X-ray diffraction data from single crystal or the powder X-ray diffraction data.
2) Molecular mass of the substance.

B. The measured crystal density is obtained as the mass to volume ratio after measuring the single crystal mass and volume.

Particle Density

The particle density takes account both the crystal density and the intraparticulate porosity (sealed and/or experimentally non-accessible open pores) as a part of the particle volume. The particle density depends on the value of the volume determined, and the volume in turn depends on the method of measurement. Particle density can be determined either by gas displacement pycnometry or mercury porosimetry, but the Japanese Pharmacopoeia specifies the pycnometry as the “Powder Particle Density Determination”.

A. The pycnometric density is obtained by assuming that the volume of the gas displaced, which is measured with the gas displacement pycnometer, is equivalent to that of a known mass of the powder. In pycnometric density measurements, any volume with the open pores accessible to the gas is not included as a part of volume of the powder, but the sealed pores or pores inaccessible to the gas is included as a part of the volume of the powder. Due to the high diffusivity of helium which can penetrate to most open pores, it is recommendable as the measurement gas of particle density. Therefore, the pycnometric particle density of a finely milled powder is generally not very different from the crystal density. Hence, the particle density by this method is the best estimate of the true density of an amorphous or partially

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crystalline sample, and can be widely used for manufacturing control of the processed pharmaceutical powder samples.

B. The mercury porosimetric density is also called granular density. This method also includes the sealed pores as a part of the volumes of the solid or the powder, but excludes the volume only from the open pores larger than some size limit. This pore size limit or minimal access diameter depends on the maximal mercury intrusion pressure applied during the measurement and under normal operating pressure, the mercury does not penetrate the finestest pores accessible to helium. Since this method is capable of measuring the density which corresponds to the pore size limit at each mercury intrusion pressure, the various granular densities can be obtained from one sample.

**Bulk Density and Tapped Density**

The bulk density of a powder includes the contribution of interparticulate void volume as a part of the volume of the powder. Therefore, the bulk density depends on both the powder particle density and the space arrangement of particles in the powder bed. Further, since the slightest disturbance of the bed may result in variation of the space arrangement, it is often very difficult to determine the bulk density with good reproducibility. Therefore, it is essential to specify how the determination was made upon reporting the bulk density.

The Japanese Pharmacopoeia specifies "Determination of Bulk and Tapped Densities". A. The bulk density is determined by measuring the apparent volume of a known mass of powder sample that has been passed through a screen in a graduated cylinder (constant mass method). Separately, the Pharmacopoeia specifies the method of determining bulk density by measuring the mass of powder in a vessel having a known volume (constant volume method).

B. The tapped density is obtained by mechanically tapping a measuring cylinder containing a powder sample. After determining the initial bulk volume, carry out tapping under a fixed measurement condition (tapping rate and drop height), and the measurement is carried out repeatedly until the bulk volume variation obtained at consecutive two measurements is within an acceptable range (constant mass method). Separately, the Pharmacopoeia specifies the method of determining the tapped density by measuring the mass of a fixed volume of the tapped powder (constant volume method).

**G3 Biotechnological/Biological Products**

**Amino Acid Analysis**

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

Amino acid analysis refers to the methodology used to determine the amino acid composition or content of proteins, peptides, and other pharmaceutical preparations. Proteins and peptides are macromolecules consisting of covalently bonded amino acid residues organized as a linear polymer. The sequence of the amino acids in a protein or peptide determines the properties of the molecule. Proteins are considered large molecules that commonly exist as folded structures with a specific conformation, while peptides are smaller and may consist of only a few amino acids. Amino acid analysis can be used to quantify protein and peptides, to determine the identity of proteins or peptides based on their amino acid composition, to support protein and peptide structure analysis, to evaluate fragmentation strategies for peptide mapping, and to detect atypical amino acids that might be present in a protein or peptide. It is necessary to hydrolyze a protein/peptide to its individual amino acid constituents before amino acid analysis. Following protein/peptide hydrolysis, the amino acid analysis procedure can be the same as that practiced for free amino acids in other pharmaceutical preparations. The amino acid constituents of the test sample are typically derivatized for analysis.

**Apparatus**

Methods used for amino acid analysis are usually based on a chromatographic separation of the amino acids present in the test sample. Current techniques take advantage of the automated chromatographic instrumentation designed for analytical methodologies. An amino acid analysis instrument will typically be a low-pressure or high-pressure liquid chromatograph capable of generating mobile phase gradients that separate the amino acid analytes on a chromatographic column. The instrument must have postcolumn derivatization capability, unless the sample is analyzed using precolumn derivatization. The detector is usually an ultraviolet-visible or fluorescence detector depending on the derivatization method used. A recording device (e.g., integrator) is used for transforming the analog signal from the detector and for quantitation. It is preferred that instrumentation be dedicated particularly for amino acid analysis.

**General Precautions**

Background contamination is always a concern for the analyst in performing amino acid analysis. High purity reagents are necessary (e.g., low purity hydrochloric acid can contribute to glycine contamination). Analytical reagents are changed routinely every few weeks using only high-pressure liquid chromatography (HPLC) grade solvents. Potential microbial contamination and foreign material that might be present in the solvents are reduced by filtering solvents before use, keeping solvent reservoirs covered, and not placing amino acid analysis instrumentation in direct sunlight. Laboratory practices can determine the quality of the amino acid analysis. Place the instrumentation in a low traffic area of the laboratory. Keep the laboratory clean. Clean and calibrate pipets according to a maintenance schedule. Keep pipet tips in a covered box; the analysts may not handle pipet tips with their hands. The analysts may wear powder-free latex or equivalent gloves. Limit the number of times a test sample vial is opened and closed because dust can contribute to elevated levels of glycine, serine, and alanine.

A well-maintained instrument is necessary for acceptable amino acid analysis results. If the instrument is used on a routine basis, it is to be checked daily for leaks, detector and lamp stability, and the ability of the column to maintain resolution of the individual amino acids. Clean or replace all instrument filters and other maintenance items on a routine
schedule.

Reference Standard Material

Acceptable amino acid standards are commercially available for amino acid analysis and typically consist of an aqueous mixture of amino acids. When determining amino acid composition, protein or peptide standards are analyzed with the test material as a control to demonstrate the integrity of the entire procedure. Highly purified bovine serum albumin has been used as a protein standard for this purpose.

Calibration of Instrumentation

Calibration of amino acid analysis instrumentation typically involves analyzing the amino acid standard, which consists of a mixture of amino acids at a number of concentrations, to determine the response factor and range of analysis for each amino acid. The concentration of each amino acid in the standard is known. In the calibration procedure, the analyst dilutes the amino acid standard to several different analyte levels within the expected linear range of the amino acid analysis technique. Then, replicates at each of the different analyte levels can be analyzed. Peak areas obtained for each amino acid are plotted versus the known concentration for each of the amino acids in the standard dilution. These results will allow the analyst to determine the range of amino acid concentrations where the peak area of a given amino acid is an approximately linear function of the amino acid concentration. It is important that the analyst prepare the samples for amino acid analysis so that they are within the analytical limits (e.g., linear working range) of the technique employed in order to obtain accurate and repeatable results.

Four to six amino acid standard levels are analyzed to determine a response factor for each amino acid. The response factor is calculated as the average peak area or peak height per nmol of amino acid present in the standard. A calibration file consisting of the response factor for each amino acid is prepared and used to calculate the concentration of each amino acid present in the test sample. This calculation involves dividing the peak area corresponding to a given amino acid by the response factor for that amino acid to give the nmol of the amino acid. For routine analysis, a single-point calibration may be sufficient; however, the calibration file is updated frequently and tested by the analysis of analytical controls to ensure its integrity.

Repeatability

Consistent high quality amino acid analysis results from an analytical laboratory require attention to the repeatability of the assay. During analysis of the chromatographic separation of the amino acids or their derivatives, numerous peaks can be observed on the chromatogram that correspond to the amino acids. The large number of peaks makes it necessary to have an amino acid analysis system that can repeatedly identify the peaks based on retention time and integrate the peak areas for quantitation. A typical repeatability evaluation involves preparing a standard amino acid solution and analyzing many replicates (i.e., six analyses or more) of the same standard solution. The relative standard deviation (RSD) is determined for the retention time and integrated peak area of each amino acid. An evaluation of the repeatability is expanded to include multiple assays conducted over several days by different analysts. Multiple assays include the preparation of standard dilutions from starting materials to determine the variation due to sample handling. Often the amino acid composition of a standard protein (e.g., bovine serum albumin) is analyzed as part of the repeatability evaluation. By evaluating the replicate variation (i.e., RSD), the laboratory can establish analytical limits to ensure that the analyses from the laboratory are under control. It is desirable to establish the lowest practical variation limits to ensure the best results. Areas to focus on to lower the variability of the amino acid analysis include sample preparation, high background spectral interference due to quality of reagents and/or laboratory practices, instrument performance and maintenance, data analysis and interpretation, and analyst performance and habits. All parameters involved are fully investigated in the scope of the validation work.

Sample Preparation

Accurate results from amino acid analysis require purified protein and peptide samples. Buffer components (e.g., salts, urea, detergents) can interfere with the amino acid analysis and are removed from the sample before analysis. Methods that utilize postcolumn derivatization of the amino acids are generally not affected by buffer components to the extent seen with precolumn derivatization methods. It is desirable to limit the number of sample manipulations to reduce potential background contamination, to improve analyte recovery, and to reduce labor. Common techniques used to remove buffer components from protein samples include the following methods: (1) injecting the protein sample onto a reversed-phase HPLC system, eluting the protein with a volatile solvent containing a sufficient organic component, and drying the sample in a vacuum centrifuge; (2) dialysis against a volatile buffer or water; (3) centrifugal ultrafiltration for buffer replacement with a volatile buffer or water; (4) precipitating the protein from the buffer using an organic solvent (e.g., acetone); and (5) gel filtration.

Internal Standards

It is recommended that an internal standard be used to monitor physical and chemical losses and variations during amino acid analysis. An accurately known amount of internal standard can be added to a protein solution prior to hydrolysis. The recovery of the internal standard gives the general recovery of the amino acids of the protein solution. Free amino acids, however, do not behave in the same way as protein-bound amino acids during hydrolysis because their rates of release or destruction are variable. Therefore, the use of an internal standard to correct for losses during hydrolysis may give unreliable results. It will be necessary to take this point under consideration when interpreting the results. Internal standards can also be added to the mixture of amino acids after hydrolysis to correct for differences in sample application and changes in reagent stability and flow rates. Ideally, an internal standard is an unnaturally occurring primary amino acid that is commercially available and inexpensive. It should also be stable during hydrolysis, its response factor should be linear with concentration, and it needs to elute with a unique retention time without overlapping other amino acids. Commonly used amino acid standards include norleucine, nitrotyrosine, and α-amino butyric acid.

Protein Hydrolysis

Hydrolysis of protein and peptide samples is necessary for amino acid analysis of these molecules. The glassware used
for hydrolysis must be very clean to avoid erroneous results. Glove powders and fingerprints on hydrolysis tubes may cause contamination. To clean glass hydrolysis tubes, boil tubes for 1 hour in 1 mol/L hydrochloric acid or soak tubes in concentrated nitric acid or in a mixture of concentrated hydrochloric acid and concentrated nitric acid (1:1). Clean hydrolysis tubes are rinsed with high-purity water followed by a rinse with HPLC grade methanol, dried overnight in an oven, and stored covered until use. Alternatively, pyrolysis of clean glassware at 500°C for 4 hours may also be used to eliminate contamination from hydrolysis tubes. Adequate disposable laboratory material can also be used.

Acid hydrolysis is the most common method for hydrolyzing a protein sample before amino acid analysis. The acid hydrolysis technique can contribute to the variation of the analysis due to complete or partial destruction of several amino acids. Tryptophan is destroyed; serine and threonine are partially destroyed; methionine might undergo oxidation; and cysteine is typically recovered as cystine (but cysteine recovery is usually poor because of partial destruction or reduction to cysteine). Application of adequate vacuum (≤ less than 200 μm of mercury or 26.7 Pa) or introduction of an inert gas (argon) in the headspace of the reaction vessel can reduce the level of oxidative destruction. In peptide bonds involving isoleucine and valine the amido bonds of Ile-Ile, Val-Val, Ile-Val, and Val-Ile are partially cleaved; and asparagine and glutamine are deamidated, resulting in aspartic acid and glutamic acid, respectively. The loss of tryptophan, asparagine, and glutamine during an acid hydrolysis limits quantitation to 17 amino acids. Some of the hydrolysis techniques described are used to address these concerns. Some of the hydrolysis techniques described (i.e., Methods 4-11) may cause modifications to other amino acids. Therefore, the benefits of using a given hydrolysis technique are weighed against the concerns with the technique and are tested adequately before employing a method other than acid hydrolysis.

A time-course study (i.e., amino acid analysis at acid hydrolysis times of 24, 48, and 72 hours) is often employed to analyze the starting concentration of amino acids that are partially destroyed or slow to cleave. By plotting the observed concentration of labile amino acids (i.e., serine and threonine) versus hydrolysis time, the line can be extrapolated to the origin to determine the starting concentration of these amino acids. Time-course hydrolysis studies are also used with amino acids that are slow to cleave (e.g., isoleucine and valine). During the hydrolysis time course, the analyst will observe a plateau in these residues. The level of this plateau is taken as the residue concentration. If the hydrolysis time is too long, the residue concentration of the sample will begin to decrease, indicating destruction by the hydrolysis conditions.

An acceptable alternative to the time-course study is to subject an amino acid calibration standard to the same hydrolysis conditions as the test sample. The amino acid in free form may not completely represent the rate of destruction of labile amino acids within a peptide or protein during the hydrolysis. This is especially true for peptide bonds that are slow to cleave (e.g., Ile-Val bonds). However, this technique will allow the analyst to account for some residue destruction. Microwave acid hydrolysis has been used and is rapid but requires special equipment as well as special precautions. The optimal conditions for microwave hydrolysis must be investigated for each individual protein/peptide sample. The microwave hydrolysis technique typically requires only a few minutes, but even a deviation of one minute may give inadequate results (e.g., incomplete hydrolysis or destruction of labile amino acids). Complete proteolysis, using a mixture of proteases, has been used but can be complicated, requires the proper controls, and is typically more applicable to peptides than proteins.

Note: During initial analyses of an unknown protein, experiments with various hydrolysis time and temperature conditions are conducted to determine the optimal conditions.

Method 1
Acid hydrolysis using hydrochloric acid containing phenol is the most common procedure used for protein/peptide hydrolysis preceding amino acid analysis. The addition of phenol to the reaction prevents the halogenation of tyrosine.

**Hydrolysis Solution** 6 mol/L hydrochloric acid containing 0.1% to 1.0% of phenol.

**Procedure—**

**Liquid Phase Hydrolysis** Place the protein or peptide sample in a hydrolysis tube, and dry. [Note: The sample is dried so that water in the sample will not dilute the acid used for the hydrolysis.] Add 200 μL of Hydrolysis Solution per 500 μg of lyophilized protein. Freeze the sample tube in a dry ice-acetone bath, and flame seal in vacuum. Samples are typically hydrolyzed at 110°C for 24 hours in vacuum or inert atmosphere to prevent oxidation. Longer hydrolysis times (e.g., 48 and 72 hours) are investigated if there is a concern that the protein is not completely hydrolyzed.

**Vapor Phase Hydrolysis** This is one of the most common acid hydrolysis procedures, and it is preferred for microanalysis when only small amounts of the sample are available.

Contamination of the sample from the acid reagent is also minimized by using vapor phase hydrolysis. Place vials containing the dried samples in a vessel that contains an appropriate amount of Hydrolysis Solution. The Hydrolysis Solution does not come in contact with the test sample. Apply an inert atmosphere or vacuum (≤ less than 200 μm of mercury or 26.7 Pa) to the headspace of the vessel, and heat to about 110°C for a 24-hour hydrolysis time. Acid vapor hydrolyzes the dried sample. Any condensation of the acid in the sample vials is minimized. After hydrolysis, dry the test sample in vacuum to remove any residual acid.

Method 2
Tryptophan oxidation during hydrolysis is decreased by using mercaptoethanesulfonic acid (MESA) as the reducing acid.

**Hydrolysis Solution** 2.5 mol/L MESA solution.

**Vapor Phase Hydrolysis** About 1 to 100 μg of the protein/peptide under test is dried in a hydrolysis tube. The hydrolysis tube is placed in a larger tube with about 200 μL of the Hydrolysis Solution. The larger tube is sealed in vacuum (about 50 μm of mercury or 6.7 Pa) to vaporize the Hydrolysis Solution. The hydrolysis tube is heated to 170°C to 185°C for about 12.5 minutes. After hydrolysis, the hydrolysis tube is dried in vacuum for 15 minutes to remove the residual acid.

Method 3
Tryptophan oxidation during hydrolysis is prevented by using thioglycolic acid (TGA) as the reducing acid.

**Hydrolysis Solution** A solution containing 7 mol/L hy-
drochloric acid, 10% of trifluoroacetic acid, 20% of thioglycolic acid, and 1% of phenol.

**Vapor Phase Hydrolysis** About 10 to 50 μg of the protein/peptide under test is dried in a sample tube. The sample tube is placed in a larger tube with about 200 μL of the Hydrolysis Solution. The larger tube is sealed in vacuum (about 50 μm of mercury or 6.7 Pa) to vaporize the TGA. The sample tube is heated to 166°C for about 15 to 30 minutes. After hydrolysis, the sample tube is dried in vacuum for 5 minutes to remove the residual acid. Recovery of tryptophan by this method may be dependent on the amount of sample present.

**Method 4**

Cysteine-cystine and methionine oxidation is performed with performic acid before the protein hydrolysis.

**Oxidation Solution** The performic acid is prepared fresh by mixing formic acid and 30 percent hydrogen peroxide (9:1), and incubated at room temperature for 1 hour.

**Procedure** The protein/peptide sample is dissolved in 20 μL of formic acid, and heated at 50°C for 5 minutes; then 100 μL of the Oxidation Solution is added. The oxidation is allowed to proceed for 10 to 30 minutes. In this reaction, cysteine is converted to cysteic acid and methionine is converted to methionine sulfone. The excess reagent is removed from the sample in a vacuum centrifuge. This technique may cause modifications to tyrosine residues in the presence of halides. The oxidized protein can then be acid hydrolyzed using Method 1 or Method 2.

**Method 5**

Cysteine-cystine oxidation is accomplished during the liquid phase hydrolysis with sodium azide.

**Hydrolysis Solution** 6 mol/L hydrochloric acid containing 0.2% of phenol, to which is added sodium azide to obtain a final concentration of 0.2% (w/v). The added phenol prevents halogenation of tyrosine.

**Liquid Phase Hydrolysis** The protein/peptide hydrolysis is conducted at about 110°C for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the sodium azide present in the Hydrolysis Solution. This technique allows better tyrosine recovery than Method 4, but it is not quantitative for methionine. Methionine is converted to a mixture of the parent methionine and its two oxidative products, methionine sulfoxide and methionine sulfone.

**Method 6**

Cysteine-cystine oxidation is accomplished with dimethyl sulfoxide (DMSO).

**Hydrolysis Solution** 6 mol/L hydrochloric acid containing 0.1% to 1.0% of phenol, to which DMSO is added to obtain a final concentration of 2% (v/v).

**Vapor Phase Hydrolysis** The protein/peptide hydrolysis is conducted at about 110°C for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the DMSO present in the Hydrolysis Solution. As an approach to limit variability and compensate for partial destruction, it is recommended to evaluate the cysteic acid recovery from oxidative hydrolyses of standard proteins containing 1 to 8 mol of cysteine. The response factors from protein/peptide hydrolysates are typically about 30%, lower than those for nonhydrolyzed cysteic acid standards. Because histidine, methionine, tyrosine, and tryptophan are also modified, a complete compositional analysis is not obtained with this technique.

**Method 7**

Cysteine-cystine reduction and alkylation is accomplished by a vapor phase pyridylethylation reaction.

**Reducing Solution** Transfer 83.3 μL of pyridine, 16.7 μL of 4-vinylpyridine, 16.7 μL of tributylphosphine, and 83.3 μL of water to a suitable container, and mix.

**Procedure** Add the protein/peptide (between 1 and 100 μg) to a hydrolysis tube, and place in a larger tube. Transfer the Reducing Solution to the large tube, seal in vacuum (about 50 μm of mercury or 6.7 Pa), and incubate at about 100°C for 5 minutes. Then remove the inner hydrolysis tube, and dry it in a vacuum desiccator for 15 minutes to remove residual reagents. The pyridylethylated protein/peptide can then be acid hydrolyzed using previously described procedures. The pyridylethylated reaction is performed simultaneously with a protein standard sample containing 1 to 8 mol of cysteine to improve accuracy in the pyridylethyl-cysteine recovery. Longer incubation times for the pyridylethyl reaction can cause modifications to the ε-amino terminal group and the ε-amino group of lysine in the protein.

**Method 8**

Cysteine-cystine reduction and alkylation is accomplished by a liquid phase pyridylethylation reaction.

**Stock Solutions** Prepare and filter three solutions: 1 mol/L Tris hydrochloride (pH 8.5) containing 4 mmol/L disodium dihydrogen ethylendiamine tetracacetate (Stock Solution A), 8 mol/L guanidine hydrochloride (Stock Solution B), and 10% of 2-mercaptoethanol in water (Stock Solution C).

**Reducing Solution** Prepare a mixture of Stock Solution B and Stock Solution A (3:1) to obtain a buffered solution of 6 mol/L guanidine hydrochloride in 0.25 mol/L Tris hydrochloride.

**Procedure** Dissolve about 10 μg of the test sample in 50 μL of the Reducing Solution, and add about 2.5 μL of Stock Solution C. Store under nitrogen or argon for 2 hours at room temperature in the dark. To achieve the pyridylethyl reaction, add about 2 μL of 4-vinylpyridine to the protein solution, and incubate for an additional 2 hours at room temperature in the dark. The protein/peptide is desalted by collecting the protein/peptide fraction from a reversed-phase HPLC separation. The collected sample can be dried in a vacuum centrifuge before acid hydrolysis.

**Method 9**

Cysteine-cystine reduction and alkylation is accomplished by a liquid phase carboxymethylation reaction.

**Stock Solutions** Prepare as directed for Method 8.

**Carboxymethylation Solution** Prepare a solution containing 100 mg of iodoacetamide per mL of ethanol (95%).

**Buffer Solution** Use the Reducing Solution, prepared as directed for Method 8.

**Procedure** Dissolve the test sample in 50 μL of the Buffer Solution, and add about 2.5 μL of Stock Solution C. Store under nitrogen or argon for 2 hours at room temperature in the dark. Add the Carboxymethylation Solution in a ratio 1.5 fold per total theoretical content of thiols, and incubate for an additional 30 minutes at room temperature in the dark. [Note: If the thiol content of the protein is
unknown, then add 5 μL of 100 mmol/L iodoacetamide for every 20 nmol of protein present. The reaction is stopped by adding excess of 2-mercaptoethanol. The protein/peptide is desalted by collecting the protein/peptide fraction from a reversed-phase HPLC separation. The collected sample can be dried in a vacuum centrifuge before acid hydrolysis. The S-carboxymethyl-cysteine and formic acid residues will be converted to S-carboxymethylcysteine during acid hydrolysis.

Method 10
Cysteine-cystine is reacted with dithiodiglycolic acid or dithiodipropionic acid to produce a mixed disulfide. [Note: The choice of dithiodiglycolic acid or dithiodipropionic acid depends on the required resolution of the amino acid analysis method.]

Reducing Solution A solution containing 10 mg of dithiodiglycolic acid (or dithiodipropionic acid) per mL of 0.2 mol/L sodium hydroxide.

Procedure Transfer about 20 μg of the test sample to a hydrolysis tube, and add 5 μL of the Reducing Solution. Add 10 μL of isopropyl alcohol, and then remove all of the sample liquid by vacuum centrifugation. The sample is then hydrolyzed using Method 1. This method has the advantage that other amino acid residues are not derivatized by side reactions, and the sample does not need to be desalted prior to hydrolysis.

Method 11
Asparagine and glutamine are converted to aspartic acid and glutamic acid, respectively, during acid hydrolysis. Asparagine and aspartic acid residues are added and represented by Asx, while glutamine and glutamic acid residues are added and represented by Glx. Proteins/peptides can be reacted with bis(1,1-trifluoroacetoxy)iodobenzene (BTI) to convert the asparagine and glutamine residues to diaminopropionic acid and diaminobutyric acid residues, respectively, upon acid hydrolysis. These conversions allow the analyst to determine the asparagine and glutamine content of a protein/peptide in the presence of aspartic acid and glutamic acid residues.

Reducing Solutions Prepare and filter three solutions: a solution of 10 mmol/L trifluoroacetic acid (Solution A), a solution of 5 mol/L guanidine hydrochloride and 10 mmol/L trifluoroacetic acid (Solution B), and a freshly prepared solution of N,N-dimethylformamide containing 36 mg of BTI per mL (Solution C).

Procedure In a clean hydrolysis tube, transfer about 200 μg of the test sample, and add 2 mL of Solution A or Solution B and 2 mL of Solution C. Seal the hydrolysis tube in vacuum. Heat the sample at 60°C for 4 hours in the dark. The sample is then dialyzed with water to remove the excess reagents. Extract the dialyzed sample three times with equal volumes of n-butyl acetate, and then lyophilize. The protein can then be acid hydrolyzed using previously described procedures. The ε,β-diaminopropionic and ε,γ-diaminobutyric acid residues do not typically resolve from the lysine residues upon ion-exchange chromatography based on amino acid analysis. Therefore, when using ion-exchange as the mode of amino acid separation, the asparagine and glutamine contents are the quantitative difference in the aspartic acid and glutamic acid content assayed with underivatized and BTI-derivatized acid hydrolysis. [Note: The threonine, methionine, cysteine, tyrosine, and histidine assayed content can be altered by BTI derivatization; a hydrolysis without BTI will have to be performed if the analyst is interested in the composition of these other amino acid residues of the protein/peptide.]

Methodologies of Amino Acid Analysis General Principles
Many amino acid analysis techniques exist, and the choice of any one technique often depends on the sensitivity required from the assay. In general, about one-half of the amino acid analysis techniques employed rely on the separation of the free amino acids by ion-exchange chromatography followed by postcolumn derivatization (e.g., with ninhydrin or o-phthalaldehyde). Postcolumn detection techniques can be used with samples that contain small amounts of buffer components, such as salts and urea, and generally require between 5 and 10 μg of protein sample per analysis. The remaining amino acid techniques typically involve pre-column derivatization of the free amino acids (e.g., phenyl isothiocyanate; 6-aminoninonicotinyl-N-hydroxysuccinimidy carbamate or o-phthalaldehyde; (dimethylamino)azobenzene-sulfonyl chloride; 9-fluorenylmethylchloroformate; and, 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole) followed by reverse-phase HPLC. Precolumn derivatization techniques are very sensitive and usually require between 0.5 and 1.0 μg of protein sample per analysis but may be influenced by buffer salts in the samples. Precolumn derivatization techniques may also result in multiple derivatives of a given amino acid, which complicates the result interpretation. Postcolumn derivatization techniques are generally influenced less by performance variation of the assay than precolumn derivatization techniques.

The following Methods may be used for quantitative amino acid analysis. Instruments and reagents for these procedures are available commercially. Furthermore, many modifications of these methodologies exist with different reagent preparations, reaction procedures, chromatographic systems, etc. Specific parameters may vary according to the exact equipment and procedure used. Many laboratories will utilize more than one amino acid analysis technique to exploit the advantages offered by each. In each of these Methods, the analog signal is visualized by means of a data acquisition system, and the peak areas are integrated for quantification purposes.

Method 1—Postcolumn Ninhydrin Detection General Principle
Ion-exchange chromatography with postcolumn ninhydrin detection is one of the most common methods employed for quantitative amino acid analysis. As a rule, a Li-based cation-exchange system is employed for the analysis of the more complex physiological samples, and the faster Na-based cation-exchange system is used for the more simplistic amino acid mixtures obtained with protein hydrolysates (typically containing 17 amino acid components). Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes in pH and cation strength. A temperature gradient is often employed to enhance separation.

When the amino acid reacts with ninhydrin, the reactant has characteristic purple or yellow color. Amino acids, except imino acid, give a purple color, and show the maximum absorption at 570 nm. The imino acids such as proline give a yellow color, and show the maximum absorption at 440 nm. The postcolumn reaction between ninhydrin and amino acid eluted from column is monitored at 440 and 570 nm, and the
chromatogram obtained is used for the determination of amino acid composition. Detection limit is considered to be 10 pmol for most of the amino acid derivatives, but 50 pmol for proline. Response linearity is obtained in the range of 20 to 500 pmol with correlation coefficients exceeding 0.999. To obtain good compositional data, samples larger than 500 ng of protein/peptide before hydrolysis are best suited for this amino acid analysis of protein/peptide.

**Method 2—Postcolumn OPA Fluorometric Detection General Principle**

\(\text{o-Phthalaldehyde (OPA)}\) reacts with primary amines in the presence of thiol compound, to form highly fluorescent isoindole products. This reaction is utilized for the postcolumn derivatization in analysis of amino acids by ion-exchange chromatography. The rule of the separation is the same as **Method 1**. Instruments and reagents for this form of amino acid analysis are available commercially. Many modifications of this methodology exist. Although OPA does not react with secondary amines (amine acids such as proline) to form fluorescent substances, the oxidation with sodium hypochlorite allows secondary amines to react with OPA. The procedure employs a strongly acidic cation-exchange column for separation of free amino acids followed by postcolumn oxidation with sodium hypochlorite and postcolumn derivatization using OPA and thiol compound such as \(\text{N-acetyl-L-cysteine}\) and 2-mercaptoethanol. The derivatization of primary amino acids are not noticeably affected by the continuous supply of sodium hypochlorite.

Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes in \(\text{pH}\) and cation strength. After postcolumn derivatization of eluted amino acids with OPA, the reactant passes through the fluorometric detector. Fluorescence intensity of OPA-derivatized amino acids are monitored with an excitation wavelength at 348 nm and an emission wavelength at 450 nm.

Detection limit is considered to be a few tens of picomole level for most of the amino acid derivatives. Response linearity is obtained in the range of 2.5 to 200 pmol with correlation coefficients exceeding 0.999. To obtain good compositional data, samples larger than 1 \(\mu\)g before hydrolysis are best suited for this amino acid analysis of protein/peptide.

**Method 3—Precolumn PTC Derivatization General Principle**

Phenylisothiocyanate (PTC) reacts with amino acids to form phenylthiocarbamyl (PTC) derivatives which can be detected with high sensitivity at 245 nm. Therefore, precolumn derivatization of amino acids with PTC followed by a reversed-phase HPLC separation with UV detection is used to analyze the amino acid composition.

After the reagent is removed under vacuum, the derivatized amino acids can be stored dry and frozen for several weeks with no significant degradation. If the solution for injection is kept cold, no noticeable loss in chromatographic response occurs after three days.

Separation of the PTC-amino acids on a reversed-phase HPLC with ODS column is accomplished through a combination of changes in concentrations of acetonitrile and buffer ionic strength. PTC-amino acids eluted from the column are monitored at 254 nm.

Detection limit is considered to be 1 pmol for most of the amino acid derivatives. Response linearity is obtained in the range of 20 to 500 pmol with correlation coefficients exceeding 0.999. To obtain good compositional data, samples larger than 500 ng of protein/peptide before hydrolysis is best suited for this amino acid analysis of proteins/peptides.

**Method 4—Precolumn AQC Derivatization General Principle**

Precolumn derivatization of amino acids with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) followed by reversed-phase HPLC separation with fluorometric detection is used.

AQC reacts with amino acids to form stable, fluorescent unsymmetric urea derivatives (AQC-amino acids) which are readily amenable to analysis by reversed-phase HPLC. Therefore, precolumn derivatization of amino acids with AQC followed by reversed-phase HPLC separation is used to analyze the amino acid composition.

Separation of the AQC-amino acids on an ODS column is accomplished through a combination of changes in concentrations of acetonitrile and salt. Selective fluorescence detection of the derivatives with excitation wavelength at 250 nm and emission wavelength at 395 nm allows for the direct injection of the reaction mixture with no significant interference from the only major fluorescent reagent by-product, 6-aminquinoline. Excess reagent is rapidly hydrolyzed \((t_{1/2} < 15\text{ seconds})\) to yield 6-aminoquinoline, \(\text{N-hydroxysuccinimide}\) and carbon dioxide, and after 1 minute no further derivatization can take place.

Peak areas for AQC-amino acids are essentially unchanged for at least 1 week at room temperature, and the derivatives have more than sufficient stability to allow for overnight automated chromatographic analysis.

Detection limit is considered to be ranging from ca. 40 to 320 fmol for each amino acid, except for Cys. Detection limit for Cys is approximately 800 fmol. Response linearity is obtained in the range of 2.5 to 200 \(\mu\)mol/L with correlation coefficients exceeding 0.999. Good compositional data could be obtained from the analysis of derivatized protein hydrolysates containing as little as 30 ng of protein/peptide.

**Method 5—Precolumn OPA Derivatization General Principle**

Precolumn derivatization of amino acids with \(\text{o-phthalaldehyde (OPA)}\) followed by reversed-phase HPLC separation with fluorometric detection is used. This technique does not detect amino acids that exist as secondary amines (e.g., proline).

OPA in conjunction with a thiol reagent reacts with primary amine groups to form highly fluorescent isoindole products. 2-Mercaptoethanol or 3-mercaptopropionic acid can be used as thiol. OPA itself does not fluoresce and consequently produces no interfering peaks. In addition, its solubility and stability in aqueous solution, along with the rapid kinetics for the reaction, make it amenable to automated derivatization and analysis using an autosampler to mix the sample with the reagent. However, lack of reactivity with secondary amino acids has been a predominant drawback. This method does not detect amino acids that exist as secondary amines (e.g., proline). To compensate for this drawback, this technique may be combined with another technique described in **Method 7** or **Method 8**.
Method 11—Precolumn BTI Derivatization General Principle

Precolumn derivatization of amino acids with BTI is followed by a reversed-phase HPLC separation. Because of the instability of the BTI-derivative, derivatization and analysis are performed immediately following derivatization. The liquid chromatograph is equipped with a fluorometric detector for the detection of BTI-derivatized amino acids. Fluorescence intensity of BTI-derivatized amino acids is monitored with an excitation wavelength of 348 nm and an emission wavelength of 450 nm.

Detection limits as low as 50 fmol via fluorescence have been reported, although the practical limit of analysis remains at 1 pmol.

Method 6—Precolumn DABS-Cl Derivatization General Principle

Precolumn derivatization of amino acids with (dimethylamino)azobenzensulfonil chloride (DABS-Cl) followed by reversed-phase HPLC separation with visible light detection is used.

DABS-Cl is a chromophoric reagent employed for the labeling of amino acids. Amino acids labeled with DABS-Cl (DABS-amino acids) are highly stable and show the maximum absorption at 436 nm.

DABS-amino acids, all 19 naturally occurring amino acids derivatives, can be separated on an ODS column of a reversed-phase HPLC by employing gradient systems consisting of acetonitrile and aqueous buffer mixture. Separated DABS-amino acids eluted from the column are detected at 436 nm in the visible region.

This Method can analyze the imino acids such as proline together with the amino acids at the same degree of sensitivity, DABS-Cl derivatization method permits the simultaneous quantification of tryptophan residues by previous hydrolysis of the protein/peptide with sulfonic acids such as mercaptoethanesulfonic acid, p-toluenesulfonic acid or methanesulfonic acid described under Method 2 in “Protein Hydrolysis”. The other acid-labile residues, asparagine and glutamine, can also be analyzed by previous conversion into diaminopropionic acid and diaminobutyric acid, respectively, by treatment of protein/peptide with BTI described under Method 2 in “Protein Hydrolysis”.

The non-proteinogenic amino acid, norleucine cannot be used as internal standard in this method, as this compound is eluted in a chromatographic region crowded with peaks of primary amino acids. Nitrotyrosine can be used as an internal standard, because it is eluted in a clean chromatographic region.

Detection limit of DABS-amino acid is about 1 pmol. As little as 2 to 5 pmol of an individual DABS-amino acid can be quantitatively analyzed with reliability, and only 10 to 30 ng of the dabsylated protein hydrolysate is required for each analysis.

Method 7—Precolumn FMOC-Cl Derivatization General Principle

Precolumn derivatization of amino acids with 9-fluorenylethyl chloroformate (FMOC-Cl) followed by reversed-phase HPLC separation with fluorometric detection is used.

FMOC-Cl reacts with both primary and secondary amino acids to form highly fluorescent products. The reaction of FMOC-Cl with amino acid proceeds under mild conditions in aqueous solution and is completed in 30 seconds. The derivatives are stable, only the histidine derivative showing any breakdown. Although FMOC-Cl is fluorescent itself, the reagent excess and fluorescent side-products can be eliminated without loss of FMOC-amino acids.

FMOC-amino acids are separated by a reversed-phase HPLC using an ODS column. The separation is carried out by gradient elution varied linearly from a mixture of acetonitrile and acetic acid buffer (10:40:50) to a mixture of acetonitrile and acetic acid buffer (50:50), and 20 amino acid derivatives are separated in 20 minutes. Each derivative eluted from the column is monitored by a fluorometric detector set at an excitation wavelength of 260 nm and an emission wavelength of 313 nm.

The detection limit is in the low fmol range. A linearity range of 0.1 to 50 μmol/L is obtained for most of the amino acids.

Method 8—Precolumn NBD-F Derivatization General Principle

Precolumn derivatization of amino acids with 7-fluoro-4-nitrobenzo-2-oxa-1.3-diazole (NBD-F) followed by reversed-phase HPLC separation with fluorometric detection is used. NBD-F reacts with both primary and secondary amino acids to form highly fluorescent products. Amino acids are derivatized with NBD-F by heating to 60°C for 5 minutes. NBD-amino acid derivatives are separated on an ODS column of a reversed-phase HPLC by employing gradient elution system consisting of acetonitrile and aqueous buffer mixture, and 17 amino acid derivatives are separated in 35 minutes. ε-Aminocaproic acid can be used as an internal standard, because it is eluted in a clean chromatographic region. Each derivative eluted from the column is monitored by a fluorometric detector set at an excitation wavelength of 480 nm and an emission wavelength of 530 nm.

The sensitivity of this method is almost the same as for precolumn OPA derivatization method (Method 5), excluding proline to which OPA is not reactive, and might be advantageous for NBD-F against OPA. The detection limit for each amino acid is about 10 fmol. Profile analysis was achieved for about 1.5 μg of protein hydrolysates in the final precolumn labeling reaction mixture for HPLC.

Data Calculation and Analysis

When determining the amino acid content of a protein/peptide hydrolysate, it should be noted that the acid hydrolysis step destroys tryptophan and cysteine. Serine and threonine are partially destroyed by acid hydrolysis, while isoleucine and valine residues may be only partially cleaved. Methionine can undergo oxidation during acid hydrolysis, and some amino acids (e.g., glycine and serine) are common contaminants. Application of adequate vacuum (≤0.0267 KPa) or introduction of inert gas (argon) in the headspace of the reaction vessel during vapor phase hydrolysis can reduce the level of oxidative destruction. Therefore, the quantitative results obtained for cysteine, tryptophan, threonine, isoleucine, valine, methionine, glycine, and serine from a protein/peptide hydrolysate may be variable and may warrant further investigation and consideration.

Calculations

Amino Acid Mole Percent This is the number of specific amino acid residues per 100 residues in a protein. This result may be useful for evaluating amino acid analysis data when the molecular weight of the protein under investigation is unknown. This information can be used to corroborate the identity of a protein/peptide and has other applications.
Carefully identify and integrate the peaks obtained as directed for each Procedure. Calculate the mole percent for each amino acid present in the test sample by the formula:

\[ 100n_m/r, \]

in which \( n_m \) is the peak response, in nmol, of the amino acid under test; and \( r \) is the sum of peak responses, in nmol, for all amino acids present in the test sample. Recalculate the mole percent of the amino acids under test to data from known proteins can help establish or corroborate the identity of the sample protein.

**Unknown Protein Samples** This data analysis technique can be used to estimate the protein concentration of an unknown protein sample using the amino acid analysis data. Calculate the mass, in \( \mu g \), of each recovered amino acid by the formula:

\[ m \times \frac{M_w}{1000}, \]

in which \( m \) is the recovered quantity, in nmol, of the amino acid under test; and \( M_w \) is the average molecular weight for that amino acid, corrected for the weight of the water molecule that was eliminated during peptide bond formation. The sum of the masses of the recovered amino acids will give an estimate of the total mass of the protein analyzed after appropriate correction for partially and completely destroyed amino acids. If the molecular weight of the unknown protein is available (i.e., by SDS-PAGE analysis or mass spectrometry), the amino acid composition of the unknown protein can be predicted. Calculate the number of residues of each amino acid by the formula:

\[ m/(1000M/M_w), \]

in which \( m \) is the recovered quantity, in nmol, of the amino acid under test; \( M \) is the total mass, in \( \mu g \), of the protein; and \( M_w \) is the molecular weight of the unknown protein.

**Known Protein Samples** This data analysis technique can be used to investigate the amino acid composition and protein concentration of a protein sample of known molecular weight and amino acid composition using the amino acid analysis data. When the composition of the protein being analyzed is known, one can exploit the fact that some amino acids are recovered well, while other amino acid recoveries may be compromised because of complete or partial destruction (e.g., tryptophan, cysteine, threonine, serine, methionine), incomplete bond cleavage (i.e., for isoleucine and valine) and free amino acid contamination (i.e., by glycine and serine).

Because those amino acids that are recovered best represent the protein, these amino acids are chosen to quantify the amount of protein. Well-recovered amino acids are, typically, aspartate-asparagine, glutamate-glutamine, alanine, leucine, phenylalanine, lysine, and arginine. This list can be modified based on experience with one’s own analysis system. Divide the quantity, in nmol, of each of the well-recovered amino acids by the expected number of residues for that amino acid to obtain the protein content based on each well-recovered amino acid. Average the protein content results calculated. The protein content determined for each of the well-recovered amino acids should be evenly distributed about the mean. Discard protein content values for those amino acids that have an unacceptable deviation from the mean. Typically \( \geq 5\% \) variation from the mean is considered unacceptable. Recalculate the mean protein content from the remaining values to obtain the protein content of the sample. Divide the content of each amino acid by the calculated mean protein content to determine the amino acid composition of the sample by analysis.

Calculate the relative compositional error, in percentage, by the formula:

\[ 100m/m_S, \]

in which \( m \) is the experimentally determined quantity, in nmol per amino acid residue, of the amino acid under test; and \( m_S \) is the known residue value for that amino acid. The average relative compositional error is the average of the absolute values of the relative compositional errors of the individual amino acids, typically excluding tryptophan and cysteine from this calculation. The average relative compositional error can provide important information on the stability of analysis run over time. The agreement in the amino acid composition between the protein sample and the known composition can be used to corroborate the identity and purity of the protein in the sample.

### Basic Requirements for Viral Safety of Biotechnological/Biological Products listed in Japanese Pharmacopoeia

**Introduction**

The primary role of specification of biotechnological/biological products listed in Japanese Pharmacopoeia (JP) is not only for securing quality control or consistency of the quality but also for assuring their efficacy and safety. In the meantime, the requirements to assure quality and safety of drugs have come to be quite strict recently, and a rigid attitude addressing safety assurance is expected for biotechnological/biological products. The key points for quality and safety assurance of biotechnological/biological products are selection and appropriate evaluation of source material, appropriate evaluation of manufacturing process and maintenance of manufacturing consistency, and control of specific physical properties of the products. Now, how to assure quality and safety of such drugs within a scope of JP has come to be questioned. This General Information describes what sorts of approaches are available to overcome these issues.

It is desired that quality and safety assurance of JP listed products are achieved by state-of-the-art methods and concepts which reflect progress of science and accumulation of experiences. This General Information challenges to show the highest level of current scientific speculation. It is expected that this information will contribute to promotion of scientific understanding of quality and safety assurance of not only JP listed products but also the other biotechnological/biological products and to promotion of active discussion of each Official Monograph in JP.

1. **Fundamental measures to ensure viral safety of JP listed biotechnological/biological products**

The biotechnological/biological product JP includes the products derived from living tissue and body fluid (urine, blood, etc.) of mammals, etc. Protein drugs derived from cell lines of human or animal origin (e.g., recombinant DNA drug, cell culture drug) are also included. The fundamental measures required for comprehensive viral safety of JP listed
biotechnological/biological products are as follows: 1) acquaintance of possible virus contamination (source of contamination); 2) careful examination of eligibility of raw materials and their sources, e.g., human/animal, and thorough analysis and screening of the sample chosen as a substrate for drug production (e.g., pooled body fluid, cell bank, etc.) to determine any virus contamination and determination of type and nature of the virus, if contaminated; 3) evaluation to determine virus titer and virus-like particles hazardous to human, if exists; 4) selection of production related material (e.g., reagent, immune antibody column) free from infectious or pathogenic virus; 5) performance of virus free test at an appropriate stage of manufacturing including the final product, if necessary; 6) adoption of effective viral clearance method in the manufacturing process to remove/inactivate virus. Combined method sometimes achieves higher level of clearance; 7) development of a deliberate viral clearance scheme; 8) performance of the test to evaluate viral removal and inactivation. It is considered that the stepwise and supplemental adoption of the said measures will contribute to ensure viral safety and its improvement.

2. Safety assurance measures described in the Official Monograph and this General Information

As mentioned in above 1, this General Information describes, in package, points to be concerned with and concrete information on the measures taken for viral safety of JP listed products. Except where any specific caution is provided in Official Monograph of a product in question, Official Monograph provides in general that “Any raw material, substrate for drug production and production related material used for production of drug should be derived from healthy animals and should be shown to be free of latent virus which is infectious or pathogenic to human”, “Cell line and culture method well evaluated in aspects of appropriateness and rationality on viral safety are used for production, and the presence of infectious or pathogenic latent virus to human in process related materials derived from living organisms should be denied”. and “biotechnological/biological drug should be produced through a manufacturing process which is capable of removing infectious or pathogenic virus”, etc., to raise awareness on viral safety and on necessity to conduct test and process evaluation for viral safety.

3. Items and contents described in this General Information

As for viral safety of protein drug derived from cell line of human or animal origin, there is a Notice in Japan entitled “Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin” (Iyakushin No. 329 issued on February 22, 2000 by Director, Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare) to reflect the internationally harmonized ICH Guideline, and as for blood plasma protein fraction preparations, there is a document entitled “Guideline for ensuring viral safety of blood plasma protein fraction preparations”. This General Information for ensuring viral safety of JP listed biotechnological/biological products has been written, referencing the contents of those guidelines, to cover general points and their details to be concerned for ensuring viral safety of not only JP listed biotechnological/biological products but also all products which would be listed in JP in future, i.e., biological products derived from living tissue and body fluid, such as urine, and protein drugs derived from cell line of human or animal origin (Table 1).

3.1. Purpose

The purpose of this document is to propose the comprehensive concepts of the measures to be taken for ensuring viral safety of biotechnological/biological products derived from living tissue or body fluid of mammals, etc. and of protein drugs derived from cell lines of human or animal origin. That is to say, this document describes the measures and the points of concern on the items, such as (1) consideration of the source of virus contamination; (2) appropriate evaluation on eligibility at selecting the raw material and on qualification of its source, e.g., human or animal; (3) virus test, and its analysis and evaluation at a stage of cell substrate for drug production; (4) appropriate evaluation to choose product related materials derived from living organisms (e.g. reagent, immune antibody column, etc.); (5) conduct of necessary virus test on the product at an appropriate stage of manufacturing; (6) development of viral clearance test scheme; (7) performance and evaluation of viral clearance test. This document is also purposed to comprehensively describe in details that supplemental and combining adoption of the said measures will contribute to secure viral safety and its improvement.

3.2. Background

One of the most important issues to be cautioned for safety of a biological product, which is directly derived from human or animal, or of a protein drug, which is derived from cell line of human or animal origin (recombinant DNA derived product, cell culture derived product, etc.), is risk of virus contamination. Virus contamination may cause serious situation at clinical use once it occurs. Virus contamination may be from a raw material or from a cell substrate for drug production, or may be from an adventitious factor introduced to the manufacturing process. JP listed biological drugs or protein drugs derived from cell line have achieved drastic contribution to the medical society, and to date, there has not been any evidence of any safety problem on them caused by virus. But, social requirement of health hazard prevention is strong, and it is now very important to prevent accidental incidence, taking security measures carefully supported by scientific rationality. It is always great concern among the persons involved that under what sort of viewpoint and to what extent we have to pursue for ensuring viral safety of a biotechnological/biological product. Before discussing these issues, two fundamental points have to be reconfirmed. One is that; we have to consider scientific, medical, and social profiles a drug has. In other words, “Medicine is a social asset which is utilized in medical practice paying attention to the risk and benefit from the standpoints of science and society”. It is the destiny and the mission of the medical/pharmaceutical society to realize prompt and stable supply of such a social asset, drug, among the medical work front to bring gospel to the patients.

The other is that; issue of viral safety is independent from safety of the components of a drug per se (narrow sense of safety). It is important to consider that this is the matter of general safety of drug (broad sense of safety). In case of a drug which has been used for a long time in the medical front, such as a JP listed product, its broad sense of safety is considered to have been established epidemiologically, and its usage past records have a great meaning. However,
difference from safety of drug per se (its components), taking into account any possibility of virus contamination, we have to say that only the results accumulated can not always assure viral safety of a drug used in future. Accordingly, the basis for securing broad sense of viral safety of JP listed biotechnological/biological products is to pay every attention to the measures to take for prevention, while evaluating the accumulated results.

Adopting strict regulations and conducting tests at maximum level to the extent theoretically considered may be the ways off assuring safety, but applying such way generally, without sufficient scientific review of the ways and evaluation of usage results, causes excessive requirement of regulation and test not having scientific rationality. As the results, effective and prompt supply of an important drug, already having enough accumulation of experiences, to the medical work front will be hampered, and the drug, a social asset, may not to be utilized effectively. Medicine is a sword used in medical field having double-edge named effectiveness and safety. Effectiveness and safety factors have to be derived as the fruits of leading edge of science, and relatively evaluated on a balance sheet of usefulness. Usefulness evaluation should not be unbalanced in a way that too much emphasis is placed on safety concern without back-up of appropriate scientific rationality. A drug can play an important role as a social asset only when well balanced appropriate scientific usefulness evaluation in addition to social concern of the age are given. In other words, drug is a common asset utilized by society for medication as a fruit of science of the age, and the key point of its utilization lies on a balance of risk and benefit produced from scientific and social evaluation. So, those factors have to be taken into account when target and pursuance levels for ensuring viral safety of a JP listed biotechnological/biological product are reviewed.

And, in general, the risk and benefit of drugs should be considered with the relative comparison to alternative drugs or medical treatment. The usefulness of certain drug should be reviewed finally after the competitive assessment on the risk and benefit on the alternative drugs, relevant drugs and/ or alternative medical treatment.

Under such background, the purpose of this article is to describe the scientific and rational measures to be taken for ensuring viral safety of JP listed biotechnological/biological products. Giving scientific and rational measures mean that; appropriate and effective measures, elaborated from the current scientific level, are given to the issues assumable under the current scientific knowledge. In other words, possible contaminant virus is assumed to have the natures of genus, morph, particle size, physical/chemical properties, etc. which are within the range of knowledge of existing virology, and is those assumed to exist in human and animal, tissue and body fluid, which are the source of biotechnological/biological product, reagent, material, additives, etc. Accordingly, viral clearance studies using a detection method which target those viruses have to be designed.

3.3. Unknown risk on the measures taken for ensuring viral safety

There are known and unknown risks.

It is easy to determine a test method and an evaluation standard on the known risk, which exists in the drug per se (pharmaceutical component) or inevitably exists due to a quality threshold, and quantification of such risk is possible. In other words, it is easy to evaluate the known risk on a balance sheet in relation to the benefit, and we can say that valuation even in this respect has been established to some extent.

On the other hand, as for the unknown risk which is inevitable for ensuring viral safety, the subject of the risk can not be defined and quantitative concept is hard to introduce,

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**Table 1** Items described in General Information for Viral Safety Assurance of JP listed Biotechnological/Biological Product

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and, therefore, taking a counter measure and evaluating its effect are not so easy. Therefore, this is the subject to be challenged calling upon wisdom of the related parties among the society of drug.

Talking about the unknown risk, there are view points that say “It is risky because it is unknown.” and “What are the unknowns, and how do we cope with them in ensuring safety?”

The view of “It is risky because it is unknown.” is already nothing but a sort of evaluation result, and directly connects to a final decision if it can be used as a drug. Such evaluation/decision has to be made based upon a rational, scientific or social judgment.

For example, in the case that “In a manufacturing process of drug, virus, virus-like particle or retrovirus was detected, but its identification could not be confirmed, and, therefore, its risk can not be denied.”, the evaluation of “It is risky because it is unknown.” is scientifically rational and reasonable. On the other hand, however, if we reach a decision of “It is risky because it is unknown.” due to the reason that “In a manufacturing process of drug, virus, virus-like particle or retrovirus was not detected, but there is a ‘concern’ that something unknown may exist.”, it can not be said that such evaluation is based upon a rational, scientific or social judgment. It goes without saying that the utmost care has to be taken for viral safety, but the substance of ‘concern’ has to be at least clearly explainable. Otherwise, the ‘concern’ may result in causing contradiction in the meaningful mission to utilize a social asset, drug, in medical practice.

From scientific viewpoint, we should not be narrow minded by saying “it is risky” because “there is a ‘concern’ that something unknown may exist”, but challenge to clarify the subject of “What is unknown, and how to cope with it for ensuring safety” using wisdom. What is important at the time is to define “what is unknown” based upon current scientific knowledge. Only through this way, is it possible for us to elaborate the measures for ensuring safety.

Once we chase up the substance of unknown risk for viral safety without premises of “what is unknown”, “unknown” will be an endless question because it theoretically remains unresolved forever. If this kind of approach is taken, the issue and the measure can not be scientifically connected to each other, which will result in the excessive requirement of regulation and of test to be conducted. Yet, it is unlikely that the measure which has no relation with science will be effective to the subject of “What is unknown is unknown.”

For example, “what is unknown” at the “evaluation of a purification process which can completely clear up every virus that contaminated in a manufacturing process” should be the subject of “what sort of existing virus that contaminated is unknown”, not on the subject of “what sort of virus that exist in the world is unknown. In the former subject, the premise of the study is based on all the knowledge on viruses including DNA/RNA-virus, virus with/without envelope, particle size, physical/chemical properties, etc. The premise is that the virus contaminated should be within range of existing wisdom and knowledge of virus such as species, type, nature, etc., even though the virus that contaminated is unknown. Under such premise, when evaluation is made on a purification process to decide its capability of clearing a derived virus, which is within the range of existing wisdom and learning, specific viral clearance studies designed to combine a few model viruses with different natures, such as type of nucleic acid, with/without envelope, particle size, physical/chemical properties, etc., would be enough to simulate every sort of the virus already known, and will be a “good measure for ensuring safety”.

The issue of “the sort of viruses that exist in the world is unknown” may be a future study item, but it is not an appropriate subject for the viral clearance test. Further, even if the subject of “unknown viruses, which have a particle size smaller than that of currently known viruses, may exists” or “unknown viruses, which have special physical/chemical properties that can not be matched to any of the currently known viruses, may exists” is set up as an armchair theory, any experimental work can not be pursued under the current scientific level, since such virus model is not available. Further, any viral clearance test performed by using the currently available methods and technologies will be meaningless “for ensuring safety”, since particle size or natures of such speculated virus are unknown. Likewise, any counter measures can not be taken on the subject of “unknown virus, which can not be detected by currently available screening method, may exist”, and conducting any virus detection test at any stage will be useless “for ensuring safety”.

The requirement of regulations or tests excessively over scientific rationality will raise human, economical and temporal burden to the pharmaceutical companies, and will adversely affect prompt, effective and economical supply of a drug to the medical front. As drug is a sort of social asset, which has to be scientifically evaluated, how to assure maximization of its safety by means of scientifically rational approach at minimum human, economical and time resources is important.

It is also important to reconfirm that achievement of those issues is on the premise that appropriate measures are taken on the supply source of drugs. For example, in a case of “In a manufacturing process of drug, virus, virus-like particle or retrovirus was not detected, but there is a ‘concern’ that something unknown may exist.”, appropriateness of the test, which resulted in the judgment that “virus, virus-like particle or retrovirus was not detected in a process of drug production”, should be a prerequisite premise when judged by science standard at the time. If there is any question on the premise, it is quite natural that the question of “there is a ‘concern’ that unknown something may exist.” will be effective.

3.4. Applicable range

This General Information is on JP listed biological products, derived from living tissue or body fluid, and protein drugs, derived from human or animal cell line, that in Japan. In the case of protein drugs derived from human or animal cell line, the products developed and approved before enforcement of the Notice Iyakushin No. 329 entitled “Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin” should have been treated under the Notice had there been one, and it is inevitable that some products approved after the Notice might not have been sufficiently treated. It is expected that such biodrug will be sufficiently examined to meet such General Information before being listed in JP. On the other hand, blood preparations listed in the biological products standard and covered by “Guideline for securing safety of blood plasma protein product fraction preparations against virus”, are out of the scope of this General Information. Further, in case of a relatively lower molecular biogenous substance, such as amino acid,
saccharide and glycerin, and of gelatin, which is even classified as infectious or pathogenic polymer, there are cases that viral contamination can not be considered due to its manufacturing or purification process, and that potent viral inactivation/removal procedure that can not be applied to protein, can be used, and, therefore, it is considered reasonable to omit such substances from the subject for application. However, some part of this General Information may be used as reference. Further, a comprehensive assurance measure for viral safety is recommendable on a biotechnological/biological product not listed in JP using this document as a reference so long as it is similar to the biotechnological/biological product JP.

3.5. Possible viral contamination to a JP listed biotechnological/biological product (source of virus contamination)

Promoting awareness of virus contamination to a JP listed biotechnological/biological product (source of virus contamination) and citing countermeasure are important for eradicating any possible virus contamination and raising probability of safety assurance. Many biotechnological/biological products are produced from a “substrate” which is derived from human or animal tissue, body fluid, etc. as an origin/raw material, and in purification or pharmaceutical processing of such products column materials or additives, which are living organism origin, are occasionally used. Accordingly, enough safety measures should be taken against diffusion of the contaminant virus. Further, as mentioned in Notice Iyakushin No. 329, any protein drug derived from cell lines of human or animal origin should be carefully examined with respect to the risk of virus contamination through the cell line, the cell substrate for drug production, and through the manufacturing process applied thereafter.

“Substrate for drug production” is defined as a starting material which is at a stage where it is deemed to be in a position to ensure quality/safety of an active substance. The “substrate for drug production” is sometimes tissue, body fluid, etc. of human or animal per se and pooled material such as urine, and sometimes a material after some treatment. In many cases, it is considered rational that starting point of full-scale test, evaluation and control should be at the stage of “substrate for drug production”. The more strict levels of test, evaluation and control achieved at the stage of “substrate for drug production” can more rationalize evaluation and control of the raw material or individual level of upper stream. On the contrary, strict evaluation and control of the raw material or individual level at an upper stream stage can rationalize tests, evaluation or quality control at the stage of “substrate for drug production”.

The measures taken for ensuring viral safety on a biotechnological/biological product currently listed in JP can be assumed from the provisions of manufacturing method, specification and test methods of each preparation. However, unitary principles or information with respect to the measures to be taken for ensuring viral safety, totally reviewing the entire process up to the final product rationally and comprehensively, including source/raw material/substrate, purification process, etc. have not been clarified. The most important thing for ensuring viral safety is to take thorough measures to eliminate the risk of virus contamination at any stage of source animal, raw material and substrate. Although not the cases of a biotechnological/biological product, known examples of a virus contamination from a raw material/substrate for drug production in old times are Hepatitis A Virus (HAV) or Hepatitis C Virus (HCV) contamination in blood protein fraction preparations. It is also well known that Human Immunodeficiency Virus (HIV) infection caused by blood plasma protein fraction preparations occurred in 1980s. The aim of this General Information is to show concrete guidelines for comprehensive viral safety assurance of the JP listed biotechnological/biological products. The pathogenic infectious viruses, currently known to contaminate to raw materials, etc. of drug and have to be cautioned, are HIV, HAV, Hepatitis B Virus (HBV), HCV, Human T-Lymphotropie Virus (HTLV-I/II), Human Parvovirus B19, Cytomegalovirus (CMV), etc. Biotechnological/biological products produced from raw material/cell substrate derived from tissue or body fluid of human or animal origin always have a risk of contamination of pathogenic or other latent virus. Therefore, safety measures should be thoroughly taken. There is also the case that a material, other than the biological component such as raw material/substrate, causes virus contamination. Using enzymatic or monoclonal antibody column or using albumin etc. as a stabilizer, is the example of the case, in which caution has to be taken on risk of virus contamination from the source animal or cell. Further, there is a possibility of contamination from environment or personnel in charge of production or at handling of the product. So, caution has to be taken on these respects as well.

In case of protein drugs derived from cell line of human or animal origin, there may be cases where latent or persistent infectious viruses (e.g., herpesvirus) or endogenous retroviruses exist in the cell. Further, adventitious viruses may be introduced through the routes such as: 1) derivation of a cell line from an infected animal; 2) use of a virus to drive a cell line; 3) use of a contaminated biological reagent (e.g., animal serum components); 4) contamination during cell handling. In the manufacturing process of drug, an adventitious virus may contaminate to the final product through the routes, such as 1) contamination through a reagent of living being origin, such as serum component, which is used for culturing, etc.; 2) use of a virus for introduction of a specific gene expression to code an objective protein; 3) contamination through a reagent used for purification such as monoclonal antibody affinity column; 4) contamination through an additive used for formulation production; 5) contamination at handling of cell and culture media, etc. It is reported that monitoring of cell culture parameter may be helpful for early detection of an adventitious viral contamination.

3.6. Basis for ensuring viral safety

Viral safety of a biotechnological/biological product produced from a raw material/substrate, which derived from tissue, body fluid, cell line, etc. of human or animal origin, can be achieved by supplemental and appropriate adoption of the following plural methods.

(1) Acquaintance of possible virus contamination (source of contamination).
(2) Careful examination of eligibility of the raw material and its source, i.e., human or animal, thorough analysis and screening of the sample chosen as the substrate for drug production to determine virus contamination and through examination of the type of virus and its nature, if contaminated.
(3) Evaluation to determine hazardous properties of the vi-
rus or virus-like particle to human, if exists.

4) Choosing a product related material of living organism origin (e.g., reagent, immune anti-body column, etc.) which is free from infectious or pathogenic virus.

5) Conduct virus free test at an appropriate stage of manufacturing including the final product, if necessary.

6) Adoption of an effective method to remove/inactivate the virus in the manufacturing process for viral clearance. Combined processes sometimes achieve higher level of viral clearance.

7) Develop a deliberate viral clearance scheme.

8) Conduct test and evaluation to confirm removal/inactivation of the virus.

Manufacturer is responsible for explaining rationality of the way of approach adopted among the comprehensive strategy for viral safety on each product and its manufacturing process. At the time, the approach described in this General Information shall be applicable as far as possible.

3.7. Limit of virus test

Virus test has to be conducted to define existence of virus, but it should be noted that virus test alone cannot reach a conclusion of inexistence of virus nor sufficient to secure safety of the product. Examples of a virus not being detected are as follows: 1) Due to statistical reason, there is an inherent quantitative limit, such as detection sensitivity at lower concentration depends upon the sample size. 2) Generally, every virus test has a detection limit, and any negative result of a virus test can not completely deny existence of a virus.

3) A virus test applied is not always appropriate in terms of specificity or sensitivity for detection of a virus which exists in the tissue or body fluid of human or animal origin.

Virus testing method is improved as science and technology progress, and it is important to apply scientifically the most advanced technology at the time of testing so that it can be possible to raise the assurance level of virus detection. It should be noted, however, that the limit as mentioned above can not always be completely overcome. Further, risk of virus contamination in a manufacturing process can not be completely denied, and, therefore, it is necessary to elaborate the countermeasure taken these effects into account.

Reliable assurance of viral free final product can not be obtained only by negative test results on the raw material/substrate for drug production or on the product in general, it is also necessary to demonstrate inactivation/removal capability of the purification process.

3.8. Roles of viral clearance studies

Under the premises as mentioned in the preceding clause that there is a limit of a virus test, that there is a possibility of existence of latent virus in a raw material/substrate for drug production and that there is a risk of entry of a non-endogenous virus in a manufacturing process, one of the important measures for viral safety is how to remove or inactivate the virus, which exists in a raw material, etc. and can not be detected, or the virus, which is continuously contaminated in a manufacturing process. The purpose of viral clearance study is to experimentally evaluate the viral removal/inactivation capability of a step that mounted in a manufacturing process. So, it is necessary to conduct an experimental scale spike test using an appropriate virus that is selected by taking account the properties, such as particle size, shape, with or without envelope, type of nucleic acid (DNA type, RNA type), heat and chemical treatment toler-

ance, etc., with an aim to determine removal/inactivation capability of the virus that can not be detected in a raw material or contingently contaminated.

As mentioned above, the role of viral clearance study is to speculate removal/inactivation capability of a process through a model test, and it contributes to give scientific basis to assure that a biotechnological/biological product of human or animal origin has reached an acceptable level in aspect of viral safety.

At a viral clearance study, it is necessary to adopt an appropriate approach method which is definitive and rational and can assure viral safety of a final product, taking into consideration the source and the properties of the raw material/substrate as well as the manufacturing process.

4. Raw material/substrate for drug production

4.1. Issues relating to animal species and its region as a source of raw material/substrate for drug production and countermeasures to be taken thereto

For manufacturing JP listed biotechnological/biological products, which require measures for viral safety, a raw material/substrate derived mainly from human, bovine, swine or equine is used, and it is obvious that such human and animal has to be healthy nature. A wild animal should be avoided, and it is recommended to use animals derived from a colony controlled by an appropriate SPF (Specific Pathogen-Free) condition and bred under a well designed hygienic control, including appropriate control for prevention of microbial contamination and contamination monitoring system. If a meat standard for food is available, an animal meeting this standard has to be used. The type of virus to be concerned about depend on animal species, but it may be possible to narrow down the virus for investigation by means of examining the hygiene control, applicability of a meat standard for food, etc. On the other hand, even with the animals of the same species, a different approach may be necessary depending upon the region where the specimen for a raw material/substrate is taken. For example, in case of obtaining raw material/substrate from blood or other specific region, it is necessary to be aware of the risk level, virus multiplication risk, etc. which may specifically exists depending upon its region. Such approach may be different from those applied to body waste such as urine, milk, etc. as a source of raw material/substrate. Further, caution has to be taken on transmissible spongiform encephalopathy (TSE) when pituitary gland, etc. is used as a raw material. This report does not include detailed explanation on TSE, but recommendations are to use raw material derived from 1) animals originated in the countries (area) where incidence of TSE has not been reported; 2) animals not infected by TSE; or 3) species of animal which has not been reported on TSE.

It is recommended to discuss the matters concerned with TSE with the regulatory authority if there is any unclear point.

Followings are the raw material/substrate used for manufacturing biotechnological/biological products in Japan.

1) Biological products derived from human

Blood plasma, placenta, urine, etc. derived from human are used as the sources of raw material of biotechnological/biological product. As for these raw materials, there are 2 cases: 1) Appropriateness can be confirmed by interview or by examination of the individual who supplies each raw ma-
tial, and 2) Such sufficient interview or examination of the individual can not be made due to type of raw material. In case that sufficient examination of individual level is not possible, it is necessary to perform test to deny virus contamination at an appropriate manufacturing stage, for example, the stage to decide it as a substrate for drug production.

(2) Biological products derived from animal besides human

Heparin, gonadotropin, etc. are manufactured from blood plasma or from various organs of bovine, swine and equine.

(3) Protein drug derived from cell line of human or animal origin

In the case of protein drugs derived from cell line of human or animal origin, a cell line of human or animal is the raw material per se, and the substrate for drug production is a cell bank prepared from cloned cell line (master cell bank or working cell bank). Examination at cell bank level is considered enough for viral safety qualification, but it goes without saying that the more appropriate and rational qualification evaluation test of cell bank can be realized when more information is available on the virus of the source animal or on prehistory of driving the cell line, the base of cell bank.

4.2. Qualification evaluation test on human or animal as a source of raw material/substrate for drug production

(1) Biological products derived from human

Body fluid etc. obtained from healthy human must be used for biological products production. Further, in case that interview or examination of the individual, who supplies the raw material, can be possible and is necessary, interview under an appropriate protocol and a serologic test well evaluated in aspects of specificity, sensitivity and accuracy have to be performed, so that only the raw material, which is denied latent HBV, HCV and HIV, will be used. In addition to the above, it is necessary to test for gene of HBV, HCV and HIV by a nucleic amplification test (NAT) well evaluated in aspects of specificity, sensitivity and accuracy.

In case of the raw material (e.g., urine), which can not be tested over the general medical examination of the individual who supplies the material, or of the raw material which is irrational to conduct individual test, the pooled raw material, as the substrate for drug production, has to be conducted at least to deny existence of HBV, HCV and HIV, using a method well evaluated in aspects of specificity, sensitivity and accuracy, such as the antigen test or NAT.

(2) Biological products derived from animal besides human

The animal used for manufacturing biological product has to be under appropriate health control, and has to be confirmed of its health by various tests. Further, it is necessary that the population, to which the animal belongs, has been under an appropriate breeding condition, and that no abnormal individual has been observed in the population. Further, it is necessary to demonstrate information or scientific basis which can deny known causes infection or disease to human, or to deny such animal inherent latent virus by serologic test or by nucleic amplification test (NAT). The infectious virus that is known to be common between human and animal, and known to cause infection in each animal are tentatively listed in Table 2. It is necessary that the table is completed under careful examination, and denial of all of them, by means of tests on individual animal, tissue, body fluid, etc. as a raw material, or on pooled raw material (as a direct substrate for drug production), is not always necessary. Table 2 can be used as reference information, in addition to the other information, such as; source of animal, health condition, health and breeding control, conformity to the meat standard for food, etc., to elaborate to which virus what kind of test has to be performed, and for which virus it is not always necessary to test for, etc. It is important to clarify and record the basis of choosing the virus and the test conducted thereof.

(3) Protein drug derived from cell line of human or animal origin

It is important to conduct thorough investigation on latent endogenous and non-endogenous virus contamination in a master cell bank (MCB), which is the cell substrate for drug production, in accordance with the Notice 329 entitled “Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin”. Further, it is necessary to conduct an appropriate adventitious virus test (e.g., in vitro and in vivo test) and a latent endogenous virus test on the cell at the limit of in vitro cell age (CAL) for drug production. Each WCB as a starting cell substrate for drug production should be tested for adventitious virus either by direct testing or by analysis of cells at the CAL, initiated from the WCB. When appropriate non-endogenous virus tests have been performed on the MCB and cells cultured up to or beyond the CAL have been derived from the WCB and used for testing for the presence of adventitious viruses, similar tests need not be performed on the initial WCB.

5. Points of concern with respect to manufacturing and virus testing

To ensure viral safety of a biological product derived from tissue, body fluid etc. of human or animal origin, it is necessary to exclude any possibility of virus contamination from a raw material, such as tissue and body fluid, or a substrate, paying attention to the source of virus contamination as mentioned in above 3.5, and to adopt appropriate manufacturing conditions and technologies in addition to enhancement of manufacturing environment, so that virus contamination in the course of process and handling and from operators, facilities and environment can be minimized.

In addition to the above, effective virus test and viral inactivation/removal technology, which are reflected by rapid progress of science, have to be introduced. Adoption of two or more steps with different principles is recommended for virus inactivation/removal process. Further, it is important to minimize any possible virus derivation by using a reagent, which quality is equivalent to that of a drug. Examples of virus inactivation/removal processes are

1. heating (It is reported that almost viruses are inactivated by heating at 55 – 60°C for 30 minutes with exceptions of hepatitis virus, etc. and that dry heating at 60°C for 10 – 24 hours is effective in case of the products of blood or urine origin.),
2. treatment with organic solvent/surfactant (S/D treatment),
3. membrane filtration (15 – 50 nm),
4. acid treatment,
5. irradiation (γ-irradiation, etc.),
6. treatment with column chromatography (e.g. affinity chromatography, ion-exchange chromatography),
7. fractionation (e.g. organic solvent or ammonium sulfate fractionation),
8. extraction.

5.1. Virus test conducted in advance of purification process

(1) Biological products derived from human

In many cases, samples for virus test before purification
Table 2  Infectious viruses known to be common between human and animal and known to cause infection to each animal

<table>
<thead>
<tr>
<th>Virus</th>
<th>bovine</th>
<th>swine</th>
<th>sheep</th>
<th>goat</th>
<th>equine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpox virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paravaccinia virus</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>Murray valley encephalitis virus</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Louping ill virus</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wesselsbron virus</td>
<td></td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot-and-mouth disease virus</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japanese encephalitis virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vesicular stomatitis virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine papular stomatitis virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orf virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borna disease virus</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabies virus</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis E virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encephalomyocarditis virus</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern equine encephalitis virus</td>
<td>●</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western equine encephalitis virus</td>
<td></td>
<td>●</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venezuelan equine encephalitis virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morbillivirus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hendra virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nipah virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmissible gastroenteritis virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine respiratory coronavirus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine epidemic diarrhea virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemagglutinating encephalomyelitis virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine respiratory and reproductive syndrome virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hog cholera virus</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

process are body fluid or tissue of individual collected as a raw material, or its pooled material or extraction as a substrate. As mentioned in 4.2 (1), it is necessary to deny latent HBV, HCV and HIV by the test evaluated enough in aspects of specificity, sensitivity and accuracy. Even in a case that a non-purified bulk before purification process is produced from a substrate, it is not always necessary to conduct virus test again at the stage before purification, so long as the presence of any latent virus can be denied at the stage of substrate by an appropriate virus test, with cases where the non-purified bulk is made from the substrate by adding any reagent etc. of living organisms origin are an exception.

(2) Biological products derived from animal besides human

Similar to (1) above, samples for virus test before purification process are, in many cases, body fluid or tissue of individual collected as a raw material, or its pooled material or extraction as a substrate. In these cases, it is necessary to have a data, which can deny latent virus of probable cause of human infection or disease as mentioned in the above 4.2 (2), or to have a result of serologic test or nucleic amplification test (NAT) evaluated enough in aspects of specificity, sensitivity and accuracy. The concept, which is applied to a case that non-purified bulk before purification process is produced from substrate, is the same as those provided in the above 4.2 (1).

(3) Protein drug derived from cell line of human or animal origin

Generally, substrate in this case is cell bank, and the sample for testing before purification process is a harvested cell after cell culturing or unprocessed bulk which consists of single or pooled complex culture broth. The unprocessed bulk may be sometimes culture broth without cell. Denial of latent virus, which is determined by virus test at a MCB or WCB level, does not always deny latent virus in unprocessed
bulk after culturing. Further, it is noted that the viral test at the CAL is meaningful as a validation but can not guarantee definite assurance of latent virus denial, since the test is generally performed only once. In case of using a serum or a component of blood origin in a culture medium, definite denial of latent virus at the level of unprocessed bulk can not be assured so long as the viral test has not been conducted on each lot at the CAL, since lot renewal can be a variable factor on viral contamination.

A representative sample of the unprocessed bulk, removed from the production reactor prior to further processing, represents one of the most suitable levels at which the possibility of adventitious virus contamination can be determined with a high probability of detection. Appropriate testing for viruses should be performed at the unprocessed bulk level unless virus testing is made more sensitive by initial partial processing (e.g., unprocessed bulk may be toxic in test cell cultures, whereas partially processed bulk may not be toxic). In certain instances it may be more appropriate to test a mixture consisting of both intact and disrupted cells and their cell culture supernatants removed from the production reactor prior to further processing.

In case of unprocessed bulk, it is required to conduct virus test on at least 3 lots obtained from pilot scale or commercial scale production. It is recommended that manufacturers develop programs for the ongoing assessment of adventitious viruses in production batches. The scope, extent and frequency of virus testing on the unprocessed bulk should be determined by taking several points into consideration including the nature of the cell lines used to produce the desired products, the results and extent of virus tests performed during the qualification of the cell lines, the cultivation method, raw material sources and results of viral clearance studies. Screening in vitro tests, using one or several cell lines, are generally employed to test unprocessed bulk. If appropriate, a NAT test or other suitable methods may be used.

Generally, harvest material in which adventitious virus has been detected should not be used to manufacture the product. If any adventitious viruses are detected at this level, the process should be carefully checked to determine the cause of the contamination, and appropriate actions taken.

5.2. Virus test as an acceptance test of an intermediate material, etc.

When a biological product is manufactured from tissue, body fluid etc. of human or animal origin, there are cases that an intermediate material, partially processed as a raw material or substrate by outside manufacturer, is purchased and used for manufacturing. In such case, if any test to meet this General Information has been conducted by such outside manufacturer, it is necessary for the manufacturer of the biological product, who purchased the intermediate material, to examine what sort of virus test has to be conducted as acceptance tests, and to keep record on the basis of rationality including the details of the test conducted.

On the other hand, if no test to meet this General Information has been conducted by such outside manufacturer of the raw material, all necessary virus free test has to be conducted to meet this General Information on the intermediate material regarding it as the direct substrate for drug production.

5.3. Virus test on a final product

Virus tests to be conducted on a final product (or on a product to reach the final product) has to be defined under comprehensive consideration of the type of raw material or substrate, the result of virus test conducted on raw material/substrate, the result of evaluation on viral removal/inactivation process, any possibility of virus contamination in the manufacturing process, etc. Comprehensive viral safety assurance can only be achieved by appropriate selection of the raw material/substrate, an appropriate virus test conducted on the raw material/substrate/intermediate material, the virus test conducted at an appropriate stage of manufacturing, an appropriate viral clearance test, etc. However, there are cases of having specific backgrounds, such as 1) use of the raw material derived from unspecified individual human, 2) possible existence of virus at window period, 3) specific detection limit of virus test, etc. and in these cases, virus contamination to the final product may occur if there is any deficiency on the manufacturing process (e.g., damage of membrane filter) or any mix-up of the raw materials, etc. To avoid such accidental virus contamination, it may be recommended to conduct nucleic amplification test (NAT) on the final product focusing on the most risky virus among those that may possibly to exist in the raw material.

6. Process evaluation on viral clearance

6.1. Rationale, objective and general items to be concerned with respect to viral clearance process evaluation

Evaluation of a viral inactivation/removal process is important for ensuring safety of a biological product derived from tissue or body fluid of human or animal origin. Conducting evaluation on viral clearance is to assure, even to some extent, elimination of the virus, which may exist in a raw material, etc. or may be derived to the process due to unexpected situation. Viral clearance studies should be made by a carefully designed appropriate method, and has to be rationally evaluated.

The objective of viral clearance studies is to assess process step(s) that can be considered to be effective in inactivating/removing viruses and to estimate quantitatively the overall level of virus reduction obtained by the process. This should be achieved by the deliberate addition (“spiking”) of significant amounts of a virus at different manufacturing/purification steps and demonstrating its removal or inactivation during the subsequent steps. It is not necessary to evaluate or characterize every step of a manufacturing process if adequate clearance is demonstrated by the use of fewer steps. It should be borne in mind that other steps in the process may have an indirect effect on the viral inactivation/removal achieved. Manufacturers should explain and justify the approach used in studies for evaluating viral clearance.

The reduction of virus infectivity may be achieved by removal of virus particles or by inactivation of viral infectivity. For each production step assessed, the possible mechanism of loss of viral infectivity should be described with regard to whether it is due to inactivation or removal. For inactivation steps, the study should be planned in such a way that samples are taken at different times and an inactivation curve constructed.

6.2. Selection of virus

To obtain broad range of information of viral inactivation/removal, it is desirable that a model virus used for viral clearance studies should be chosen from the viruses with broad range of characteristics in aspects of DNA/RNA, with or without envelope, particle size, significant resistance to
### Table 3  
Example of viruses which have been used for viral clearance studies

<table>
<thead>
<tr>
<th>Virus</th>
<th>Family</th>
<th>Genus</th>
<th>Natural host</th>
<th>Genome</th>
<th>Env</th>
<th>Size (nm)</th>
<th>Shape</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesicular Stomatitis Virus</td>
<td>Rhabdo</td>
<td>Vesiculovirus</td>
<td>Equine</td>
<td>RNA</td>
<td>yes</td>
<td>70 × 150</td>
<td>Bullet</td>
<td>Low</td>
</tr>
<tr>
<td>Parainfluenza Virus</td>
<td>Paramyx</td>
<td>Type 1,3</td>
<td>Various</td>
<td>RNA</td>
<td>yes</td>
<td>100 – 200+</td>
<td>Pleo-Spher</td>
<td>Low</td>
</tr>
<tr>
<td>MuLV</td>
<td>Retro</td>
<td>Type C oncovirus</td>
<td>Mouse</td>
<td>RNA</td>
<td>yes</td>
<td>80 – 110</td>
<td>Spherical</td>
<td>Low</td>
</tr>
<tr>
<td>Sindbis Virus</td>
<td>Toga</td>
<td>Alphavirus</td>
<td>Human</td>
<td>RNA</td>
<td>yes</td>
<td>60 – 70</td>
<td>Spherical</td>
<td>Low</td>
</tr>
<tr>
<td>BVDV</td>
<td>Flavi</td>
<td>Pestivirus</td>
<td>Bovine</td>
<td>RNA</td>
<td>yes</td>
<td>50 – 70</td>
<td>Pleo-Spher</td>
<td>Low</td>
</tr>
<tr>
<td>Pseudorabies Virus</td>
<td>Herpes</td>
<td>Varicellovirus</td>
<td>Swine</td>
<td>DNA</td>
<td>yes</td>
<td>120 – 200</td>
<td>Spherical</td>
<td>Med</td>
</tr>
<tr>
<td>Poliovirus Sabin Type 1</td>
<td>Picorna</td>
<td>Enterovirus</td>
<td>Human</td>
<td>RNA</td>
<td>no</td>
<td>25 – 30</td>
<td>Icosahedral</td>
<td>Med</td>
</tr>
<tr>
<td>Encephalomyocarditis Virus</td>
<td>Picorna</td>
<td>Cardiovirus</td>
<td>Mouse</td>
<td>RNA</td>
<td>no</td>
<td>25 – 30</td>
<td>Icosahedral</td>
<td>Med</td>
</tr>
<tr>
<td>Reovirus Type 3</td>
<td>Reo</td>
<td>Orthoreovirus</td>
<td>Various kind</td>
<td>RNA</td>
<td>no</td>
<td>60 – 80</td>
<td>Spherical</td>
<td>Med</td>
</tr>
<tr>
<td>SV 40</td>
<td>Papova</td>
<td>Polyomavirus</td>
<td>Monkey</td>
<td>DNA</td>
<td>no</td>
<td>40 – 50</td>
<td>Icosahedral</td>
<td>Very high</td>
</tr>
<tr>
<td>Parovirus: canine, porcine</td>
<td>Parvo</td>
<td>Parvovirus</td>
<td>Canine</td>
<td>DNA</td>
<td>no</td>
<td>18 – 24</td>
<td>Icosahedral</td>
<td>Very high</td>
</tr>
</tbody>
</table>

Physical/chemical treatment, etc. and it is necessary to combine about 3 model viruses to cover these characteristics.

At choice of a model virus, there are also the ways to choose a virus closely related to or having the same characteristics of the virus known to exist in the raw material. In such case, it is in principle recommendable to choose a virus which demonstrates a higher resistance to inactivation/removal treatment if two or more candidate viruses are available for choice. Further, a virus which can grow at a high titer is desirable for choice, although this may not always be possible. In addition to the above, choosing a virus, which will provide effective and reliable assay result at each step, is necessary, since sample condition to be tested at each step of a production process may influence the detection sensitivity. Consideration should also be given to health hazard which may pose to the personnel performing the clearance studies.

For the other items taken for consideration at choice of virus, the Notice, Iyakushin No. 329 can be used as a reference. Examples of the virus which have been used for viral clearance studies are shown in Table 3 which was derived from Iyakushin No. 329. However, the Notice, Iyakushin No. 329, is on viral safety of a product derived cell line of human or animal origin, and a more appropriate model virus has to be chosen taking into account the origin/raw material of biological products.

### 6.3. Design of viral clearance studies

The purpose of viral clearance studies is to quantitatively evaluate removal or inactivation capability of a process, in which a virus is intentionally spiked to a specific step of a manufacturing process.

Following are the precautions to be taken at planning viral clearance studies.

1. Care should be taken in preparing the high-titer virus to avoid aggregation which may enhance physical removal and decrease inactivation thus distorting the correlation with actual production.

2. Virus detection method gives great influence to viral clearance factor. Accordingly, it is advisable to gain detection sensitivity of the methods available in advance, and use a method with a detection sensitivity as high as possible. Quantitative infectivity assays should have adequate sensitivity and reproducibility in each manufacturing process, and should be performed with sufficient replicates to ensure adequate statistical validity of the result. Quantitative assays not associated with infectivity may be used if justified. Appropriate virus controls should be included in all infectivity assays to ensure the sensitivity of the method. Also, the statistics of sampling virus when at low concentrations (for example, number of virus is 1-1000/L) should be considered.

3. Viral clearance studies are performed in a miniature size system that simulates the actual production process of the biotechnological/biological product used by the manufacturer. It is inappropriate to introduce any virus into a production facility because of GMP constraints. Therefore, viral clearance studies should be conducted in a separate laboratory equipped for virological work and performed by staff with virological expertise in conjunction with production personnel involved in designing and preparing a scaled-down version of the purification process. The viral clearance studies should be performed under the basic concept of GLP.

4. Each factor on a viral clearance study of a process, which is performed in miniature size, should reflect that of actual manufacturing as far as possible, and its rationality should be clarified. In case of chromatograph process, length of column bed, linear velocity, ratio of bed volume per velocity (in other words, contact time), buffer, type of column packing, pH, temperature, protein concentration, salt concentration and concentration of the objective product are all correspondent to those of the actual produc-
tion. Further, similarity of elution profile should be achieved. For the other process, similar concept should be applied. If there is any factor which can not reflect the actual production, its effect to the result should be examined.

(5) It is desirable that two or more inactivation/removal processes of different principles are selected and examined.

(6) As for the process which is expected to inactivate/remove virus, each step should be evaluated in aspect of clearance capability, and carefully determined if it is the stage of inactivation, removal or their combination for designing the test. Generally, in viral clearance test, a virus is spiked in each step which is the object of the test, and after passing through the process in question, the reduction level of infectivity is evaluated. But, in some case, it is accepted that a high potential virus is spiked at a step of the process, and virus concentration of each succeeding step is carefully monitored. When removal of virus is made by separation or fractionation, it is desirable to investigate how the virus is separated or fractionated (mass balance).

(7) For assessment of viral inactivation, unprocessed crude material or intermediate material should be spiked with infectious virus and the reduction factor calculated. It should be recognized that virus inactivation is not a simple, first order reaction and is usually more complex, with a fast "phase 1" and a slow "phase 2". The study should, therefore, be planned in such a way that samples are taken at different times and an inactivation curve constructed. It is recommended that studies for inactivation include at least one time point less than the minimum exposure time and greater than zero, in addition to the minimum exposure time. The reproducible clearance should be demonstrated in at least two independent studies. When there is a possibility that the virus is a human pathogen, it is very important that the effective inactivation process is designed and additional data are obtained. The initial virus load should be determined from the virus which can be detected in the spiked starting material. If this is not possible, the initial virus load may be calculated from the titer of the spiking virus preparation. Where inactivation is too rapid to plot an inactivation curve using process conditions, appropriate controls should be performed to demonstrate that infectivity is indeed lost by inactivation.

(8) If antibody against virus exists in an unprocessed material, caution should be taken at clearance studies, since it may affect the behavior of virus at viral removal or inactivation process.

(9) Virus spiked in unprocessed material should be sufficient enough to evaluate viral removal or inactivation capability of the process. However, the virus "spike" to be added to the unprocessed material should be as small as possible in comparison with the sample volume of the unprocessed material so as not to cause characteristic change of the material by addition of the virus nor to cause behavioral change of the protein in the material.

(10) It is desirable that the virus in the sample is subject for quantitative determination without applying ultracentrifuge, dialysis, storage, etc. as far as possible. However, there may be a case that any handling before quantitative test, such as remove procedure of inhibitor or toxic substance, storage for a period to realize test at a time, etc., is inevitable. If any manipulation, such as dilution, concentration, filtration, dialysis, storage, etc., is applied for preparation of the sample for testing, a parallel control test, which passes through a similar manipulation, should be conducted to assess infectivity variance at the manipulation.

(11) Buffers and product (desired protein or other component contained therein) should be evaluated independently for toxicity or interference in assays used to determine the virus titer, as these components may adversely affect the indicator cells. If the solutions are toxic to the indicator cells, dilution, adjustment of the pH, or dialysis of the buffer containing spiked virus might be necessary. If the product itself has anti-viral activity, the clearance study may need to be performed without the product in a "mock" run, although omitting the product or substituting a similar protein that does not have anti-viral activity could affect the behavior of the virus in some production steps.

(12) Many purification schemes use the same or similar buffers or columns, repetitively. The effects of this approach should be taken into account when analyzing the data. The effectiveness of virus elimination by a particular process may vary with the stage in manufacture at which it is used.

(13) Overall reduction factors may be underestimated where production conditions or buffers are too cytotoxic or virucidal and should be discussed on a case-by-case basis. Overall reduction factors may also be overestimated due to inherent limitations or inadequate design of viral clearance studies.

(14) It has to be noted that clearance capability of viral removal/inactivation process may vary depending upon the type of virus. The viral removal/inactivation process, which displays viral clearance by specific principle or mechanism, may be quite effective to the virus, which meets such mechanism of action, but not effective to the other type of viruses. For example, S/D treatment is generally effective to the virus with lipid membrane, but not effective to the non-enveloped virus. Further, some virus is resistant to the general heating process (55 – 60°C, 30 minutes). When clearance is expected for such virus, introduction of a further severe condition or process, which has different principle or mechanism, is necessary. Virus removal by membrane filtration, which is different from S/D or heat treatment in aspect of principle, is effective to a broad range of viruses that can not pass through the membrane. Affinity chromatography process, which specifically absorbs the objective protein, can thoroughly wash out the materials other than the objective protein including virus etc. and is generally effective for viral removal. Separation/fractionation of a virus from an objective protein is sometimes very difficult, but there are not so rare that ion exchange chromatography, ethanol fractionation, etc. is effective for clearance of a virus which can not be sufficiently inactivated or removed by the other process.

(15) Effective clearance may be achieved by any of the following: multiple inactivation steps, multiple complementary separation steps, or combinations of inactivation and separation steps. Separation methods may be dependent on the extremely specific physico-chemical properties of a virus which influence its interaction with gel matrices and precipitation properties. However, despite these potential variables, effective removal can be obtained by a combination of complementary separation steps or combinations of inactivation and separation steps. Well designed separation steps, such as chromatographic procedures, filtration steps and extractions, can be also effective virus removal steps provided that they are performed under appropriately controlled conditions.
6.4. Interpretation of viral clearance studies

6.4.1. Evaluation on viral clearance factor

Viral clearance factor is a logarithm of reduction ratio of viral amount (infectious titer) between each step applied for viral clearance of a manufacturing process. Total viral clearance factor throughout the process is sum of the viral clearance factor of each step appropriately evaluated.

Whether each and total viral clearance factor obtained are acceptable or should not be evaluated in aspects of every virus that can be realistically anticipated to derive into the raw material or the manufacturing process, and its rationality should be recorded.

In case that existence of any viral particle is recognized in a substrate for drug production, e.g., a substrate of rodent origin for biodrug production, it is important not only to demonstrate removal or inactivation of such virus, but also to demonstrate that the purification process has enough capability over the required level to assure safety of the final product at an appropriate level. The virus amount removed or inactivated in a manufacturing process should be compared with the virus amount assumed to exist in the substrate etc. used for manufacturing drug, and for this purpose, it is necessary to obtain the virus amount in the raw materials/substrate, etc. Such figure can be obtained by measuring infectious titer or by the other method such as transmission electron microscope (TEM). For evaluation of overall process, a virus amount, far larger than that assumed to exist in the amount of the raw materials/substrate which is inactivated in a manufacturing process after several uses may provide support for repeated use of such columns.

(18) The Notice, Iyakushin No. 329, would be used as a reference when viral clearance studies on biological products are designed.

6.4.2. Calculation of viral clearance index

Viral clearance factor, \( R \), for viral removal/inactivation process can be calculated by the following formula.

\[
R = \log\left(\frac{V_1 \times T_1}{V_2 \times T_2}\right)
\]

In which

- \( R \): Logarithm of reduction ratio
- \( V_1 \): Sample volume of the unprocessed material
- \( T_1 \): Virus amount (titer) of the unprocessed material
- \( V_2 \): Sample volume of the processed material
- \( T_2 \): Virus amount (titer) of the processed material

At the calculation of viral clearance factor, it is recommendable to use the virus titer detected in the sample preparation of the unprocessed material after addition of virus, not the viral titer added to the sample preparation wherever possible. If this is not possible, loaded virus amount is calculated from virus titer of the solution used for spike.

6.4.3. Interpretation of results and items to be concerned at evaluation

At the interpretation and the evaluation of the data on effectiveness of viral inactivation/removal process, there are various factors to be comprehensively taken into account, such as (1) appropriateness of the virus used for the test, (2) design of the viral clearance studies, (3) virus reduction ratio shown in logarithm, (4) time dependence of inactivation, (5) factors/items which give influence to the inactivation/removal process, (6) sensitivity limit of virus assay method, (7) possible effect of the inactivation/removal process which is specific to certain class of viruses.

Additional items to be concerned at appropriate interpretation and evaluation of the viral clearance data are as follows:

1. Behavior of virus used to the test

   At interpretation of the viral clearance results, it is necessary to recognize that clearance mechanism may differ depending upon the virus used for the test. Virus used for a test is generally produced in tissue culture, but behavior of the virus prepared in the tissue culture may be different from that of the native virus. Examples are possible differences of purity and degree of aggregation between the native and the cultured viruses. Further, change of surface properties of a virus, e.g., addition of a sucrose chain which is ascribed to specific nature of a separation process, may give effect to the separation. These matters should be also considered at interpretation of the results.

2. Design of test

   Viral clearance test should have been designed taking into account variation factors of the manufacturing process and scaling down, but there still remain some variance from actual production scale. It is necessary to consider such variance at the interpretation of the data and limitation of the test.

3. Acceptability of viral reduction data

   Total viral clearance factor is expressed as a sum of logarithm of reduction ratio obtained at each step. The summation of the reduction factor of multiple steps, particularly of steps with little reduction (e.g., below 1 log10), may overestimate viral removal/inactivation capability of the overall process. Therefore, virus titer of the order of 1 log10 or less has to be ignored unless justified. Further, viral clearance factor achieved by repeated use of the same or similar method should be ignored for calculation unless justified.

4. Time dependence of inactivation

   Inactivation of virus infectivity frequently shows biphasic curve, which consists of a rapid initial phase and subsequent slow phase. It is possible that a virus not inactivated in a step may be more resistant to the subsequent step. For example, if an inactivated virus forms coagulation, it may be resistant to any chemical treatment and heating.

5. Evaluation of viral reduction ratio shown logarithm

   Viral clearance factor shown in logarithm of reduction ratio of virus titer can demonstrate drastic reduction of residual infectious virus, but there is a limit that infectious titer can never be reduced to zero. For example, reduction in infectivity of a preparation containing 8 log_{10} infectious unit per mL by a factor of 8 log_{10} leaves zero log_{10} per mL or one
infectious unit per mL, taking into account the detection limit of assay.

(6) Variable factor of manufacturing process

Minor variance of a variation factor of a manufacturing process, e.g., contact time of a spiked sample to a buffer or a column, will sometimes give influence to viral removal or inactivation effect. In such case, it may be necessary to investigate to what extent such variance of the factor has given influence to the process concerned in aspect of viral inactivation.

(7) Existence of anti-viral antiserum

Anti-viral antiserum that exists in the sample preparation used for a test may affect sensitivity of distribution or inactivation of a virus, which may result in not only defusing the virus titer but complicating interpretation of the test result. So, existence of anti-viral antiserum is one of the important variable factors.

(8) Introduction of a new process for removal/inactivation

Viral clearance is an important factor for securing safety of drug. In case that an achievement level of infective clearance of a process is considered insufficient, a process which is characterized by inactivation/removal mechanism to meet the purpose or an inactivation/removal process which can mutually complement to the existence process has to be introduced.

(9) Limit of viral clearance studies

Viral clearance studies are useful for contributing to the assurance that an acceptable level of safety in the final product is achieved but do not by themselves establish safety. However, a number of factors in the design and execution of viral clearance studies may lead to an incorrect estimate of the ability of the process to remove virus infectivity, as described above.

7. Statistics

The viral clearance studies should include the use of statistical analysis of the data to evaluate the results. The study results should be statistically valid to support the conclusions reached.

7.1. Statistical considerations for assessing virus assays

Virus titrations suffer the problems of variation common to all biological assay systems. Assessment of the accuracy of the virus titrations and reduction factors derived from them and the validity of the assays should be performed to define the reliability of a study. The objective of statistical evaluation is to establish that the study has been carried out to an acceptable level of virological competence.

Assay

1. Assay methods may be either semiquantitative or quantitative. Both semiquantitative and quantitative assays are amenable to statistical evaluation.

2. Variation can arise within an assay as a result of dilution errors, statistical effects and differences within the assay system which are either unknown or difficult to control. These effects are likely to be greater when different assay runs are compared (between-assay variation) than when results within a single assay run are compared (within-assay variation).

3. The 95% confidence limits for results of within-assay variation normally should be on the order of \( \pm 0.5 \log_{10} \) of the mean. Within-assay variation can be assessed by standard textbook methods. Between-assay variation can be monitored by the inclusion of a reference preparation, the estimate of whose potency should be within approximately 0.5 log_{10} of the mean estimate established in the laboratory for the assay to be acceptable. Assays with lower precision may be acceptable with appropriate justification.

7.2. Reproducibility and confidence limit of viral clearance studies

An effective virus inactivation/removal step should give reproducible reduction of virus load shown by at least two independent studies. The 95% confidence limits for the reduction factor observed should be calculated wherever possible in studies of viral clearance. If the 95% confidence limits for the viral assays of the starting material are \( \pm s \), and for the viral assays of the material after the step are \( \pm a \), the 95% confidence limits for the reduction factor are \( \pm \sqrt{s^2 + a^2} \).

8. Re-evaluation of viral clearance

Whenever significant changes in the production or purification process are made, the effect of that change, both direct and indirect, on viral clearance should be re-evaluated as needed. Changes in process steps may also change the extent of viral clearance.

9. Measurement for viral clearance studies

9.1. Measurement of virus infective titer

Assay methods may be either semiquantitative or quantitative. Semiquantitative methods include infectivity assays in animals or in cultured cell infections dose (CCID) assays, in which the animal or cell culture is scored as either infected or not. Infectivity titers are then measured by the proportion of animals or culture infected. In quantitative methods, the infectivity measured varies continuously with the virus input. Quantitative methods include plaque assays where each plaque counted corresponds to a single infectious unit. Both quantal and quantitative assays are amenable to statistical evaluation.

9.2. Testing by nucleic-acid-amplification test (NAT)

NAT can detect individual or pooled raw material/cell substrate or virus genome at a high sensitivity even in a stage that serum test on each virus is negative. Further, it can detect HBV or HCV gene, which can not be measured in culture system. Window period can be drastically shortened at the test on HBV, HCV and HIV, and the method is expected to contribute as an effective measure for ensuring viral safety. However, depending upon a choice of primer, there may be a case that not all the subtype of objective virus can be detected by this method, and, therefore, it is recommendable to evaluate, in advance, if subtype of a broad range can be detected.

NAT will be an effective evaluation method for virus removal capability for viral clearance. However, in case of viral inactivation process, viral inactivation obtained by this method may be underrated, since there is a case that inactivated virus still shows positive on nucleic acid. Further, at introduction of NAT, cautions should be taken on rationality of detection sensitivity, choice of a standard which is used as run-control, quality assurance and maintenance of a reagent used for primer, interpretation of positive and negative results, etc.

10. Reporting and preservation

All the items relating to virus test and viral clearance studies should be reported and preserved.
Capillary Electrophoresis

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

Capillary electrophoresis is a physical method of analysis based on the migration, inside a capillary, of charged analytes dissolved in an electrolyte solution, under the influence of a direct-current electric field.

The migration velocity of an analyte under an electric field of intensity \( E \), is determined by the electrophoretic mobility of the analyte and the electroosmotic mobility of the buffer inside the capillary. The electrophoretic mobility of a solute \( (\mu_n) \) depends on the characteristics of the solute (electric charge, molecular size and shape) and those of the buffer in which the migration takes place (type and ionic strength of the electrolyte, pH, viscosity and additives). The electrophoretic velocity \( (v_{ep}) \) of a solute, assuming a spherical shape, is given by the equation:

\[
v_{ep} = \mu_n E = \left( \frac{q}{6\pi \eta r} \right) \frac{V}{L}
\]

\( q \): effective charge of the solute,
\( \eta \): viscosity of the electrolyte solution,
\( r \): Stoke’s radius of the solute,
\( V \): applied voltage,
\( L \): total length of the capillary.

When an electric field is applied through the capillary filled with buffer, a flow of solvent is generated inside the capillary, called electroosmotic flow. The velocity of the electroosmotic flow depends on the electroosmotic mobility \( (\mu_{eo}) \) which in turn depends on the charge density on the capillary internal wall and the buffer characteristics. The electroosmotic velocity \( (v_{eo}) \) is given by the equation:

\[
v_{eo} = \mu_{eo} E = \left( \frac{\zeta e}{\eta} \right) \left( \frac{V}{L} \right)
\]

\( \zeta \): zeta potential of the capillary surface,
\( e \): dielectric constant of the buffer.

The velocity of the solute \( (v) \) is given by:

\[
v = v_{ep} + v_{eo}
\]

The electrophoretic mobility of the analyte and the electroosmotic mobility may act in the same direction or in opposite directions, depending on the charge of the solute. In normal capillary electrophoresis, anions will migrate in the opposite direction to the electroosmotic flow and their velocities will be smaller than the electroosmotic velocity. Cations will migrate in the same direction as the electroosmotic flow and their velocities will be greater than the electroosmotic velocity. Under conditions in which there is a fast electroosmotic velocity with respect to the electrophoretic velocity of the solutes, both cations and anions can be separated in the same run.

The time \( (t) \) taken by the solute to migrate the distance \( (l) \) from the injection end of the capillary to the detection point (capillary effective length) is given by the expression:

\[
t = \frac{l}{v_{ep} + v_{eo}} = \frac{l \times L}{(\mu_{eo} + \mu_n)V}
\]

In general, uncoated fused-silica capillaries above pH 3 have negative charge due to ionized silanol groups in the inner wall. Consequently, the electroosmotic flow is from anode to cathode. The electroosmotic flow must remain constant from run to run if good reproducibility is to be obtained in the migration velocity of the solutes. For some applications, it may be necessary to reduce or suppress the electroosmotic flow by modifying the inner wall of the capillary or by changing the concentration, composition and/or pH of the buffer solution.

After the introduction of the sample into the capillary, each analyte ion of the sample migrates within the background electrolyte as an independent zone, according to its electrophoretic mobility. Zone dispersion, that is the spreading of each solute band, results from different phenomena. Under ideal conditions the sole contribution to the solute-zone broadening is molecular diffusion of the solute along the capillary (longitudinal diffusion). In this ideal case the efficiency of the zone, expressed as the number of theoretical plates \( (N) \), is given by:

\[
N = \frac{(\mu_{eo} + \mu_n) \times V \times l}{2 \times D \times L}
\]

\( D \): molecular diffusion coefficient of the solute in the buffer.

In practice, other phenomena such as heat dissipation, sample adsorption onto the capillary wall, mismatched conductivity between sample and buffer, length of the injection plug, detector cell size and unlevelled buffer reservoirs can also significantly contribute to band dispersion.

Separation between two bands (expressed as the resolution, \( R_s \)) can be obtained by modifying the electrophoretic mobility of the analytes, the electroosmotic mobility induced
in the capillary and by increasing the efficiency for the band of each analyte, according to the equation:

\[ R_m = \frac{1}{4} \frac{\mu_{ep_a} - \mu_{ep_b}}{\mu_{ep_a} + \mu_{ep_b}} \]

\[ \mu_{ep_a} \text{ and } \mu_{ep_b}: \text{electrophoretic mobilities of the two analytes separated,} \]
\[ \mu_{ep_a}: \text{mean electrophoretic mobility of the two analytes} \]
\[ \mu_{ep} = \frac{1}{2} (\mu_{ep_a} + \mu_{ep_b}). \]

**Apparatus**

An apparatus for capillary electrophoresis is composed of:

1. A high-voltage, controllable direct-current power supply,
2. Two buffer reservoirs, held at the same level, containing the prescribed anodic and cathodic solutions,
3. Two electrode assemblies (the cathode and the anode), immersed in the buffer reservoirs and connected to the power supply,
4. A separation capillary (usually made of fused-silica) which, when used with specific types of detectors, has an optical viewing window aligned with the detector. The ends of the capillary are placed in the buffer reservoirs. The capillary is filled with the solution prescribed in the monograph,
5. A suitable injection system,
6. A detector able to monitor the amount of substances of interest passing through a segment of the separation capillary at a given time. It is usually based on absorption spectrophotometry (UV and visible) or fluorometry, but conductimetric, amperometric, or mass spectrometric detection can be useful for specific applications. Indirect detection is an alternative method used to detect non-UV-absorbing and non-fluorescent compounds,
7. A thermostatic system able to maintain a constant temperature inside the capillary and by increasing the efficiency for the band of each analyte, according to the equation:

\[ R_m = \frac{1}{4} \frac{\mu_{ep_a} - \mu_{ep_b}}{\mu_{ep_a} + \mu_{ep_b}} \]

\[ \mu_{ep_a} \text{ and } \mu_{ep_b}: \text{electrophoretic mobilities of the two analytes separated,} \]
\[ \mu_{ep_a}: \text{mean electrophoretic mobility of the two analytes} \]
\[ \mu_{ep} = \frac{1}{2} (\mu_{ep_a} + \mu_{ep_b}). \]

**Optimization**

Optimization of the separation is a complex process where several separation parameters can play a major role. The main factors to be considered in the development of separations are instrumental and electrolytic solution parameters.

**Instrumental parameters**

1. **Voltage:** A Joule heating plot is useful in optimizing the applied voltage and column temperature. Separation time is inversely proportional to applied voltage. However, an increase in the voltage used can cause excessive heat production, giving rise to temperature and, as a result thereof, viscosity gradients in the buffer inside the capillary. This effect causes band broadening and decreases resolution.
2. **Polarity:** Electrode polarity can be normal (anode at the inlet and cathode at the outlet) and the electroosmotic flow will move toward the cathode. If the electrode polarity is reversed, the electroosmotic flow is away from the outlet and only charged analytes with electroosmotic mobilities greater than the electroosmotic flow will pass to the outlet.
3. **Temperature:** The main effect of temperature is observed on buffer viscosity and electrical conductivity, and therefore on migration velocity. In some cases, an increase in capillary temperature can cause a conformational change in proteins, modifying their migration time and the efficiency of the separation.
4. **Capillary:** The dimensions of the capillary (length and internal diameter) contribute to analysis time, efficiency of separations and load capacity. Increasing total length can decrease the electric fields (working at constant voltage), and increasing both effective length and total length increase migration time. For a given buffer and electric field, heat dissipation, and hence sample band-broadening, depend on the internal diameter of the capillary. The latter also affects the detection limit, depending on the sample volume injected and the detection system employed.

Since the adsorption of the sample components on the capillary wall limits efficiency, methods to avoid these interactions should be considered in the development of a separation method. In the specific case of proteins, several strategies have been devised to avoid adsorption on the capillary wall. Some of these strategies (use of extreme pH and adsorption of positively charged buffer additives) only require modification of the buffer composition to prevent protein adsorption. In other strategies, the internal wall of the capillary is coated with a polymer, covalently bonded to the silica, that prevents interaction between the proteins and the negatively charged silica surface. For this purpose, ready-to-use capillaries with coatings consisting of neutral-hydrophilic, cationic and anionic polymers are available.

**Electrolytic solution parameters**

1. **Buffer type and concentration:** Suitable buffers for
capillary electrophoresis have an appropriate buffer capacity in the pH range of choice and low mobility to minimize current generation.

Matching buffer-ion mobility to solute mobility, whenever possible, is important for minimizing band distortion. The type of sample solvent used is also important to achieve on-column sample focusing, which increases separation efficiency and improves detection.

An increase in buffer concentration (for a given pH) decreases electroosmotic flow and solute velocity.

(2) Buffer pH: The pH of the buffer can affect separation by modifying the charge of the analyte or additives, and by changing the electroosmotic flow. In protein and peptide separation, changing the pH of the buffer from above to below the isoelectric point (pI) changes the net charge of the solute from negative to positive. An increase in the buffer pH generally increases the electroosmotic flow.

(3) Organic solvents: Organic modifiers (methanol, acetonitrile, etc.) may be added to the aqueous buffer to increase the solubility of the solute or other additives and/or to affect the degree of ionization of the sample components. The addition of these organic modifiers to the buffer generally causes a decrease in the electroosmotic flow.

(4) Additives for chiral separations: For the separation of optical isomers, a chiral selector is added to the separation buffer. The most commonly used chiral selectors are cyclodextrins, but crown ethers, polysaccharides and proteins may also be used. Since chiral recognition is governed by the different interactions between the chiral selector and each of the enantiomers, the resolution achieved for the chiral compounds depends largely on the type of chiral selector used. In this regard, for the development of a given separation it may be useful to test cyclodextrins having a different cavity size (α, β, or γ-cyclodextrin) or modified cyclodextrins with neutral (methyl, ethyl, hydroxalkyl, etc.) or ionizable (aminomethyl, carboxymethyl, sulfobutyl ether, etc.) groups. When using modified cyclodextrins, batch-to-batch variations in the degree of substitution of the cyclodextrins must be taken into account since it will influence the selectivity. Other factors controlling the resolution in chiral separations are concentration of chiral selector, composition and pH of the buffer and temperature. The use of organic additives, such as methanol or urea can also modify the resolution achieved.

2. Capillary Gel Electrophoresis

In capillary gel electrophoresis, separation takes place inside a capillary filled with a gel that acts as a molecular sieve. Molecules with similar charge-to-mass ratios are separated according to molecular size since smaller molecules move more freely through the network of the gel and therefore migrate faster than larger molecules. Different biological macromolecules (for example, proteins and DNA fragments), which often have similar charge-to-mass ratios, can thus be separated according to their molecular mass by capillary gel electrophoresis.

Characteristics of Gels

Two types of gels are used in capillary electrophoresis: permanently coated gels and dynamically coated gels. Permanently coated gels, such as cross-linked polyacrylamide, are prepared inside the capillary by polymerization of the monomers. They are usually bonded to the fused-silica wall and cannot be removed without destroying the capillary. If the gels are used for protein analysis under reducing conditions, the separation buffer usually contains sodium dodecyl sulfate and the samples are denatured by heating a mixture of sodium dodecyl sulfate and 2-mercaptoethanol or dithiothreitol before injection. When non-reducing conditions are used (for example, analysis of an intact antibody), 2-mercaptoethanol and dithiothreitol are not used. Separation in cross-linked gels can be optimized by modifying the separation buffer (as indicated in the capillary zone electrophoresis section) and controlling the gel porosity during the gel preparation. For cross-linked polyacrylamide gels, the porosity can be modified by changing the concentration of acrylamide and/or the proportion of cross-linker. As a rule, a decrease in the porosity of the gel leads to a decrease in the mobility of the solutes. Due to the rigidity of these gels, only electrokinetic injection can be used.

Dynamically coated gels are hydrophilic polymers, such as linear polyacrylamide, cellulose derivatives, dextran, etc., which can be dissolved in aqueous separation buffers giving rise to a separation medium that also acts as a molecular sieve. These separation media are easier to prepare than cross-linked polymers. They can be prepared in a vial and filled by pressure in a wall-coated capillary (with no electroosmotic flow). Replacing the gel before every injection generally improves the separation reproducibility. The porosity of the gels can be increased by using polymers of higher molecular mass (at a given polymer concentration) or by decreasing the polymer concentration (for a given polymer molecular mass). A reduction in the gel porosity leads to a decrease in the mobility of the solute for the same buffer. Since the dissolution of these polymers in the buffer gives low viscosity solutions, both hydrodynamic and electrokinetic injection techniques can be used.

3. Capillary Isoelectric Focusing

In isoelectric focusing, the molecules migrate under the influence of the electric field, so long as they are charged, in a pH gradient generated by ampholytes having pI values in a wide range (poly-aminocarboxylic acids), dissolved in the separation buffer.

The three basic steps of isoelectric focusing are loading, focusing and mobilization.

(i) Loading step: Two methods may be employed:

(ii) Sequential loading: a leading buffer, then the ampholytes, then the sample mixed with ampholytes, again ampholytes alone and finally the terminating buffer are introduced into the capillary. The volume of the sample must be small enough not to modify the pH gradient.

(2) Focusing step: When the voltage is applied, ampholytes migrate toward the cathode or the anode, according to their net charge, thus creating a pH gradient from anode (lower pH) to cathode (higher pH). During this step the components to be separated migrate until they reach a pH corresponding to their isoelectric point (pI) and the current drops to very low values.

(3) Mobilization step: If mobilization is required for detection, use one of the following methods. Three methods are available:

(i) in the first method, mobilization is accomplished
During the focusing step under the effect of the electroosmotic flow; the electroosmotic flow must be small enough to allow the focusing of the components;

(ii) in the second method, mobilization is accomplished by applying positive pressure after the focusing step;

(iii) in the third method, mobilization is achieved after the focusing step by adding salts to the cathode reservoir or the anode reservoir (depending on the direction chosen for mobilization) in order to alter the pH in the capillary when the voltage is applied. As the pH is changed, the proteins and ampholytes are mobilized in the direction of the reservoir which contains the added salts and pass the detector.

The separation achieved, expressed as $\Delta pI$, depends on the pH gradient ($d\mu / d\mu$), the number of ampholytes having different $pI$ values, the molecular diffusion coefficient ($D$), the intensity of the electric field ($E$) and the variation of the electrophoretic mobility of the analyte with the pH ($-\delta \mu / d\mu$):

\[
\Delta pI = 3 \frac{d(pH/dx)}{E(-\delta \mu / d\mu)}
\]

Optimization

The main parameters to be considered in the development of separations are:

1. Voltage: Capillary isoelectric focusing utilises very high electric fields, 300 V/cm to 1000 V/cm in the focusing step.

2. Capillary: The electroosmotic flow must be reduced or suppressed depending on the mobilization strategy (see above). Coated capillaries tend to reduce the electroosmotic flow.

3. Solutions: The anode buffer reservoir is filled with a solution with a pH lower than the $pI$ of the most acidic ampholyte and the cathode reservoir is filled with a solution with a pH higher than the $pI$ of the most basic ampholyte. Phosphoric acid for the anode and sodium hydroxide for the cathode are frequently used.

Addition of a polymer, such as methylcellulose, in the ampholyte solution tends to suppress convective forces (if any) and electroosmotic flow by increasing the viscosity. Commercial ampholytes are available covering many pH ranges and may be mixed if necessary to obtain an expanded pH range. Broad pH ranges are used to test the isoelectric point whereas narrower ranges are employed to improve accuracy. Calibration can be done by correlating migration time with isoelectric point for a series of protein markers.

During the focusing step precipitation of proteins at their isoelectric point can be prevented, if necessary, using buffer additives such as glycerol, surfactants, urea or zwitterionic buffers. However, depending on the concentration, urea denatures proteins.

4. Micellar Electrokinetic Chromatography (MEKC)

In micellar electrokinetic chromatography, separation takes place in an electrolyte solution which contains a surfactant at a concentration above the critical micellar concentration (cmc). The solute molecules are distributed between the aqueous buffer and the pseudo-stationary phase composed of micelles, according to the partition coefficient of the solute. The technique can therefore be considered as a hybrid of electrophoresis and chromatography. It is a technique that can be used for the separation of both neutral and charged solutes, maintaining the efficiency, speed and instrumental suitability of capillary electrophoresis. One of the most widely used surfactants in MEKC is the anionic surfactant sodium dodecyl sulfate, although other surfactants, for example cationic surfactants such as cetyltrimethylammonium salts, are also used.

The separation mechanism is as follows. At neutral and alkaline pH, a strong electroosmotic flow is generated and moves the separation buffer ions in the direction of the cathode. If sodium dodecyl sulfate is employed as the surfactant, the electrophoretic migration of the anionic micelle is in the opposite direction, towards the anode. As a result, the overall micelle migration velocity is slowed down compared to the bulk flow of the electrolytic solution. In the case of neutral solutes, since the analyte can partition between the micelle and the aqueous buffer, and has no electrophoretic mobility, the analyte migration velocity will depend only on the partition coefficient between the micelle and the aqueous buffer. In the electropherogram, the peaks corresponding to each uncharged solute are always between that of the electroosmotic flow marker and that of the micelle (the time elapsed between these two peaks is called the separation window). For electrically charged solutes, the migration velocity depends on both the partition coefficient of the solute between the micelle and the aqueous buffer, and on the electrophoretic mobility of the solute in the absence of micelle.

Since the mechanism in MEKC of neutral and weakly ionized solutes is essentially chromatographic, migration of the solute and resolution can be rationalized in terms of the retention factor of the solute ($k^*$), also referred to as mass distribution ratio ($D_{os}$), which is the ratio of the number of moles of solute in the micelle to those in the mobile phase. For a neutral compound, $k^*$ is given by:

\[
k^* = \frac{t_b - t_o}{t_o(1 - \frac{t_b}{t_{mc}})} = \frac{V_S}{V_M}
\]

t_b: migration time of the solute,

t_o: analysis time of an unretained solute (determined by injecting an electroosmotic flow marker which does not enter the micelle, for instance methanol),

t_{mc}: micelle migration time (measured by injecting a micelle marker, such as Sudan III, which migrates while continuously associated in the micelle),

$K$: partition coefficient of the solute,

$V_S$: volume of the micellar phase,

$V_M$: volume of the mobile phase.

Likewise, the resolution between two closely-migrating solutes ($R_S$) is given by:

\[
R_S = \frac{\sqrt[N]{4}}{\alpha} \frac{1}{\alpha} \frac{1}{k_b' + 1} \frac{1 - \frac{t_o}{t_{mc}}}{1 + \frac{t_o}{t_{mc}}} k_s^* \]

$N$: number of theoretical plates for one of the solutes,

$\alpha$: selectivity,

$k_b'$ and $k_s^*$: retention factors for both solutes, respectively ($k_b' > k_s^*$).

Similar, but not identical, equations give $k^*$ and $R_S$ values for electrically charged solutes.
Optimization

The main parameters to be considered in the development of separations by MEKC are instrumental and electrolytic solution parameters.

Instrumental parameters

1) Voltage: Separation time is inversely proportional to applied voltage. However, an increase in voltage can cause excessive heat production that gives rise to temperature gradients and viscosity gradients of the buffer in the cross-section of the capillary. This effect can be significant with high conductivity buffers such as those containing micelles. Poor heat dissipation causes band broadening and decreases resolution.

2) Temperature: Variations in capillary temperature affect the partition coefficient of the solute between the buffer and the micelles, the critical micellar concentration and the viscosity of the buffer. These parameters contribute to the migration time of the solutes. The use of a good cooling system improves the reproducibility of the migration time for the solutes.

3) Capillary: As in capillary zone electrophoresis, the dimensions of the capillary (length and internal diameter) contribute to analysis time and efficiency of separations. Increasing both effective length and total length can decrease the electric fields (working at constant voltage), increase migration time and improve the separation efficiency. The internal diameter controls heat dissipation (for a given buffer and electric field) and consequently the sample band broadening.

Electrolytic solution parameters

1) Surfactant type and concentration: The type of surfactant, in the same way as the stationary phase in chromatography, affects the resolution since it modifies separation selectivity. Also, the log k' of a neutral compound increases linearly with the concentration of surfactant in the mobile phase. Since resolution in MEKC reaches a maximum when k' approaches the value of \( \sqrt{w/w_0} \), modifying the concentration of surfactant in the mobile phase changes the resolution obtained.

2) Buffer pH: Although pH does not modify the partition coefficient of non-ionized solutes, it can modify the electroosmotic flow in uncoated capillaries. A decrease in the buffer pH decreases the electroosmotic flow and therefore increases the resolution of the neutral solutes in MEKC, resulting in a longer analysis time.

3) Organic solvents: To improve MEKC separation of hydrophobic compounds, organic modifiers (methanol, propanol, acetoniitrile, etc.) can be added to the electrolytic solution. The addition of these modifiers usually decreases migration time and the selectivity of the separation. Since the addition of organic modifiers affects the critical micellar concentration, a given surfactant concentration can be used only within a certain percentage of organic modifier before the micellization is inhibited or adversely affected, resulting in the absence of micelles and, therefore, in the absence of partition. The dissociation of micelles in the presence of a high content of organic solvent does not always mean that the separation will no longer be possible; in some cases the hydrophobic interaction between the ionic surfactant monomer and the neutral solutes forms solvophobic complexes that can be separated electrophoretically.

4) Additives for chiral separations: For the separation of enantiomers using MEKC, a chiral selector is included in the micellar system, either covalently bound to the surfactant or added to the micellar separation electrolyte. Micelles that have a moiety with chiral discrimination properties include salts of N-dodecyl-L-amino acids, bile salts, etc. Chiral resolution can also be achieved using chiral discriminators, such as cyclodextrins, added to the electrolytic solutions which contain micellized achiral surfactants.

5) Other additives: Several strategies can be carried out to modify selectivity, by adding chemicals to the buffer. The addition of several types of cyclodextrins to the buffer can also be used to reduce the interaction of hydrophobic solutes with the micelle, thus increasing the selectivity for this type of compound.

The addition of substances able to modify solute-micelle interactions by adsorption on the latter, is used to improve the selectivity of the separations in MEKC. These additives may be a second surfactant (ionic or non-ionic) which gives rise to mixed micelles or metallic cations which dissolve in the micelle and form co-ordination complexes with the solutes.

Quantification

Peak areas must be divided by the corresponding migration time to give the corrected area in order to:

1) compensate for the shift in migration time from run to run, thus reducing the variation of the response,

2) compensate for the different responses of sample constituents with different migration times.

Where an internal standard is used, verify that no peak of the substance to be examined is masked by that of the internal standard.

Calculations

From the values obtained, calculate the content of the component or components being examined. When prescribed, the percentage content of one or more components of the sample to be examined is calculated by determining the corrected area(s) of the peak(s) as a percentage of the total of the corrected areas of all peaks, excluding those due to solvents or any added reagents (normalization procedure). The use of an automatic integration system (integrator or data acquisition and processing system) is recommended.

System Suitability

In order to check the behavior of the capillary electrophoresis system, system suitability parameters are used. The choice of these parameters depends on the mode of capillary electrophoresis used. They are: retention factor (k') (only for micellar electrokinetic chromatography), apparent number of theoretical plates (N), symmetry factor (A3) and resolution (R5). In previous sections, the theoretical expressions for N and R5 have been described, but more practical equations that allow these parameters to be calculated from the electropherograms are given below.

Apparent Number of Theoretical Plates

The apparent number of theoretical plates (N) may be calculated using the expression:

\[
N = 5.54 \left( \frac{t_m}{w_m} \right)^2
\]

where:
- \( t_m \) is the migration time or distance along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to the component,
- \( w_m \) is the width of the peak at half-height.
\[ R = \frac{1.18(t_{R2} - t_{R1})}{w_{h1} + w_{h2}} \]

\[ t_{R1} > t_{R2} \]

\( t_{R1} \) and \( t_{R2} \): migration times or distances along the baseline from the point of injection to the perpendiculars dropped from the maxima of two adjacent peaks,

\( w_{h1} \) and \( w_{h2} \): peak widths at half-height.

When appropriate, the resolution may be calculated by measuring the height of the valley corresponding to the component under consideration, in the electropherogram obtained with the prescribed reference solution, measured from the maximum

\[ \frac{p}{v} = \frac{H_r}{H_c} \]

Symmetry Factor

The symmetry factor (\( A_s \)) of a peak may be calculated using the expression:

\[ A_s = \frac{w_{0.05}}{2d} \]

\( w_{0.05} \): width of the peak at one-twentieth of the peak height,

\( d \): distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

Tests for area repeatability (standard deviation of areas or of the area/migration-time ratio) and for migration time repeatability (standard deviation of migration time) are introduced as suitability parameters. Migration time repeatability provides a test for the suitability of the capillary washing procedures. An alternative practice to avoid the lack of repeatability of the migration time is to use migration time relative to an internal standard.

A test for the verification of the signal-to-noise ratio for a standard preparation (or the determination of the limit of quantification) may also be useful for the determination of related substances.

Signal-to-noise Ratio

The detection limit and quantification limit correspond to signal-to-noise ratios of 3 and 10 respectively. The signal-to-noise ratio (\( S/N \)) is calculated using the expression:

\[ S/N = \frac{2H}{h} \]

\( H \): height of the peak corresponding to the component concerned, in the electropherogram obtained with the prescribed reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to twenty times the width at half-height,

\( h \): range of the background in an electropherogram obtained after injection of a blank, observed over a distance equal to twenty times the width at the half-height of the peak in the electropherogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

### Isoelectric Focusing

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (\( * \), \( * \)).

#### General Principles

Isoelectric focusing (IEF) is a method of electrophoresis that separates proteins according to their isoelectric point. Separation is carried out in a slab of polyacrylamide or agarose gel that contains a mixture of ampholytes (ampholytes). When subjected to an electric field, the ampholytes migrate in the gel to create a pH gradient. In some cases gels containing an immobilized pH gradient, prepared by incorporating weak acids and bases to specific regions of the gel network during the preparation of the gel, are used. When the applied proteins reach the gel fraction that has a pH that is the same as their isoelectric point (\( \text{pI} \)), their charge is neutralized and migration ceases. Gradients can be made over various ranges of pH, according to the mixture of ampholytes chosen.

#### Theoretical Aspects

When a protein is at the position of its isoelectric point, it has no net charge and cannot be moved in a gel matrix by the electric field. It may, however, move from that position by diffusion. The pH gradient forces a protein to remain in its isoelectric point position, thus concentrating it; this concentrating effect is called “focusing”. Increasing the applied voltage or reducing the sample load result in improved separation of bands. The applied voltage is limited by the heat generated, which must be dissipated. The use of thin gels and an efficient cooling plate controlled by a thermostatic circulator prevents the burning of the gel whilst allowing sharp focusing. The separation \( R \) is estimated by determining the minimum \( \text{pI} \) difference \( (\Delta \text{pI}) \), which is necessary to separate 2 neighboring bands:

\[ R: \Delta \text{pI} = 3 \sqrt{\frac{D}{E(-\frac{\mu}{d\text{pI}})}} \]

\( D \): Diffusion coefficient of the protein \( d\text{pH}/dx \), \( \text{pH} \) gradient

\( E \): Intensity of the electric field, in volts per centimeter

\( -\frac{\mu}{d\text{pI}} \): Variation of the solute mobility with the \( \text{pH} \) in the region close to the \( \text{pI} \)

Since \( D \) and \( -\frac{\mu}{d\text{pI}} \) for a given protein cannot be altered, the separation can be improved by using a narrower \( \text{pH} \) range and by increasing the intensity of the electric field. Resolution between protein bands on an IEF gel prepared with carrier ampholytes can be quite good. Improvements in resolution may be achieved by using immobilized \( \text{pH} \) gradients where the buffering species, which are analogous to carrier ampholytes, are copolymerized within the gel matrix. Proteins exhibiting \( \text{pIs} \) differing by at least as little as 0.02 \( \text{pH} \) units may be resolved using a gel prepared with carrier ampholytes while immobilized \( \text{pH} \) gradients can resolve proteins differing by approximately 0.001 \( \text{pH} \) units.
Practical Aspects

Special attention must be paid to sample characteristics and/or preparation. Having salt in the sample can be problematic and it is best to prepare the sample, if possible, in deionized water or 2 per cent ampholytes, using dialysis or gel filtration if necessary.

The time required for completion of focusing in thin-layer polyacrylamide gels is determined by placing a colored protein (e.g., hemoglobin) at different positions on the gel surface and by applying the electric field: the steady state is reached when all applications give an identical band pattern. In some protocols the completion of the focusing is indicated by the time elapsed after the sample application.

The IEF gel can be used as an identity test when the migration pattern on the gel is compared to a suitable standard preparation and IEF calibration proteins, the IEF gel can be used as a limit test when the density of a band on IEF is compared subjectively with the density of bands appearing in a standard preparation, or it can be used as a quantitative test when the density is measured using a densitometer or similar instrumentation to determine the relative concentration of protein in the bands subject to validation.

Apparatus

An apparatus for IEF consists of:

— a controllable generator for constant power, current and potential. Potentials of 2500 V have been used and are considered optimal under a given set of operating conditions. Supply of up to 30 W of constant power is recommended,

—a rigid plastic IEF chamber that contains a cooled plate, of suitable material, to support the gel,

—a plastic cover with platinum electrodes that are connected to the gel by means of paper wicks of suitable width, length and thickness, impregnated with solutions of anodic and cathodic electrolytes.

Isoelectric Focusing in Polyacrylamide Gels: Detailed Procedure

The following method is a detailed description of an IEF procedure in thick polyacrylamide slab gels, which is used unless otherwise stated in the monograph.

Preparation of the Gels

Mould The mould (see Figure) is composed of a glass plate (A) on which a polyester film (B) is placed to facilitate handling of the gel, one or more spacers (C), a second glass plate (D) and clamps to hold the structure together.

7.5% Polyacrylamide gel Dissolve 29.1 g of acrylamide and 0.9 g of N,N′-methylenebisacrylamide in 100 mL of water. To 2.5 volumes of this solution, add the mixture of ampholytes specified in the monograph and dilute to 10 volumes with water. Mix carefully and degas the solution.

Preparation of the mould Place the polyester film on the lower glass plate, apply the spacer, place the second glass plate and fit the clamps. Place 7.5% polyacrylamide gel prepared before use on a magnetic stirrer, and add 0.25 volumes of a solution of ammonium persulfate (1 in 10) and 0.25 volumes of N,N′,N″,N‴-tetramethylethylenediamine. Immediately fill the space between the glass plates of the mould with the solution.

Method

Dismantle the mould and, making use of the polyester film, transfer the gel onto the cooled support, wetted with a few millilitres of a suitable liquid, taking care to avoid forming air bubbles. Prepare the test solutions and reference solutions as specified in the monograph. Place strips of paper for sample application, about 10 mm × 5 mm in size, on the gel and impregnate each with the prescribed amount of the test and reference solutions. Also apply the prescribed quantity of a solution of proteins with known isoelectric points as pH markers to calibrate the gel. In some protocols the gel has pre-cast slots where a solution of the sample is applied instead of using impregnated paper strips. Cut 2 strips of paper to the length of the gel and impregnate them with the electrolyte solutions: acid for the anode and alkaline for the cathode. The compositions of the anode and cathode solutions are given in the monograph. Apply these paper wicks to each side of the gel several millimetres from the edge. Fit the cover so that the electrodes are in contact with the wicks (respecting the anodic and cathodic poles). Proceed with the isoelectric focusing by applying the electrical parameters described in the monograph. Switch off the current when the migration of the mixture of standard proteins has stabilized. Using forceps, remove the sample application strips and the 2 electrode wicks. Immers the gel in fixing solution for isoelectric focusing in polyacrylamide gel. Incubate with gentle shaking at room temperature for 30 minutes. Drain off the solution and add 200 mL of destaining solution. Incubate with shaking for 1 hour. Drain the gel, add coomassie staining TS. Incubate for 30 minutes. Destain the gel by passive diffusion with destaining solution until the bands are well visualized against a clear background. Locate the position and intensity of the bands in the electropherogram as prescribed in the monograph.

Variations to the Detailed Procedure (Subject to Validation)

Where reference to the general method on isoelectric focusing is made, variations in methodology or procedure may be made subject to validation. These include:

(1) the use of commercially available pre-cast gels and of commercial staining and destaining kits,
(2) the use of immobilized pH gradients,
(3) the use of rod gels,
(4) the use of gel cassettes of different dimensions, including ultra-thin (0.2 mm) gels,
(5) variations in the sample application procedure, including different sample volumes or the use of sample application masks or wicks other than paper,
(6) the use of alternate running conditions, including variations in the electric field depending on gel dimensions and equipment, and the use of fixed migration times...
rather than subjective interpretation of band stability,

(7) the inclusion of a pre-focusing step,

(8) the use of automated instrumentation,

(9) the use of agarose gels.

Validation of Iso-Electric Focusing Procedures

Where alternative methods to the detailed procedure are employed they must be validated. The following criteria may be used to validate the separation:

(1) formation of a stable pH gradient of desired characteristics, assessed for example using colored pH markers of known isoelectric points,

(2) comparison with the electropherogram provided with the chemical reference substance for the preparation to be examined,

(3) any other validation criteria as prescribed in the monograph.

Specified Variations to the General Method

Variations to the general method required for the analysis of specific substances may be specified in detail in monographs. These include:

(1) the addition of urea in the gel (3 mol/L concentration is often satisfactory to keep protein in solution but up to 8 mol/L can be used): some proteins precipitate at their isoelectric point. In this case, urea is included in the gel formulation to keep the protein in solution. If urea is used, only fresh solutions should be used to prevent carbamylation of the protein,

(2) the use of alternative staining methods,

(3) the use of gel additives such as non-ionic detergents (e.g., octylglucoside) or zwitterionic detergents (e.g., 3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO)), or 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) or 3-[3-Cholamidopropyl]dimethy lammonio]-2-hydroxy-1-propanesulfonate (CHAPSO)), and the addition of ampholyte to the sample, to prevent proteins from aggregating or precipitating.

Points to Consider

Samples can be applied to any area on the gel, but to protect the proteins from extreme pH environments samples should not be applied close to either electrode. During method development the analyst can try applying the protein in 3 positions on the gel (i.e. middle and both ends); the pattern of a protein applied at opposite ends of the gel may not be identical.

A phenomenon known as cathodic drift, where the pH gradient decays over time, may occur if a gel is focused too long. Although not well understood, electroendoosmosis and absorption of carbon dioxide may be factors that lead to cathodic drift. Cathodic drift is observed as focused protein migrating off the cathode end of the gel. Immobilized pH gradients may be used to address this problem.

Efficient cooling (approximately 4°C) of the bed that the gel lies on during focusing is important. High field strengths used during isoelectric focusing can lead to overheating and affect the quality of the focused gel.

Reagents and Solutions—

Fixing solution for isoelectric focusing in polyacrylamide gel Dissolve 35 g of 5-sulfosalicylic acid dihydrate and 100 g of trichloroacetic acid in water to make 1000 mL.

Coomassie staining TS Dissolve 125 mg of coomassie brilliant blue R-250 in 100 mL of a mixture of water, methanol and acetic acid (100) (5:4:1), and filter.

Destaining solution A mixture of water, methanol and acetic acid (100) (5:4:1).

Mass Spectrometry of Peptides and Proteins

Mass spectrometry (MS) is based on the ionization of molecules and separation of the electrically charged ions according to the m/z. The results are expressed as a mass spectrum with m/z values of the ions on the x-axis and signal intensity of the ions on the y-axis. The mass of the molecule calculated from the m/z values is expressed in unified atomic mass units (u) or daltons (Da). Tandem mass spectrometry (MS/MS) is based on the selection of the precursor ion in the first stage analyzer, fragmentation of the precursor ion and measurement of the product ions in the second stage mass analyzer. This technique provides useful information for structural analysis of the molecule. Information obtained in MS is qualitative and is sometimes used for qualification. MS and MS/MS are useful for measuring masses of peptides and proteins and for confirming amino acid sequences and post-translational modifications. Both methods are therefore used for identification of pharmaceutical peptides and proteins.

1. Instrument

A mass spectrometer is composed of an ion source, an analyzer, an ion detector, and a data system (Figure 1). A peptide or protein sample introduced into the ion source is ionized by soft-ionization methods, such as matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). The charged and gas-phased ions are sorted according to the m/z under a vacuum in the analyzer, which may be a quadrupole, time-of-flight, ion trap or Fourier transform ion cyclotron resonance analyzer. The ion flux collected in the detector is converted to an electric signal. Then the signal is recorded as a mass spectrum. MS/MS is carried out by using two mass spectrometers connected in series, an ion-trap mass spectrometer and Fourier transform ion cyclotron resonance mass spectrometer. The precursor ions are generally fragmented by collision-induced dissociation, post-source decay, electron capture dissociation, etc.

2. Analytical mode

2.1. MS

There are two useful modes for MS:

(1) Full scan mode

The signals of the entire ion are acquired over the chosen range of m/z. This mode provides information on the masses of the molecule of interest and different species.

(2) Selected ion monitoring

The signals of the ion at chosen m/z are acquired. This mode is useful for the sensitive measurement of the chosen molecule.

2.2. MS/MS

There are four essential modes for MS/MS:

(1) Product ion analysis

The signals of all the product ions produced from the precursor at chosen m/z are acquired. This mode provides structural information on the analyte and various co-existing species.
2. Precursor ion scan mode
The precursor that yields the product ion at chosen m/z are monitored. This mode is used for sorting the molecules containing a component of interest.

3. Constant neutral loss scan mode
The precursor that loses the fragment at chosen m/z are monitored. This mode is useful to sort the molecules containing a component of interest.

4. Selected reaction monitoring
The product ions at chosen m/z that are produced from the precursor at chosen m/z are monitored. This mode allows for sensitive and selective measurement and is used for quantification of a molecule in a complex mixture.

3. Analytical procedure

3.1. MS
System suitability is tested by measuring the mass of a test sample specified in the monograph. The system performance should be confirmed based on the difference between the calculated mass and observed mass of the test sample. If the detectability and system performance do not meet the criteria, the system should be optimized by adjustment of the ion source, analyzer and detector, as well as by calibration using appropriate molecules. MS is performed according to the sample preparation and operating conditions indicated in the monograph. The general procedure is described as follows.

1. Matrix-assisted laser desorption/ionization (MALDI)
A desalted peptide or protein sample is dissolved in an appropriate solvent, e.g., an aqueous solution of trifluoroacetic acid. A suitable matrix, such as α-ciano-4-hydroxycinnamic acid, 2,5-dihydroxybenzoic acid, or sinapic acid, is dissolved in an aqueous solution containing acetonitrile and trifluoroacetic acid. A mixture of sample solution and matrix solution is deposited on a sample plate and dried. The sample on the plate is set in the ion source, and ionized by a laser beam at suitable intensity.

2. Electrospray ionization (ESI)
A desalted peptide or protein sample is dissolved in a suitable solvent, such as an aqueous solution containing acetic acid and methanol or acetonitrile. The sample solution is sprayed through a capillary and held at a potential of several kilovolts. The sample is introduced by using a syringe or HPLC.

3.2. MS/MS
System suitability is tested by MS/MS of the test sample specified in the monograph. The detectability and system performance should be confirmed based on the detection of the product ions specified in the monograph. The sample is ionized in the same way as for MS, and the chosen precursor is fragmented by the suitable conditions specified in the monograph. The signals are recorded as a mass spectrum. A peptide containing disulfide bonds is generally reduced by dithiothreitol, 2-mercaptethanol and tris (2-carboxyethyl) phosphine. The reduced peptides are derivatized with monoiodoacetic acid, iodoacetamide, and 4-binyldipyrine.

4. Identification test

4.1. Mass of the molecule
The monoisotopic mass of the peptide is preferably acquired. If the monoisotopic peak is not detectable, the average mass is calculated using the weighed average of isotopic masses. Deconvolution is effective for calculating the average mass of multiply-charged proteins. The mass should meet the criteria specified in the monograph.

4.2. Amino acid sequence
After measuring the mass of the sample peptide, the presence of the specified product ions that arise from the selected precursor is confirmed according to the conditions indicated in the monograph. Digestion of sample proteins with a suitable enzyme followed by MS/MS is sometimes effective for sequencing of the high-molecular weight proteins which provide insufficient product ions. Details of the diges-
tion procedure are provided in the section on peptide mapping.

5. **Glossary**

**Ion-trap (IT)**  
Ion-trap refers to the quadrupole ion trap mass analyzer in a restricted sense. Ions stored in the analyzer by applying radio frequency voltage to ring electrodes are separated by subsequent ejection of the ions from the analyzer by varying the voltage on the ring electrodes. This allows multiple stage MS in which a selected ion is repeatedly trapped, fragmented and ejected.

**Electrospray ionization (ESI)**  
The sample in solution is sprayed through a capillary and held at high-voltage at atmospheric pressure. The sample is ionized by a formation of charged liquid droplets. High-molecular weight proteins are detected as multiply-charged ions. The analyzer can be connected with HPLC.

**Quadrupole (Q)**  
The analyzer is composed of four parallel electrodes which have a hyperboloidal or cylindrical cross-section. The ions transmitted to the analyzer are separated by varying the potential of direct and radio frequency components applied to the rods so that the filter for sorting the \( m/z \) values of ions is changed.

**Collision-induced dissociation (CID)**  
When an ion collides with a neutral atom or molecule (He, Ar, \( N_2 \), and so on), some of the translational energy of the collision is converted into internal energy, thereby causing dissociation. The terms low-energy CID and high-energy CID refer to those CIDs for which the translational energy of the precursor ions is lower than 1000 eV and higher than 1000 eV, respectively.

**Electron capture dissociation (ECD)**  
Multiply-charged positive ions interact with low energy electrons producing charge-reduced radical ions, which readily dissociate. This method is primarily used for MS/MS in FT-ICR MS or IT MS.

**Time-of-flight (TOF)**  
The ionized sample is accelerated at high-voltage and separated based on the time required for an ion to travel to the detector. There are two types of analyzer, a linear type in which ions travel linearly from the ion source to the detector, and a reflectron type where ions are inverted by a reflectron. The latter type allows high-resolution measurement by correction of the variation in the initial energy of ions.

**Fourier transform ion cyclotron resonance (FT-ICR)**  
The analyzer is based on the principle that the cyclotron frequency of the ions in a magnetic field is inversely proportional to its \( m/z \) value. Ions are excited to a larger radius orbit using radio frequency energy and their image current is detected on receiver plates. The resulting data are devolved by applying a Fourier transform to give a mass spectrum.

**Matrix-assisted laser desorption/ionization (MALDI)**  
The sample, which is mixed with a suitable matrix and deposited on a target plate, is ionized by irradiation with nanosecond laser pulses. Proteins, carbohydrates, oligonucleotides, and lipids can be ionized without any dissociation. Singly-charged ions are mainly detected.

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**Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/Biological Products**

This document describes the currently available methods of mycoplasma testing that should be performed for cell substrates that are used in the manufacture of biotechnological/biological products.

Methods suggested for detection of mycoplasma are, A. culture method, B. indicator cell culture method, and C. polymerase chain reaction (PCR) method.

Mycoplasma testing should be performed on the master cell bank (MCB) and the working cell bank (WCB), as well as on the cell cultures used during the manufacturing process of the product. For the assessment of these cells, mycoplasma testing should be performed using both methods A and B. Method B, however, does not detect only DNA derived from mycoplasma. Therefore, if a positive result is obtained only from method B, method C can be used to determine whether mycoplasma is actually present. When method C is used, it is necessary to demonstrate the rationale for determining a negative result. In such a case, the sensitivity and specificity of the method, the appropriateness of the sample preparation, and the suitability of the selection of the test method, including selection of reagents, reaction conditions and primers should be taken into account.

Prior to mycoplasma testing, the sample should be tested to detect the presence of any factors inhibiting the growth of mycoplasma. If such growth-inhibiting factors are detected they should be neutralized or eliminated by an appropriate method, such as centrifugation or cell passage.

If the test will be performed within 24 hours of obtaining the sample, the sample should be stored at a temperature between 2°C and 8°C. If more than 24 hours will elapse before the test is performed, the sample should be stored at \(-60^\circ C\) or lower.

If mycoplasma is detected, additional testing to identify the species may be helpful in determining the source of contamination.

A. **Culture Method**

1. **Culture Medium**

Both agar plates and broth are used. Each lot of agar and broth medium should be free of antibiotics except for penicillin. Refer to the Minimum Requirements for Biological Products regarding selection of the culture media. Other culture media may be used if they fulfill the requirements described in the following section 2.

2. **Suitability of Culture Medium**

Each lot of medium should be examined for mycoplasma growth-promoting properties. To demonstrate the capacity of the media to detect known mycoplasma, each test should
include control cultures of at least two known species or strains of mycoplasma, one of which should be a dextrose fermenter (i.e., *M. pneumoniae* ATCC15531 or equivalent species or strains) and one of which should be an arginine hydrolyser (i.e., *M. orale* ATCC23714 or equivalent species or strains). The mycoplasma strains used for the positive control tests should be those with a low number of passages obtained from an official or suitably accredited agency, and handled appropriately. Inoculate the culture medium with 100 colony-forming units (CFU) or 100 color-changing units (CCU) or less.

3. Culture and Observation

1) Inoculate no less than 0.2 mL of test sample (cell suspension) in evenly distributed amounts over the surface of each of two or more agar plates. After the surfaces of the inoculated plates are dried, the plates should be incubated under microaerophilic conditions in an atmosphere of nitrogen containing 5 to 10 percent carbon dioxide and adequate humidity at 36 ± 1°C for no less than 14 days.

2) Inoculate no less than 10 mL of the test sample (cell suspension) into each of one or more vessels containing 100 mL of broth medium, and incubate at 36 ± 1°C.

If the culture medium for the sample cells contains any growth-inhibiting factors, such as antibiotics, these factors must be removed. A method such as centrifugation is recommended for this purpose. Refer to the Validation tests for growth-inhibiting factors described in the Minimum Requirements for Biological Products for the detection of growth-inhibiting factors.

3) Subculture 0.2 mL of broth culture from each vessel on the 3rd, 7th, and 14th days of incubation onto two or more agar plates. The plates should be inoculated microaerophilically at 36 ± 1°C for no less than 14 days.

4) Examination of all plates for mycoplasma colonies should be done microscopically on the 7th and 14th day at 100 times magnification or greater.

B. Indicator Cell Culture Method

Using Vero cell culture substrate, pretest the suitability of the method using an inoculum of 100 CFU or 100 CCU or less of *M. hyorhinis* (ATCC29052, ATCC17981 or equivalent species or strains) and *M. orale* (ATCC23714 or equivalent species or strains).

An equivalent indicator cell substrate and suitable mycoplasma strains may be acceptable if data demonstrate at least equal sensitivity for the detection of known mycoplasma contaminants. The mycoplasma strains should be those with a low number of passages obtained from an official or suitably accredited agency, and handled appropriately, and the unit of inoculation should be determined before use. The cell substrate used should be obtained from a qualified cell bank and certified to be mycoplasma free. The acquired cells should be carefully cultured and propagated, and sufficient volumes of seed stock should be prepared with the proper precautions to avoid mycoplasma contamination. The stock should be tested for mycoplasma contamination using at least one of the methods described in this document, then frozen for storage. For each test a new container from the stock should be thawed and used within 6 passages.

Indicator cell cultures should be grown on cover slips submerged in culture dishes or equivalent containers for one day. Inoculate no less than 1 mL of the test sample (cell culture supernatant) into two or more of the culture dishes.

The test should include a negative (non-infected) control and two positive mycoplasma controls, such as *M. hyorhinis* (ATCC29052, ATCC17981 or equivalent species or strains) and *M. orale* (ATCC23714 or equivalent species or strains). Use an inoculum of 100 CFU or 100 CCU or less for the positive controls.

Incubate the cell cultures for 3 to 6 days at 36 ± 1°C in an atmosphere of air containing 5 percent carbon dioxide.

Examine the cell cultures after fixation for the presence of mycoplasma by epifluorescence microscopy (400 to 600 times magnification or greater) using a DNA-binding fluorochrome, such as bisbenzimidaole or an equivalent stain. Compare the microscopical appearance of the test cultures with that of the negative and positive controls.

**Procedure**

1) Aseptically place a sterilized glass cover slip into each cell culture dish (35 mm diameter).

2) Prepare Vero cell suspension in Eagle’s minimum essential medium containing 10 percent bovine calf serum at a concentration of 1 × 10^6 cells per 1 mL. The bovine calf serum should be tested and confirmed to be free from mycoplasma prior to use.

3) Inoculate aliquots of 2 mL of the Vero cell suspension into each culture dish. Ensure that the cover slips are completely submerged, and not floating on the surface of the culture medium. Incubate the cultures at 36 ± 1°C in an atmosphere of air containing 5 percent carbon dioxide for one day, so that the cells are attached to the glass cover slip.

4) Replace 2 mL of the culture medium with fresh medium, then add 0.5 mL of the test sample (cell culture supernatant) to each of two or more culture dishes. Perform the same procedure for the positive (2 types of mycoplasma, such as *M. hyorhinis* (ATCC29052, ATCC17981 or equivalent species or strains) and *M. orale* (ATCC23714 or equivalent species or strains) and negative controls.

5) Incubate the cultures for 3 to 6 days at 36 ± 1°C in an atmosphere of air containing 5 percent carbon dioxide.

6) Remove the culture medium from the culture dishes, and add 2 mL of a mixture of acetic acid (100) and methanol (1:3) (fixative) to each dish; then, allow them to stand for 5 minutes.

7) Remove the fixative from each dish, then add the same amount of fixative again, and leave the dishes to stand for 10 minutes.

8) Remove the fixative and then completely air-dry all the dishes.

9) Add 2 mL of bisbenzamide fluorochrome staining solution to each culture dish. Cover the dishes and let them stand at room temperature for 30 minutes.

10) Aspirate the staining solution and rinse each dish with 2 mL of distilled water 3 times. Take out the glass cover slips and dry them.

11) Mount each cover slip with a drop of a mounting fluid. Blot off surplus mounting fluid from the edges of the cover slips.

12) Examine by epifluorescence microscopy at 400 to 600 times magnification or greater.

13) Compare the microscopical appearance of the test sample with that of the negative and positive controls.

14) The test result is judged to be positive if there are more than 5 cells per 1000 (0.5%) that have minute fluorescent spots that appear to surround, but are outside, the cell nucleus.
C. Polymerase Chain Reaction (PCR) Detection Method

The PCR method is a highly specific method that enables the detection of trace amounts of mycoplasma DNA, and has come to be widely used in recent years as a means of detecting mycoplasma contamination. However, the sensitivity and specificity depend on the procedure employed, and a positive result from PCR does not always indicate the presence of viable mycoplasma.

The PCR method is based on amplifying DNA extracted from the cell culture with specific primers so that the presence of the target DNA is detected. A two-step PCR (nested PCR) is recommended in order to increase sensitivity and specificity. The tests should include both a positive control (such as M. hyorhinis (ATCC29052, ATCC17981 or equivalent species or strains) of 100 CFU or 100 CCU or less) and a negative control.

Mycoplasma DNA from the sample of cells or cell cultures is amplified using primers which should be able to amplify some commonly conserved mycoplasma DNA sequence. The amplification should be performed using an appropriate heat-resistant DNA polymerase, and suitable conditions. The amplified DNA can be identified after agarose gel electrophoresis, followed by ethidium bromide staining and UV irradiation of the gel.

For this method, it is important to use primers that are specific to mycoplasma by choosing base sequences that are well-conserved for a wide range of mycoplasma species, for example, the spacer region between the 16S-23S ribosome genes. It is recommended that a two-step PCR using nested primers should be performed to increase the sensitivity and specificity, if the one-step PCR is negative.

The primers to be selected for the second stage of a two-step PCR are nested primers from the inner portion of the sequence. The outer and inner primers should have proven effectiveness and specificity as described in publications or be validated experimentally.

It is possible to increase the accuracy of the detection of mycoplasma DNA by performing PCR tests after cultivation of mycoplasma that may be present in samples using Vero cells.

The following is an example of a two-step PCR procedure. The reagents and reaction conditions in this example are not exclusive. If the suitability of other reagents and conditions is verified, they may be used. If another procedure is used, the procedure should be justified and documented in detail, and the information provided should include the sensitivity and specificity of the method.

Example Procedure

1. Preparation of template

1) Place 600 μL of the test cell suspension (if necessary, subcultured with Vero cells) in a tube and dissolve the cells with 0.1% SDS or an equivalent. Add an equal volume (600 μL) of TE (10 mmol/L tris-hydrochloric acid (pH 8.0), 1 mmol/L EDTA) buffer-saturated phenol, and mix.

2) Centrifuge at 15,000 min⁻¹ for 5 minutes at room temperature.

3) Transfer 400 μL of the supernatant to another tube, and add 10 μL of 3 mol/L sodium acetate.

4) Add 1 mL (2.5 volumes) of ethanol (95%) and stir thoroughly. Ice the mixture for 15 minutes, then centrifuge at 15,000 min⁻¹ for 10 minutes at 4°C.

5) Discard the supernatant and rinse the precipitate once or twice with 200 to 300 μL of 80% ethanol. Remove the rinse solution using a pipette. Centrifuge at 15,000 min⁻¹ for 10 minutes at 4°C, then remove the supernatant thoroughly and dry up the precipitate.

6) Dissolve the precipitate in 40 μL of distilled water.

2. Perform the same procedure for the positive and negative controls

3. First stage of a two-step PCR

1) Make a mixture of the heat-resistant DNA polymerase, dNTP solution, outer primer, and reaction buffer solution (including Mg ions), and place 90 μL in each tube.

2) Add 10 μL of the template prepared as above to each tube containing the first stage PCR solution (90 μL).

3) Perform the DNA amplification by repeating 30 cycles of denaturation at 94°C for 30 seconds, annealing at an appropriate temperature for the primer (55°C for the primer in this example), and elongation at 72°C for 2 minutes.

4. Second stage of a two-step PCR

1) Make a mixture of the heat-resistant DNA polymerase, dNTP solution, inner primer, and reaction buffer solution (including Mg ions), and place 99 μL in each tube.

2) Add 1 μL of the first stage PCR product from each tube to a tube containing the second stage PCR solution (99 μL).

3) Perform the DNA amplification by repeating 30 cycles of denaturation at 94°C for 30 seconds, annealing at an appropriate temperature for the primer (55°C for the primer in this example), and elongation at 72°C for 2 minutes.

5. Agarose gel electrophoresis

1) Mix 10 μL of each of the first stage and second stage PCR products with 2 μL of an appropriate dye as a migration marker, and perform 1% agarose gel electrophoresis.

2) Stain the gel with ethidium bromide and take a photograph under UV irradiation.

3) The test is judged to be positive if a DNA band is detected.

[An Example of Primer]

For mycoplasma detection
Outer primer
F1 : 5’-ACACCATGGAG(C/T)TGGAAT-3’
R1 : 5’-CTTC(A/T)TGACTC(T/C)CAAGG-CAT-3’

Inner primer
F2 : 5’-GT(G)/GG(A/C)TGGAATCCTT-3’
R2 : 5’-GATCCACAA(A/T)AC(A/T)AC/C/TCTT-3’

( ) indicates a mixture.

[PCR reaction solution]

<table>
<thead>
<tr>
<th>[First stage]</th>
<th>[Second stage]</th>
</tr>
</thead>
<tbody>
<tr>
<td>dNTP solution (1.25 mmol/L)</td>
<td>16 μL</td>
</tr>
<tr>
<td>Primer (10 pmol/μL)</td>
<td>F1</td>
</tr>
<tr>
<td>Primer (10 pmol/μL)</td>
<td>R1</td>
</tr>
<tr>
<td>Heat-resistant DNA polymerase (1 U/μL)</td>
<td>2 μL</td>
</tr>
<tr>
<td>Reaction buffer solution</td>
<td>25 mmol/L magnesium chloride hexahydrate</td>
</tr>
<tr>
<td></td>
<td>10-fold buffer solution*</td>
</tr>
<tr>
<td></td>
<td>Sterile distilled water</td>
</tr>
</tbody>
</table>

*Composition of 10-fold buffer solution 2-amino-2-hydroxymethyl-1,3-
propanediol-hydrochloric acid
\( \text{pH 8.4} \) 100 mmol/L
Potassium chloride 500 mmol/L
Gelatin 0.1 g/L

[Method of cultivating mycoplasma within Vero cells]
1) Use at least two cell culture dishes for each of the test sample, positive control and negative control.
2) Into each cell culture dish (35 mm diameter), inoculate 2 mL of the Vero cell suspension \((1 \times 10^6 \text{ cells per mL})\) in Eagle’s minimum essential medium containing 10 percent bovine calf serum (tested in advance using the PCR method to verify that it does not contain any detectable mycoplasma DNA). Incubate the cultures at 36° C in an atmosphere of air containing 5 percent carbon dioxide for one day.
3) Replace the culture media with fresh media, and add 0.5 mL of the test sample (cell culture supernatant) to each of two or more Vero cell culture dishes. Perform the same procedure for the positive (such as 100 CFU or 100 CCU or less of two or more Vero cell culture dishes. Perform the same procedure for the positive (such as 100 CFU or 100 CCU or less of \( M. \) hyorhinis (ATCC29052, ATCC17981 or equivalent species or strains)) and negative controls.
4) Incubate the Vero cell culture dishes for the test sample, positive and negative controls for 3 to 6 days at 36 ± 1°C in an atmosphere of air containing 5 percent carbon dioxide.

**Peptide Mapping**

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

Peptide mapping is an identity test for proteins, especially those obtained by r-DNA technology. It involves the chemical or enzymatic treatment of a protein, resulting in the formation of peptide fragments, followed by separation and identification of the fragments in a reproducible manner. It is a powerful test that is capable of identifying single amino acid changes resulting from events such as errors in the reading of complementary DNA (cDNA) sequences or point mutations. Peptide mapping is a comparative procedure because the information obtained, compared to a reference standard or reference material similarly treated, confirms the primary structure of the protein, is capable of detecting whether alterations in structure have occurred, and demonstrates process consistency and genetic stability. Each protein presents unique characteristics which must be well understood so that the scientific and analytical approaches permit validated development of a peptide map that provides sufficient specificity.

This chapter provides detailed assistance in the application of peptide mapping and its validation to characterize the desired protein product, to evaluate the stability of the expression construct of cells used for recombinant DNA products, to evaluate the consistency of the overall process and to assess product stability as well as to ensure the identity of the protein product, or to detect the presence of protein variant.

1. The Peptide Map

Peptide mapping is not a general method, but involves developing specific maps for each unique protein. Although the technology is evolving rapidly, there are certain methods that are generally accepted. Variations of these methods will be indicated, when appropriate, in specific monographs.

A peptide map may be viewed as a fingerprint of a protein and is the end product of several chemical processes that provide a comprehensive understanding of the protein being analyzed. Four major steps are necessary for the development of the procedure: isolation and purification of the protein, if the protein is part of a formulation; selective cleavage of the peptide bonds; chromatographic separation of the peptides; and analysis and identification of the peptides. A test sample is digested and assayed in parallel with a reference standard or a reference material. Complete cleavage of peptide bonds is more likely to occur when enzymes such as endoproteases (e.g., trypsin) are used, instead of chemical reagents. A map should contain enough peptides to be meaningful. On the other hand, if there are too many fragments, the map might lose its specificity because many proteins will then have the same profiles.

2. Isolation and Purification

Isolation and purification are necessary for analysis of bulk drugs or dosage forms containing interfering excipients and carrier proteins and, when required, will be specified in the monograph. Quantitative recovery of protein from the dosage form should be validated.

3. Selective Cleavage of Peptide Bonds

The selection of the approach used for the cleavage of peptide bonds will depend on the protein under test. This selection process involves determination of the type of cleavage to be employed—enzymatic or chemical—and the type of cleavage agent within the chosen category. Several cleavage agents and their specificity are shown in Table 1.

This list is not all-inclusive and will be expanded as other cleavage agents are identified.

3.1. Pretreatment of Sample

Depending on the size or the configuration of the protein, different approaches in the pretreatment of samples can be used. For monoclonal antibodies, the heavy and light chains will need to be separated before mapping. If trypsin is used as a cleavage agent for proteins with a molecular mass greater than 100,000 Da, lysine residues must be protected by acetylation or maleylation; otherwise, too many peptides will be generated.

3.2. Pretreatment of the Cleavage Agent

Pretreatment of cleavage agents—especially enzymatic agents—might be necessary for purification purposes to ensure reproducibility of the map. For example, trypsin used as a cleavage agent will have to be treated with tosyl-L-phenylalanine chloromethyl ketone to inactivate chymotrypsin. Other methods, such as purification of trypsin by HPLC, may be necessary. Other pretreatments, such as the addition of chaotropic agents (e.g., urea) can be used to unfold the protein prior to mapping. To allow the enzyme to have full access to cleavage sites and permit
somesome unfolding of the protein, it is often necessary to reduce and alkylate the disulfide bonds prior to digestion.

Digestion with trypsin can introduce ambiguities in the tryptic map due to side reactions occurring during the digestion reaction, such as nonspecific cleavage, deamidation, disulfide isomerization, oxidation of methionine residues, or formation of pyroglutamic groups created from the deamidation of glutamine at the N-terminal side of a peptide. Furthermore, peaks may be produced by autohydrolysis of trypsin. Their intensities depend on the ratio of trypsin to protein. To avoid autohydrolysis, solutions of proteases may be prepared at a pH that is not optimal (e.g., at pH 5 for trypsin), which would mean that the enzyme would not become active until diluted with the digest buffer.

### 3.4. Establishment of Optimal Digestion Conditions

Factors that affect the completeness and effectiveness of digestion of proteins are those that could affect any chemical or enzymatic reactions.

(i) **pH**: The pH of the digestion mixture is empirically determined to ensure the optimal performance of the given cleavage agent. For example, when using cyanogen bromide as a cleavage agent, a highly acidic environment (e.g., pH 2, formic acid) is necessary; however, when using trypsin as a cleavage agent, a slightly alkaline environment (pH 8) is optimal. As a general rule, the pH of the reaction milieu should not alter the chemical integrity of the protein during the digestion and should not change during the course of the fragmentation reaction.

(ii) **Temperature**: A temperature between 25°C and 37°C is adequate for most digestions. The temperature used is intended to minimize chemical side reactions. The type of protein under test will dictate the temperature of the reaction milieu, because some proteins are more susceptible to denaturation as the temperature of the reaction increases. For example, digestion of recombinant bovine somatropin is conducted at 4°C, because at higher temperatures it will precipitate during digestion.

(iii) **Time**: If sufficient sample is available, a time course study is considered in order to determine the optimum time to obtain a reproducible map and avoid incomplete digestion. Time of digestion varies from 2 to 30 hours. The reaction is stopped by the addition of an acid which does not interfere in the tryptic map or by freezing.

(iv) **Amount of Cleavage Agent**: Although excessive amounts of cleavage agent are used to accomplish a reasonably rapid digestion time (i.e., 6 to 20 hours), the amount of cleavage agent is minimized to avoid its contribution to the chromatographic map pattern. A protein to protease ratio between 20:1 and 200:1 is generally used. It is recommended that the cleavage agent can be added in two or more stages to optimize cleavage. Nonetheless, the final reaction volume remains small enough to facilitate the next step in peptide mapping — the separation step. To sort out digestion artifacts that might be interfering with the subsequent analysis, a blank determination is performed, using a digestion control with all the reagents, except the test protein.

### 4. Chromatographic Separation

Many techniques are used to separate peptides for mapping. The selection of a technique depends on the protein being mapped. Techniques that have been successfully used for separation of peptides are shown in Table 2. In this section, a most widely used reverse-phase High Performance Liquid Chromatographic (RP-HPLC) method is described as one of the procedures of chromatographic separation.

The purity of solvents and mobile phases is a critical factor in HPLC separation. HPLC-grade solvents and water that are commercially available are recommended for RP-HPLC. Dissolved gases present a problem in gradient systems where the solubility of the gas in a solvent may be less in a mixture than in a single solvent. Vacuum degassing and agitation by sonication are often used as useful degassing procedures. When solid particles in the solvents are drawn into the HPLC system, they can damage the sealing of pump valves or clog the top of the chromatographic column. Both pre- and post-pump filtration is also recommended.

#### 4.1. Chromatographic Column

The selection of a chromatographic column is empirically determined for each protein. Columns with 100 Å or 300 Å pore size with silica support can give optimal separation. For smaller peptides, octylsilane chemically bonded to totally porous silica articles, 3 to 10 μm in diameter (L7) and octadecylsilane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 μm in diameter (L1) column packings are more efficient than the butyl silane chemically bonded to totally porous silica particles, 5 to 10 μm in diameter (L26) packing.

#### 4.2. Solvent

The most commonly used solvent is water with acetonitrile as the organic modifier to which less than 0.1% trifluoro-
Table 2 Techniques Used for the Separation of Peptides

<table>
<thead>
<tr>
<th>Technique</th>
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<tbody>
<tr>
<td>Reverse-Phase High Performance Liquid Chromatography (RP-HPLC)</td>
</tr>
<tr>
<td>Ion-Exchange Chromatography (IEC)</td>
</tr>
<tr>
<td>Hydrophobic Interaction Chromatography (HIC)</td>
</tr>
<tr>
<td>Polyacrylamide Gel Electrophoresis (PAGE), non-denaturating</td>
</tr>
<tr>
<td>SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE)</td>
</tr>
<tr>
<td>Capillary Electrophoresis (CE)</td>
</tr>
<tr>
<td>Paper Chromatography-High Voltage (PCHV)</td>
</tr>
<tr>
<td>High-Voltage Paper Electrophoresis (HVPE)</td>
</tr>
</tbody>
</table>

Cetic acid is added. If necessary, add isopropyl alcohol or n-propyl alcohol to solubilize the digest components, provided that the addition does not unduly increase the viscosity of the components.

4.3. Mobile Phase
Buffered mobile phases containing phosphate are used to provide some flexibility in the selection of pH conditions, since shifts of pH in the 3.0 to 5.0 range enhance the separation of peptides containing acidic residues (e.g., glutamic and aspartic acids). Sodium or potassium phosphates, ammonium acetate, phosphoric acid, and a pH between 2 and 7 (or higher for polymer-based supports) have also been used with acetonitrile gradients. Acetonitrile containing trifluoroacetic acid is used quite often.

4.4. Gradient Selection
Gradients can be linear, nonlinear, or include step functions. A shallow gradient is recommended in order to separate complex mixtures. Gradients are optimized to provide clear resolution of one or two peaks that will become “marker” peaks for the test.

4.5. Isocratic Selection
Isocratic HPLC systems using a single mobile phase are used on the basis of their convenience of use and improved detector responses. Optimal composition of a mobile phase to obtain clear resolution of each peak is sometimes difficult to establish. Mobile phases for which slight changes in component ratios or in pH significantly affect retention times of peaks in peptide maps should not be used in isocratic HPLC systems.

4.6. Other Parameters
Temperature control of the column is usually necessary to achieve good reproducibility. The flow rates for the mobile phases range from 0.1 to 2.0 mL per minute, and the detection of peptides is performed with a UV detector at 200 to 230 nm. Other methods of detection have been used (e.g., postcolumn derivatization), but they are not as robust or versatile as UV detection.

4.7 Validation
This section provides an experimental means for measuring the overall performance of the test method. The acceptance criteria for system suitability depend on the identification of critical test parameters that affect data interpretation and acceptance. These critical parameters are also criteria that monitor peptide digestion and peptide analysis. An indicator that the desired digestion endpoint was achieved is by the comparison with a reference standard or reference material, which is treated exactly as the article under test. The use of a reference standard or reference material in parallel with the protein under test is critical in the development and establishment of system suitability limits. In addition, a specimen chromatogram should be included with the reference standard or reference material for additional comparison purposes. Other indicators may include visual inspection of protein or peptide solubility, the absence of intact protein, or measurement of responses of a digestion-dependent peptide. The critical system suitability parameters for peptide analysis will depend on the particular mode of peptide separation and detection and on the data analysis requirements.

When peptide mapping is used as an identification test, the system suitability requirements for the identified peptides cover selectivity and precision. In this case, as well as when identification of variant proteins is done, the identification of the primary structure of the peptide fragments in the peptide map provides both a verification of the known primary structure and the identification of protein variants by comparison with the peptide map of the reference standard/reference material for the specified protein. The use of a digested reference standard or reference material for a given protein in the determination of peptide resolution is the method of choice. For an analysis of a variant protein, a characterized mixture of a variant and a reference standard or reference material can be used, especially if the variant peptide is located in a less-resolved region of the map. The index of pattern consistency can be simply the number of major peptides detected. Peptide pattern consistency can be best defined by the resolution of peptide peaks. Chromatographic parameters—such as peak-to-peak resolution, maximum peak width, peak area, peak tailing factors, and column efficiency—may be used to define peptide resolution. Depending on the protein under test and the method of separation used, single peptide or multiple peptide resolution requirements may be necessary.

The replicate analysis of the digest of the reference standard or reference material for the protein under test yields measures of precision and quantitative recovery. Recovery of the identified peptides is generally ascertained by the use of internal or external peptide standards. The precision is expressed as the relative standard deviation (RSD). Differences in the recovery and precision of the identified peptides are expected; therefore, the system suitability limits will have to be established for both the recovery and the precision of the identified peptides. These limits are unique for a given protein and will be specified in the individual monograph.

Visual comparison of the relative retention times, the peak responses (the peak area or the peak height), the number of peaks, and the overall elution pattern is completed initially. It is then complemented and supported by mathematical analysis of the peak response ratios and by the chromatographic profile of a 1:1 (v/v) mixture of sample and reference standard or reference material digest. If all peaks in the sample digest and in the reference standard or reference material digest have the same relative retention times and peaks response ratios, then the identity of the sample under test is confirmed.

If peaks that initially eluted with significantly different relative retention times are then observed as single peaks in the 1:1 mixture, the initial difference would be an indication of system variability. However, if separate peaks are observed in the 1:1 mixture, this would be evidence of the nonequivalence of the peptides in each peak. If a peak in the
1:1 mixture is significantly broader than the corresponding peak in the sample and reference standard or reference material digest, it may indicate the presence of different peptides. The use of computer-aided pattern recognition software for the analysis of peptide mapping data has been proposed and applied, but issues related to the validation of the computer software preclude its use in a compendial test in the near future. Other automated approaches have been used that employ mathematical formulas, models, and pattern recognition. Such approaches are, for example, the automated identification of compounds by IR spectroscopy and the application of diode-array UV spectral analysis for identification of peptides. These methods have limitations due to inadequate resolutions, co-elution of fragments, or absolute peak response differences between reference standard or reference material and sample fragments.

The numerical comparison of the retention times and peak areas or peak heights can be done for a selected group of relevant peaks that have been correctly identified in the peptide maps. Peak areas can be calculated using one peak showing relatively small variation as an internal reference, keeping in mind that peak area integration is sensitive to baseline variation and likely to introduce error in the analysis. Alternatively, the percentage of each peptide peak height relative to the sum of all peak heights can be calculated for the sample under test. The percentage is then compared to that of the corresponding peak of the reference standard/reference material. The possibility of auto-hydrolysis of trypsin is monitored by producing a blank peptide map, that is, the peptide map obtained when a blank solution is treated with trypsin. The minimum requirement for the qualification of peptide mapping is an approved test procedure that includes system suitability as a test control. In general, early in the regulatory process, qualification of peptide mapping for a protein is sufficient. As the regulatory approval process for the protein progresses, additional qualifications of the test can include a partial validation of the analytical procedure to provide assurance that the method will perform as intended in the development of a peptide map for the specified protein.

5. Analysis and Identification of Peptides

This section gives guidance on the use of peptide mapping during development in support of regulatory applications. The use of a peptide map as a qualitative tool does not require the complete characterization of the individual peptide peaks. However, validation of peptide mapping in support of regulatory applications requires rigorous characterization of each of the individual peaks in the peptide map. Methods to characterize peaks range from N-terminal sequencing of each peak followed by amino acid analysis to the use of mass spectroscopy (MS).

For characterization purposes, when N-terminal sequencing and amino acids analysis are used, the analytical separation is scaled up. Since scale-up might affect the resolution of peptide peaks, it is necessary, using empirical data, to assure that there is no loss of resolution due to scale-up. Eluates corresponding to specific peptide peaks are collected, vacuum-concentrated, and chromatographed again, if necessary. Amino acid analysis of fragments may be limited by the peptide size. If the N-terminus is blocked, it may need to be cleared before sequencing. C-terminal sequencing of proteins in combination with carboxypeptidase digestion and MALDI-TOF MS can also be used for characterization purposes.

The use of MS for characterization of peptide fragments is by direct infusion of isolated peptides or by the use of online LC-MS for structure analysis. In general, it includes electrospray and MALDI-TOF MS analyzer as well as fast atom bombardment (FAB). Tandem MS has also been used to sequence a modified protein and to determine the type of amino acid modification that has occurred. The comparison of mass spectra of the digests before and after reduction provides a method to assign the disulfide bonds to the various sulfhydryl-containing peptides.

If regions of the primary structure are not clearly demonstrated by the peptide map, it might be necessary to develop a secondary peptide map. The goal of a validated method of characterization of a protein through peptide mapping is to reconcile and account for at least 95% of the theoretical composition of the protein structure.

Qualification of Animals as Origin of Animal-derived Medicinal Products provided in the General Notices of Japanese Pharmacopoeia and Other Standards

Introduction

The Official Gazette issued on March 29, 2002 announced that General Notices of the Japanese Pharmacopoeia and other standards were amended to add a provision that “When a drug product or a drug substance which is used to manufacture a drug product, is manufactured from a raw material of animal origin, the animal in question should be in principle a healthy subject, if not otherwise provided.”.

The Notice Iyaku-hatsu No. 0329001, which was issued on the same date, provided that “Healthy subject herein provided is the animal which does not cause any disease or any infection to human being at an appropriate production process and use of the drug product, and as for the oral or external drug for example, the animal, as its raw material of animal origin, should be confirmed at this stage to meet the Food Standard. It has to be noted that this standard of healthy subject has to be revised timely taking into account the up-to-date information with respect to the amphixenosis infections common between human beings and animals.”.

This General Information describes safety assurance against infection associated with the use of drugs, which are manufactured from raw materials of animal origin, to follow up the Notice as mentioned above.

1. Basic concept

When drugs derived from raw materials of animal origin including human are used, it is important to take into account any possibility that communicable disease agents such as virus may cause infectious disease or any possible hazards to patients. In such case, it goes without saying that the primary subject that has to be considered is the absence of any infectious agents such as virus in the raw materials of animal origin including human as the source of the drug. More important points are whether the drugs derived from such raw
materials are free of such infectious agents and whether there is any possibility of transmission of infectious agents when the drugs are administered to patient. The eligibility of animals including human, as the source of raw materials of drugs, in other words “the subject which is free from any disease or transmission of infectious agents that is infectious to human being at an appropriate production process and use of the drug product” is that “The drug should be entirely free from any risk of infections by means of whole procedures which include evaluation of appropriateness of the animals including human as the source of their raw materials, establishment of appropriate production processes and their appropriate control, and strict adherence to the clinical indications of the final product.”

2. Animals including human as the source of raw materials of drugs

What is the most clear and appropriate preventive measures against infection to human being due to administration of drugs which are derived from animals including human is to assure the absence of any infectious agents such as virus in its raw materials or an appropriate critical raw material by each of the following: (1) the use of raw materials of healthy animal origin, which are proved to be free from communicable disease agents to human, or (2) the use of appropriate critical raw materials (e.g., cell substrate, blood plasma, pooled urine after some treatments) for drug production, which are proved to be free from communicable disease agents after certain appropriate processing on raw materials of animal origin.

As for raw materials of drugs of human origin, cell, tissue, blood, placenta, urine, etc. are used. Whenever it is sufficient and possible each donor, as the origin of such raw materials, should be asked his (her) health condition and undergoes his (her) medical examination at this stage, so that the appropriateness as a donor can be confirmed from the standpoint of safety concerning communicable disease agents such as virus.

For example, “Basic concept on handling and use of a drug product, etc. which is derived from cell/tissue” (Attachment 1 of the Notice Iyaku-Hatsu No. 1314 dated December 26, 2000) issued by the Director-General of the Medicinal Safety Bureau, Ministry of Health and Welfare, states that since the cell/tissue supplied by a human donor comes to be applied to patients without processing through any sufficient inactivation or removal of communicable disease agents, the selection and qualification criteria on such donor has to be established. These criteria are to be composed with the respect to the check items on the case history and the physical conditions as well as the test items on the various transmission of infectious agents through cell/tissue, and that the appropriateness of these criteria has to be clarified. Hepatitis Type-B (HBV), Hepatitis Type-C (HCV), Human Immune Deficiency Viral infections (HIV), Adult T-Cell Leukemia and Parvovirus B19 Infections should be denied through the interview to the donor and the tests (serologic test, nucleic-acid amplification test, etc.). Further, if necessary, Cytomegalovirus infection and EB Virus infection should be denied by tests. “Infections caused by bacteria such as Treponema pallidum, Chlamydia, Gonococci, Tubercule bacillus, etc.”, “septicemia and its suspicious case”, “vicious tumor”, “serious metabolic or endocrine-related disorders”, “collagenosis and haematological disorders”, “hepatic disease” and “dementia (transmissible spongiform encephalopathies and its suspicious case)” should be checked on the case history or by the interview, etc. and the experience of being transfused or/and transplanted should be checked to confirm eligibility as a donor. The most appropriate check items and test methods then available are to be used, which need to be reconsidered at appropriate times taking into account the updated knowledge and the progress of the science and the technologies. At screening of a donor, reexaminations has to be made at appropriate timing using the eligible check items and the test methods taking into account the window period (Initial period after infection, in which antibody against bacteria, fungi or virus is not detected.)

In the case of plasma derivatives produced from the donated blood in Japan, the donor should be checked by means of self-assessed report about health conditions, and a serologic check and a nucleic acid amplification test (NAT) on mini pooled plasma should be performed at the stage of donated blood. Further, the plasma material (i.e., critical raw material) for fractionation should be stored 4 months in minimum so that the arrangement could be taken based on the information available after collection of the blood and the blood infusion to exclude the possibility of using any critical raw material which might cause infection to patients. On the other hand, as for the materials such as urine which are taken from the unspecified number of the donors and come to be critical raw materials for drug production after some treatments, it is unrealistic and not practical to conduct the tests of virus infection, etc. on the individual donor. Consequently, appropriate tests such as virus test has to be performed on such critical raw materials for drug production.

In the case of the animals besides human, the wild ones should be excluded. Only the animals, which are raised under well sanitarily controlled conditions taken to prevent bacterial contamination or under the effective bacterial pollution monitoring systems, have to be used, and it is recommended that the animals from a colony appropriately controlled under specific pathogen-free (SPF) environment are to be used as far as possible. Further, for the animals regulated under the Food Standard, only the animals that met this standard should be used. It should be confirmed by appropriate tests that the animals were free from pathogen, if necessary.

The concrete measures to avoid transmittance or spread of infectivity of prion, which is considered to be the pathogen of transmissible spongiform encephalopathies (TSEs), as far as possible are the followings: 1) avoidance of use of animals, which are raised in the areas where high incidence or high risk of TSEs (Scrapie in sheep and goat, bovine spongiform encephalopathies (BSE) in cattle, chronic wasting disease (CWD) in deer, new type of Creutzfeldt-Jacob Disease (CJD) in human, etc.) is reported, and humans, who have stayed long time (more than 6 months) in such areas, as raw materials or related substances of drugs; 2) avoidance of use of any substances that are derived from the individual infected with scrapie, BSE, CJD, etc.; 3) avoidance of using a material derived from organ, tissue and cell, etc. of high risk of TSEs; and 4) taking appropriate measures basing on the information collected, which includes incidence of TSEs, the results of epidemiological investigation and the experimental research on prion, and incidence of tardive infection on
3. Human or animal cells which are used as critical raw materials for drug production

Cell substrates derived from humans or animals are used for drug production. In such case, it is desirable that the humans or the animals, which are the origins of the cell substrates, are healthy subjects. However, it is considered practical that viral safety of the drugs derived from the cell substrates are evaluated on the cells, which are so called critical raw materials for production of such drugs. In such case, the safety should be confirmed through the test and analysis on established cell bank thoroughly with respect to virus etc., as far as possible. The items and the methods of the tests that have been followed in this case are described in detail in the Notice of Japanese version on the internationally accepted ICH Guideline entitled “Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin” (Iyakushin No. 329 issued on February 22, 2000 by Director, Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare). In the meantime, it is important how to handle the cell in case that any virus has been detected under the cell level tests. This Notice describes how to cope with this situation as follows: “It is recognised that some cell lines used for the manufacture of product will contain endogenous retroviruses, other viruses or viral sequences. In such circumstances, the action plan recommended for manufacturer is described in Section V (Rationale and action plan for viral clearance studies and virus tests on purified bulk) of the Notice. The acceptability of cell lines containing viruses other than endogenous retroviruses will be considered on an individual basis by the regulatory authorities, by taking into account a risk/benefit analysis based on the benefit of the product and its intended clinical use, the nature of the contaminating viruses, their potential for infecting humans or for causing disease in humans, the purification process for the product (e.g., viral clearance evaluation data), and the extent of the virus tests conducted on the purified bulk.”

For example, it is well known that Type A-, R- and C-endogenous particles like retrovirus are observed in the cells of the rodents used most often for drug production. It is also known that they are not infectious to human and is not dangerous, and CHO cells are generally used for drug production. The established cell lines (e.g., NAMALWA Cell, BALL-1 Cell, etc.) derived from cancer patients are sometimes used, but through the thorough virus tests, etc., their safety are confirmed. The established cell lines are assumed to be safer than the primary cultured cells which are hard to conduct the thorough virus test.

4. Establishment and control of appropriate production process and adherence to the clinical indication of final product for safety assurance

Safety assurance against potential infections at only the level of animals that are source of raw materials of drugs is limited. Further, “health of animal” can not be defined univocally, and the various factors have to be taken into account. The final goal of this subject is to protect human from any infectious disease caused by drugs. Achieving this goal, the establishment and control of appropriate production processes of each drug and the adherence to the clinical indications of the final product are important.

As mentioned above, the rodent cells used most often for the production of the drugs are known to have endogenous retrovirus-like particles sometimes. The reason why such cells can be used for the production of the drugs is that multiple measures are applied for safety in the purification stages which include appropriate inactivation or removal processes. There are cases in which the production procedure involves intentional use of a virus or a microorganism. In this case, relevant measures capable of removing or inactivating of such virus or microorganism are appropriately incorporated in the purification process, so that the risk of infection to human can be fully denied and its safety can be assured when it is used as a drug. Further, even in the case that it is difficult to clarify the risk of contamination of the infectious agents or that the raw material are contaminated by viruses etc., the raw material in question may be used for the production of drugs so long as appropriate inactivation or removal processes are introduced, their effectiveness can be confirmed and the safety can be assured by appropriate control of the manufacturing processes under GMP, etc.

5. Conclusion

The qualification of animals including human, as the source of raw materials of drugs, in other words “the subject which does not cause any infectious diseases to human beings at an appropriate production process and use of the drug product” is that “the drug has to be entirely free from any risk of infections by means of whole procedures which include evaluation of appropriateness of the animal including human as the source of their raw materials, establishment of appropriate production processes and their appropriate control, and strict adherence to the clinical indication of the final product.”

To cope with this subject, the advanced scientific measures, which actually reflect the updated knowledge and progress of the science and the technology about infectious diseases in human and infection of animal origin, have to be taken into account timely.

SDS-Polyacrylamide Gel Electrophoresis

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The SDS-Polyacrylamide Gel Electrophoresis is used for the characterization of proteins in biotechnological and biological products and for control of purity and quantitative determinations. This technique is a suitable analytical method with which to identify and to assess the homogeneity of proteins in biotechnological and biological products. The method is also routinely used for the estimation of protein subunit molecular masses and for determining the subunit compositions of purified proteins.

Ready-to-use gels and reagents are widely available on the market and can be used instead of those described in this text, provided that they give equivalent results and that they meet the validity requirements given below under Validation of the Test.

1. Characteristics of Polyacrylamide Gels

The sieving properties of polyacrylamide gels are afforded
by the three-dimensional network of fibers and pores which is formed as the bifunctional bisacrylamide cross-links adjacent polyacrylamide chains. Polymerization is catalyzed by a free radical-generating system composed of ammonium persulfate and $N,N',N''$-tetramethylethylenediamine (TEMED).

As the acrylamide concentration of a gel increases, its effective pore size decreases. The effective pore size of a gel is operationally defined by its sieving properties; that is, by the resistance it imparts to the migration of macromolecules. There are limits on the acrylamide concentration that can be used. At high acrylamide concentrations, gels break much more easily and are difficult to handle. As the pore size of a gel decreases, the migration rate of a protein through the gel decreases. By adjusting the pore size of a gel, through manipulating the acrylamide concentration, the resolution of the method can be optimized for a given protein product. Thus, the physical characteristics of a given gel are determined by the relative concentrations of acrylamide and bisacrylamide, used in its preparation.

In addition to the composition of the gel, the state of the protein is an important determinant of the electrophoretic mobility. In the case of proteins, the electrophoretic mobility is dependent on the $pK$ values of the charged groups and the size of the molecule. It is also influenced by the type, concentration and $pH$ of the buffer, the temperature and the field strength, as well as by the nature of the support material.

2. Polyacrylamide Gel Electrophoresis under Denaturing Conditions

The method cited as an example is limited to the analysis of monomeric polypeptides with a mass range of 14,000 to 100,000 daltons. It is possible to extend this mass range by various techniques (e.g., by using gradient gels, particular buffer systems, etc.), but those techniques are not discussed in this chapter.

Analysis by electrophoresis on sodium dodecyl sulfate (SDS) polyacrylamide gel (SDS-Polyacrylamide Gel Electrophoresis) under denaturing conditions is the most common mode of electrophoresis used in assessing the quality of proteins in biotechnological and biological products, and will be the focus of the example described here. Typically, analytical electrophoresis of proteins is carried out in polyacrylamide gels under conditions that ensure dissociation of the proteins into their individual polypeptide subunits and that minimize aggregation. Most commonly, the strongly anionic detergent SDS is used in combination with heat to dissociate the proteins before they are loaded on the gel. The denatured polypeptides bind to SDS, become negatively charged and exhibit a consistent charge-to-mass ratio regardless of protein type. Because the amount of SDS bound is almost always proportional to the molecular mass of the polypeptide and is independent of its amino acid sequence, SDS-polypeptide complexes migrate through polyacrylamide gels with mobilities that are dependent on the size of the polypeptides.

The electrophoretic mobilities of the resultant SDS-polypeptide complexes all assume the same functional relationship to their molecular masses. Migration of SDS-complexes occurs toward the anode in a predictable manner, with low-molecular-mass complexes migrating faster than larger ones. The molecular mass of a protein can therefore be estimated from its relative mobility calibrated in SDS-Polyacrylamide Gel Electrophoresis and the occurrence of a single band in such a gel is a criterion of purity.

However, modifications to the polypeptide backbone, such as N- or O-linked glycosylation, have a significant impact on the apparent molecular mass of a protein, since SDS does not bind to a carbohydrate moiety in a manner similar to a polypeptide. Thus, a consistent charge-to-mass ratio is not maintained. The apparent molecular masses of proteins that have undergone post-translational modifications do not truly reflect the masses of the polypeptides.

2.1. Reducing conditions

Polypeptide subunits and three-dimensional structure of proteins are often fixed, at least in part, by the presence of disulfide bonds. A goal of SDS-Polyacrylamide Gel Electrophoresis under reducing conditions is to disrupt this structure by reducing the disulfide bonds. Complete denaturation and dissociation of proteins by treatment with 2-mercaptoethanol or dithiothreitol (DTT) will result in unfolding of the polypeptide backbone and subsequent complexation with SDS. Under these conditions, the molecular masses of the polypeptide subunits can be calculated by linear regression in the presence of suitable molecular-mass standards.

2.2. Non-reducing conditions

For some analyses, complete dissociation of the protein of interest into subunit peptides is not desirable. In the absence of treatment with reducing agents such as 2-mercaptoethanol or DTT, disulfide covalent bonds remain intact, preserving the oligomeric form of the protein. Oligomeric SDS-protein complexes migrate more slowly than their SDS-polypeptide subunits. In addition, non-reduced proteins may not be completely saturated with SDS and, hence, may not bind the detergent in the expected mass ratio. This makes molecular-mass determinations of these molecules by SDS-Polyacrylamide Gel Electrophoresis less straightforward than analyses of fully denatured polypeptides, since it is necessary that both standards and unknown proteins be in similar configurations for valid comparisons. However, the staining of a single band in such a gel is a criterion of purity.

3. Characteristics of Discontinuous Buffer System Gel Electrophoresis

The most widely used electrophoretic method for the analysis of complex mixtures of proteins involves the use of a discontinuous buffer system consisting of two contiguous, but distinct gels: a resolving (lower) gel and a stacking (upper) gel. The two gels are cast with different porosities, $pH$, and ionic strengths. In addition, different mobile ions are used in the gel and electrode buffers. The buffer discontinuity acts to concentrate large-volume samples in the stacking gel, resulting in improved resolution. When power is applied, a voltage drop develops across the sample solution which drives the proteins into the stacking gel. Glycinate ions from the electrode buffer follow the proteins into the stacking gel. A moving boundary region is rapidly formed with the highly mobile chloride ions in the front and the relatively slow glycinate ions in the rear. A localized high-voltage gradient is formed between the leading and trailing ion fronts, causing the SDS-protein complex to form into a very thin zone (called the stack) and to migrate between the chloride and glycinate phases. Regardless of the height of the applied sample solution in the wells, all SDS-protein complexes condense within a very narrow range and enter the resolving
gel as a well-defined, thin zone of high protein density. The large-pore stacking gel does not retard the migration of most proteins and serves mainly as an anticonvective medium. At the interface of the stacking and resolving gels, the proteins experience a sharp increase in retardation due to the smaller pore size of the resolving gel. Once the proteins are in the resolving gel, their mobility continues to be slowed down by the molecular sieving effect of the matrix. The glycinate ions overtake the proteins, which then move in a space of uniform pH formed by the tris(hydroxymethyl)aminomethane and glycine. Molecular sieving causes the SDS-polypeptide complexes to separate on the basis of their molecular masses.

4. Preparing Vertical Discontinuous Buffer SDS-Polyacrylamide Gels

4.1. Assembling of the gel moulding cassette

Clean the two glass plates (size: e.g. 10 cm × 8 cm), the sample comb made of polytetrafluoroethylene, the two spacers and the silicone rubber tubing (diameter, e.g. 0.6 mm) away from the long side corresponding to the bottom of the gel. Begin to lay the silicone rubber tubing on the glass plate by using one spacer as a guide. Carefully twist the silicone rubber tubing at the bottom of the spacer and follow the long side of the glass plate. While holding the silicone rubber tubing with one finger along the long side again twist the tubing and lay it on the second short side of the glass plate, using the spacer as a guide. Place the second glass plate in perfect alignment and hold the mould together by hand pressure. Apply two clamps on each of the two short sides of the glass plate. Carefully apply four clamps on the longer side of the gel mould, thus forming the bottom of the gel plate, using the spacer as a guide. Verify that the silicone rubber tubing is running along the edge of the glass plates and has not been extruded while placing the clamps.

4.2. Preparation of the gel

In a discontinuous buffer SDS polyacrylamide gel, it is recommended to pour the resolving gel, let the gel set, and then pour the stacking gel, since the compositions of the two gels in acrylamide-bisacrylamide, buffer and pH are different.

4.2.1. Preparation of the resolving gel

In a conical flask, prepare the appropriate volume of solution containing the desired concentration of acrylamide for the resolving gel, using the values given in Table 1. Mix the components in the order shown. Where appropriate, before adding the ammonium persulfate solution and TEMED as indicated in Table 2, swirl and pour immediately into the gap between the two glass plates of the mould directly onto the surface of the polymerized resolving gel. Immediately insert a clean sample comb into the stacking gel solution, taking care to avoid trapping air bubbles. Add more stacking gel solution to fill completely the spaces of the sample comb. Leave the gel in a vertical position and allow to polymerize at room temperature.

4.3. Mounting the gel in the electrophoresis apparatus and electrophoretic separation

After polymerization is complete (about 30 minutes), remove the sample comb carefully. Rinse the wells immediately with water or with the running buffer for SDS-Polyacrylamide Gel Electrophoresis to remove any unpolymerized acrylamide. If necessary, straighten the teeth of the sample comb of the stacking gel with a blunt hypodermic needle attached to a syringe. Remove the clamps on one short side, carefully pull out the silicone rubber tubing and replace the clamps. Proceed similarly on the other short side. Remove the silicone rubber tubing from the bottom part of the gel. Mount the gel in the electrophoresis apparatus. Add the electrophoresis buffers to the top and bottom reservoirs. Remove any bubbles that become trapped at the bottom of the gel between the glass plates. This is best done with a bent hypodermic needle attached to a syringe. Never pre-run the gel before loading solutions, such as samples, since this will destroy the discontinuity of the buffer systems. Before loading solutions, such as samples, carefully rinse the stacking gel wells with the running buffer for SDS-Polyacrylamide Gel Electrophoresis. Prepare the test and reference solutions in the recommended sample buffer and treat as specified in the individual monograph. Apply the appropriate volume of each solution to the stacking gel wells. Start the electrophoresis using suitable operating conditions for the electrophoresis equipment to be used. There are commercially available gels of different surface area and thickness that are appropriate for various types of electrophoresis equipment. Electrophoresis running time and current/voltage may need to be altered depending on the type of apparatus used, in order to achieve optimum separation. Check that the dye front is moving into the resolving gel. When the dye is reaching the bottom of the gel, stop the electrophoresis. Remove the gel assembly from the apparatus and separate the glass plates. Remove the spacers, cut off and discard the stacking gel and immediately proceed with staining.
5. Detection of Proteins in Gels

Coomassie staining is the most common protein staining method, with a detection level of the order of 1 μg to 10 μg of protein per band. Silver staining is the most sensitive method for staining proteins in gels and a band containing 10 ng to 100 ng can be detected. All of the steps in gel staining are done at room temperature with gentle shaking in any convenient container. Gloves must be worn when staining gels, since fingerprints will stain.

5.1. Coomassie staining

Immerse the gel in a large excess of Coomassie staining TS and allow to stand for at least 1 hour. Remove the staining solution.

Destain the gel with a large excess of destaining TS. Change the destaining solution several times, until the stained protein bands are clearly distinguishable on a clear background. The more thoroughly the gel is destained, the smaller is the amount of protein that can be detected by the method. Destaining can be speeded up by including 2 to 3 g of anion-exchange resin or a small sponge in the destaining solution.

NOTE: the acid-alcohol solutions used in this procedure do not completely fix proteins in the gel. This can lead to losses of some low-molecular-mass proteins during the staining and destaining of the gel. Permanent fixation is obtainable by allowing the gel to stand in trichloroacetic acid TS for fixing for 1 hour before it is immersed in Coomassie staining TS.

5.2. Silver staining

Immerse the gel in a large excess of fixing TS and allow to stand for 1 hour. Remove the fixing solution, add fresh fixing solution and incubate either for at least 1 hour or overnight, if convenient. Discard the fixing solution and wash the gel in water for 1 hour. Soak the gel for 15 minutes in a 1 vol% glutaraldehyde solution. Wash the gel twice for 15 minutes in water. Soak the gel in fresh silver nitrate TS for silver staining for 15 minutes, in darkness. Wash the gel three times for 5 minutes in water. Immerse the gel for about 1 minute in developer TS until satisfactory staining has been obtained. Stop the development by incubation in blocking TS for 15 minutes. Rinse the gel with water.

6. Drying of Stained SDS-Polyacrylamide Gels

Depending on the staining method used, gels are pretreated in a slightly different way. For Coomassie staining, after the destaining step, allow the gel to stand in a diluted solution of concentrated glycerin (1 in 10) for at least 2 hours (overnight incubation is possible). For silver staining, after the final rinse, allow the gel to stand in a diluted solution of concentrated glycerin (1 in 50) for 5 minutes.

Immerse two sheets of porous cellulose film in water and incubate for 5 to 10 minutes. Place one of the sheets on a drying frame. Carefully lift the gel and place it on the cellulose film. Remove any trapped air bubbles and pour 2 to 3 mL of water around the edges of the gel. Place the second sheet on top and remove any trapped air bubbles. Complete the assembly of the drying frame. Place in an oven or leave at room temperature until dry.

7. Molecular-Mass Determination

Molecular masses of proteins are determined by comparison of their mobilities with those of several marker proteins of known molecular mass. Mixtures of proteins with precisely known molecular masses blended for uniform staining are commercially available for calibrating gels. They are obtainable in various molecular mass ranges. Concentrated stock solutions of proteins of known molecular mass are diluted in the appropriate sample buffer and loaded on the same gel as the protein sample to be studied.

Immediately after the gel has been run, the position of the bromophenol blue tracking dye is marked to identify the leading edge of the electrophoretic ion front. This can be done by cutting notches in the edges of the gel or by inserting a needle soaked in India ink into the gel at the dye front. After staining, measure the migration distances of each protein band (markers and unknowns) from the top of the resolving gel. Divide the migration distance of each protein by the distance traveled by the tracking dye. The normalized migration distances so obtained are called the relative mobilities of the proteins (relative to the dye front) and conventionally denoted as Rf. Construct a plot of the logarithm of the relative molecular masses (MR) of the protein standards as a function of the Rf values. Note that the graphs are slightly sigmoid. Unknown molecular masses can be estimated by linear regression analysis or interpolation from the curves of log MR against Rf as long as the values obtained for the unknown samples are positioned along the linear part of the graph.

8. Suitability of the Test (Validation)

The test is not valid unless the proteins of the molecular mass marker are distributed along 80% of the length of the gel and over the required separation range (e.g., the range covering the product and its dimer or the product and its related impurities) the separation obtained for the relevant protein bands shows a linear relationship between the logarithm of the molecular mass and the Rf as described in 7. Additional requirements with respect to the solution under test may be specified in individual monographs.

9. Quantification of Impurities

Where the impurity limit is specified in the individual monograph, a reference solution corresponding to that level of impurity should be prepared by diluting the test solution. For example, where the limit is 5%, a reference solution would be a 1:20 dilution of the test solution. No impurity (any band other than the main band) in the electrophoretogram obtained with the test solution may be more intense than the main band obtained with the reference solution.

Under validated conditions, impurities may be quantified by normalization to the main band, using an integrating densitometer. In this case, the responses must be validated for linearity.

10. Test solutions:

(i) Coomassie staining TS: Dissolve 125 mg of coomassie brilliant blue R-250 in 100 mL of a mixture of water, methanol and acetic acid (100) (5:4:1), and filter.

(ii) Developer TS: Dissolve 2 g of citric acid monohydrate in water to make 100 mL. To 2.5 mL of this solution add 0.27 mL of formaldehyde solution and water to make 500 mL.

(iii) Fixing TS: To 250 mL of methanol add 0.27 mL of formaldehyde solution and water to make 500 mL.

(iv) Silver nitrate TS for silver staining: To 40 mL of sodium hydroxide TS add 3 mL of ammonia solution (28), then add dropwise 8 mL of a solution of silver nitrate (1 in 5) while stirring, and add water to make 200 mL.
Table 1  Preparation of resolving gel

<table>
<thead>
<tr>
<th>Solution components</th>
<th>Component volumes (mL) per gel mould volume of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 mL</td>
</tr>
<tr>
<td>6% Acrylamide</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>2.6</td>
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<td>Acrylamide solution</td>
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<tr>
<td>1.5 mol/L Tris solution (pH 8.8)</td>
<td>1.3</td>
</tr>
<tr>
<td>100 g/L SDS</td>
<td>0.05</td>
</tr>
<tr>
<td>100 g/L APS</td>
<td>0.05</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.004</td>
</tr>
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<td>8% Acrylamide</td>
<td></td>
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<td>Water</td>
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<td>Acrylamide solution</td>
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</tr>
<tr>
<td>1.5 mol/L Tris solution (pH 8.8)</td>
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</tr>
<tr>
<td>100 g/L SDS</td>
<td>0.05</td>
</tr>
<tr>
<td>100 g/L APS</td>
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</tr>
<tr>
<td>TEMED</td>
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</tr>
<tr>
<td>100 g/L SDS</td>
<td>0.05</td>
</tr>
<tr>
<td>100 g/L APS</td>
<td>0.05</td>
</tr>
<tr>
<td>TEMED</td>
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<td>12% Acrylamide</td>
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</tr>
<tr>
<td>1.5 mol/L Tris solution (pH 8.8)</td>
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</tr>
<tr>
<td>100 g/L SDS</td>
<td>0.05</td>
</tr>
<tr>
<td>100 g/L APS</td>
<td>0.05</td>
</tr>
<tr>
<td>TEMED</td>
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<tr>
<td>14% Acrylamide</td>
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<tr>
<td>100 g/L APS</td>
<td>0.05</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.002</td>
</tr>
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</table>

(1) Acrylamide solution: 30% acrylamide/bisacrylamide (29:1) solution
(2) 1.5 mol/L Tris solution (pH 8.8): 1.5 mol/L tris-hydrochloride buffer solution, pH 8.8
(3) 100 g/L SDS: 100 g/L solution of sodium dodecyl sulfate
(4) 100 g/L APS: 100 g/L solution of ammonium persulfate. Ammonium persulfate provides the free radicals that drive polymerization of acrylamide and bisacrylamide. Since ammonium persulfate solution decomposes slowly, fresh solutions must be prepared before use.
(5) TEMED: N,N,N’,N’-tetramethylethylenediamine

(v) Destaining TS: A mixture of water, methanol and acetic acid (100) (5:4:1).
(vi) Blocking TS: To 10 mL of acetic acid (100) add water to make 100 mL.
(vii) Trichloroacetic acid TS for fixing: Dissolve 10 g of trichloroacetic acid in a mixture of water and methanol (5:4) to make 100 mL.
**Total Protein Assay**

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (*). The following procedures are provided as illustrations of the determination of total protein content in pharmacopeial preparations. Other techniques, such as HPLC, are also acceptable if total protein recovery is demonstrated. Many of the total protein assay methods described below can be performed successfully using kits from commercial sources.

**Method 1 (UV method)**

Protein in solution absorbs UV light at a wavelength of 280 nm, due to the presence of aromatic amino acids, mainly tyrosine and tryptophan. This property is the basis of this method. Protein determination at 280 nm is mainly a function of the tyrosine and tryptophan content of the protein. If the buffer used to dissolve the protein has a high absorbance relative to that of water, there is an interfering substance in the buffer. This interference can be compensated for when the spectrophotometer is adjusted to zero buffer absorbance. If the interference results in a large absorbance that challenges the limit of sensitivity of the spectrophotometer, the results may be compromised. Furthermore, at low concentrations protein can be absorbed onto the cuvette, thereby reducing the content in solution. This can be prevented by preparing samples at higher concentrations or by using a nonionic detergent in the preparation.

**Standard Solution**

Unless otherwise specified in the individual monograph, prepare a solution of the reference standard or reference material for the protein under test in the same buffer and at the same concentration as the Test Solution.

**Test Solution**

Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration of 0.2 to 2 mg per mL.

**Procedure**

Concomitantly determine the absorbances of the Standard Solution and the Test Solution in quartz cells at a wavelength of 280 nm, with a suitable spectrophotometer, using the buffer as the blank. To obtain accurate results, the response should be linear in the range of protein concentrations to be assayed.

**Light-Scattering**

The accuracy of the UV spectroscopic determination of protein can be decreased by the scattering of light by the test specimen. If the proteins in solution exist as particles comparable in size to the wavelength of the measuring light (250 to 300 nm), scattering of the light beam results in an apparent increase in absorbance of the test specimen. To calculate the absorbance at 280 nm due to light-scattering, determine the absorbances of the Test Solution at wavelengths of 320, 325, 330, 335, 340, 345, and 350 nm. Using the linear regression method, plot the log of the observed absorbance versus the log of the wavelength, and determine the standard curve best fitting the plotted points. From the graph so obtained, extrapolate the absorbance value due to light-scattering at 280 nm. Subtract the absorbance from light-scattering from the total absorbance at 280 nm to obtain the absorbance value of the protein in solution. Filtration with a filter having a 0.2-μm porosity or clarification by centrifugation may be performed to reduce the effect of light-scattering, especially if the solution is noticeably turbid.

**Calculations**

Calculate the concentration, \( C_U \), of protein in the test specimen by the formula:

\[
C_U = \frac{C_S (A_U/A_S)}
\]

in which \( C_S \) is the concentration of the Standard Solution; and \( A_U \) and \( A_S \) are the corrected absorbances of the Test Solution and the Standard Solution, respectively.

**Method 2 (Lowry method)**

This method, commonly referred to as the Lowry assay, is based on the reduction by protein of the phosphomolybdic-tungstic mixed acid chromogen in the Folin-Ciocalteu's phenol reagent, resulting in an absorbance maximum at 750 nm. The Folin-Ciocalteu’s phenol reagent (Folin’s TS) reacts primarily with tyrosine residues in the protein, which can lead to variation in the response of the assay to different proteins. Because the method is sensitive to interfering sub-

---

**Table 2** Preparation of stacking gel

<table>
<thead>
<tr>
<th>Solution components</th>
<th>Component volumes (mL) per gel mould volume of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mL</td>
</tr>
<tr>
<td>Water</td>
<td>0.68</td>
</tr>
<tr>
<td>Acrylamide solution*</td>
<td>0.17</td>
</tr>
<tr>
<td>1.0 mol/L Tris solution (pH 6.8)*</td>
<td>0.13</td>
</tr>
<tr>
<td>100 g/L SDS*</td>
<td>0.01</td>
</tr>
<tr>
<td>100 g/L APS*</td>
<td>0.01</td>
</tr>
<tr>
<td>TEMED*</td>
<td>0.001</td>
</tr>
</tbody>
</table>

(1) Acrylamide solution: 30% acrylamide/bisacrylamide (29:1) solution
(2) 1.0 mol/L Tris solution (pH 6.8): 1 mol/L tris-hydrochloride buffer solution, pH 6.8
(3) 100 g/L SDS: 100 g/L solution of sodium dodecyl sulfate
(4) 100 g/L APS: 100 g/L solution of ammonium persulfate. Ammonium persulfate provides the free radicals that drive polymerization of acrylamide and bisacrylamide. Since ammonium persulfate solution decomposes slowly, fresh solutions must be prepared before use.
(5) TEMED: N,N,N',N'-tetramethylethylenediamine
stances, a procedure for precipitation of the protein from the test specimen may be used. Where separation of interfering substances from the protein in the test specimen is necessary, proceed as directed below for Interfering Substances prior to preparation of the Test Solution. The effect of interfering substances can be minimized by dilution provided the concentration of the protein under test remains sufficient for accurate measurement. Variations in the Lowry test that are indicated in national regulatory documents can be substituted for the method described below.

**Standard Solutions** Unless otherwise specified in the individual monograph, dissolve the reference standard or reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 5 and 100 μg of protein per mL, the concentrations being evenly spaced.

**Test Solution** Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions. An appropriate buffer will produce a pH in the range of 10 to 10.5.

**Blank** Use the buffer used for the Test Solution and the Standard Solutions.

**Reagents and Solutions—**

- **Copper Sulfate Reagent** Dissolve 100 mg of copper (II) sulfate pentahydrate and 200 mg of sodium tetraborate decahydrate in water, dilute with water to 50 mL, and mix.** Dissolve 10 g of anhydrous sodium carbonate in water to a final volume of 50 mL, and mix. Slowly pour the sodium carbonate solution into the copper sulfate solution with mixing. Prepare this solution fresh daily.

- **5% SDS TS** Dissolve 5 g of sodium dodecyl sulfate in water, and dilute with water to 100 mL.

- **Alkaline Copper Reagent** Prepare a mixture of 5% SDS TS, Copper Sulfate Reagent, and Sodium Hydroxide Solution (4 in 125) (2:1:1). This reagent may be stored at room temperature for up to 2 weeks.

- **Diluted Folin’s TS** Mix 10 mL of Folin’s TS with 50 mL of water. Store in an amber bottle, at room temperature.

**Procedure** To 1 mL of each Standard Solution, the Test Solution, and the Blank, add 1 mL of Alkaline Copper Reagent, and mix. Allow to stand at room temperature for 10 minutes. Add 0.5 mL of the Diluted Folin’s TS to each solution, and mix each tube immediately after the addition, and allow to stand at room temperature for 30 minutes. Determine the absorbances of the solutions from the Standard Solutions and the Test Solution at the wavelength of maximum absorbance at 750 nm, with a suitable spectrophotometer, using the solution from the Blank to set the instrument to zero.

**Calculations** [Note: The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using the linear regression method, plot the absorbances of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the Test Solution, determine the concentration of protein in the Test Solution.

**Interfering Substances** In the following procedure, deoxycholate-trichloroacetic acid is added to a test specimen to remove interfering substances by precipitation of the protein before testing. This technique also can be used to concentrate proteins from a dilute solution.

**Sodium Deoxycholate Reagent** Prepare a solution of sodium deoxycholate in water having a concentration of 150 mg in 100 mL.

**Trichloroacetic Acid Reagent** Prepare a solution of trichloroacetic acid in water having a concentration of 72 g in 100 mL.

**Procedure** Add 0.1 mL of Sodium Deoxycholate Reagent to 1 mL of a solution of the protein under test. Mix on a vortex mixer, and allow to stand at room temperature for 10 minutes. Add 0.1 mL of Trichloroacetic Acid Reagent, and mix on a vortex mixer. Centrifuge at 3000 × g for 30 minutes, decant the liquid, and remove any residual liquid with a pipet. Redissolve the protein pellet in 1 mL of Alkaline Copper Reagent. Proceed as directed for the Test Solution. [Note: Color development reaches a maximum in 20 to 30 minutes during incubation at room temperature, after which there is a gradual loss of color. Most interfering substances cause a lower color yield; however, some detergents cause a slight increase in color. A high salt concentration may cause a precipitate to form. Because different protein species may give different color response intensities, the standard protein and test protein should be the same.]

**Method 3 (Bradford method)**

This method, commonly referred to as the Bradford assay, is based on the absorption shift from 470 nm to 595 nm observed when Coomassie brilliant blue G-250 binds to protein. The Coomassie brilliant blue G-250 binds most readily to arginyl and lysyl residues in the protein, which can lead to variation in the response of the assay to different proteins.

**Standard Solutions** Unless otherwise specified in the individual monograph, dissolve the reference standard or the reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 100 μg and 1 mg of protein per mL, the concentrations being evenly spaced.

**Test Solution** Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions.

**Blank** Use the buffer used to prepare the Test Solution and the Standard Solutions.

**Coomassie Reagent** Dissolve 100 mg of Coomassie brilliant blue G-250 in 50 mL of ethanol (95). [Note: Not all dyes have the same brilliant blue G content, and different products may give different results.] Add 100 mL of phosphoric acid, dilute with water to 1000 mL, and mix. Filter the solution through filter paper (Whatman No.1 or equivalent), and store the filtered reagent in an amber bottle at room temperature. [Note: Slow precipitation of the dye will occur during storage of the reagent. Filter the reagent before use.]

**Procedure** Add 5 mL of the Coomassie Reagent to 100 μL of each Standard Solution, the Test Solution, and the Blank, and mix by inversion. Avoid foaming, which will lead to poor reproducibility. Determine the absorbances of the solutions from the Standard Solutions and the Test Solution at 595 nm, with a suitable spectrophotometer, using the Blank to set the instrument to zero.
Incubate the solutions at 37°C. Dilute the Standard Solutions by mixing 0.1 mL of each Standard Solution, the Test Solution, and the Blank with 2 mL of the Copper-BCA Reagent.

Mix 0.1 mL of each Standard Solution, the Test Solution, and the Blank with 2 mL of the Copper-BCA Reagent. Incubate the solutions at 37°C for 30 minutes, note the time, and allow to come to room temperature. Within 60 minutes following the incubation time, determine the absorbance of the solutions from the Standard Solutions and the Test Solution in quartz cells at 562 nm, with a suitable spectrophotometer, using the Blank to set the instrument to zero. After the solutions are cooled to room temperature, the color intensity continues to increase gradually. If substances that will cause interference in the test are present, proceed as directed for Interfering Substances under Method 2. Because different protein species may give different color response intensities, the standard protein and test protein should be the same.

Calculations [Note: The relationship of absorbance to protein concentration is nonlinear; therefore, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using the linear regression method, plot the absorbances of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the Test Solution, determine the concentration of protein in the Test Solution.

Method 4 (Bicinchoninic acid method)

This method, commonly referred to as the bicinchoninic acid or BCA assay, is based on the reduction of the cupric (Cu²⁺) ion to cuprous (Cu⁺) ion by protein. The bicinchoninic acid reagent is used to detect the cuprous ion. The method has few interfering substances. When interfering substances are present, their effect may be minimized by dilution, provided that the concentration of the protein under test remains sufficient for accurate measurement.

Standard Solutions Unless otherwise specified in the individual monograph, prepare a solution of Albumin Human for which the protein content has been previously determined by nitrogen analysis (using the nitrogen to protein conversion factor of 6.25) or of the reference standard or reference material for the protein under test in sodium chloride solution (9 in 1000). Dilute portions of this solution with sodium chloride solution (9 in 1000) to obtain not fewer than three Standard Solutions having concentrations between 0.5 and 10 mg per mL, the concentrations being evenly spaced.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use the buffer used to prepare the Test Solution and the Standard Solutions.

Reagents and Solutions—

BCA Reagent Dissolve about 10 g of bicinchoninic acid, 20 g of sodium carbonate monohydrate, 1.6 g of sodium tartrate dihydrate, 4 g of sodium hydroxide, and 9.5 g of sodium hydrogen carbonate in water. Adjust, if necessary, with sodium hydroxide or sodium hydrogen carbonate to a pH of 11.25. Dilute with water to 1000 mL, and mix.

Copper Sulfate Reagent Dissolve about 2 g of copper (II) sulfate pentahydrate in water to a final volume of 50 mL.

Copper-BCA Reagent Mix 1 mL of Copper Sulfate Reagent and 50 mL of BCA Reagent.

Procedure Mix 0.1 mL of each Standard Solution, the Test Solution, and the Blank with 2 mL of the Copper-BCA Reagent. Incubate the solutions at 37°C for 30 minutes, note the time, and allow to come to room temperature. Within 60 minutes following the incubation time, determine the absorbances of the solutions from the Standard Solutions and the Test Solution in quartz cells at 562 nm, with a suitable spectrophotometer, using the Blank to set the instrument to zero. After the solutions are cooled to room temperature, the color intensity continues to increase gradually. If substances that will cause interference in the test are present, proceed as directed for Interfering Substances under Method 2. Because different protein species may give different color response intensities, the standard protein and test protein should be the same.

Calculations [Note: The relationship of absorbance to protein concentration is nonlinear; therefore, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using the linear regression method, plot the absorbances of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the Test Solution, determine the concentration of protein in the Test Solution.

Method 5 (Biuret method)

This method, commonly referred to as the Biuret assay, is based on the interaction of cupric (Cu²⁺) ion with protein in an alkaline solution and the resultant development of absorbance at 545 nm.

Standard Solutions Unless otherwise specified in the individual monograph, prepare a solution of Albumin Human for which the protein content has been previously determined by nitrogen analysis (using the nitrogen to protein conversion factor of 6.25) or of the reference standard or reference material for the protein under test in sodium chloride solution (9 in 1000). Dilute portions of this solution with sodium chloride solution (9 in 1000) to obtain not fewer than three Standard Solutions having concentrations between 0.5 and 10 mg per mL, the concentrations being evenly spaced. [Note: Low responses may be observed if the sample under test has significantly different level of proline than that of Albumin Human. A different standard protein may be employed in such cases.]

Test Solution Prepare a solution of the test protein in sodium chloride solution (9 in 1000) having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use sodium chloride solution (9 in 1000).

Biuret Reagent Dissolve about 3.46 g of copper (II) sulfate pentahydrate in 10 mL of water, with heating if necessary, and allow to cool (Solution A). Dissolve about 34.6 g of sodium citrate dihydrate and 20.0 g of anhydrous sodium carbonate in 80 mL of water, with heating if necessary, and allow to cool (Solution B). Mix Solutions A and B, and dilute with water to 200 mL. This Biuret Reagent is stable at room temperature for 6 months. Do not use the reagent if it develops turbidity or contains any precipitate.

Procedure To one volume of the Standard Solutions and a solution of the Test Solution add an equal volume of sodium hydroxide solution (6 in 100), and mix. Immediately add a volume of Biuret Reagent equivalent to 0.4 volume of the Test Solution, and mix. Allow to stand at a temperature between 15°C and 25°C for not less than 15 minutes. Within 90 minutes after the addition of the Biuret Reagent, determine the absorbances of the Standard Solutions and the solution from the Test Solution at the wavelength of maximum absorbance at 545 nm, with a suitable spectrophotometer, using the Blank to set the instrument to zero. [Note: Any solution that develops turbidity or contains any precipitate is not acceptable for calculation of protein concentration.]

Calculations Using the least-squares linear regression method, plot the absorbances of the Standard Solutions ver-
sus the protein concentrations, and determine the standard curve best fitting the plotted points, and calculate the correlation coefficient for the line. [Note: Within the given range of the standards, the relationship of absorbance to protein concentration is approximately linear.] A suitable system is one that yields a line having a correlation coefficient of not less than 0.99. From the standard curve and the absorbance of the Test Solution, determine the concentration of protein in the test specimen, making any necessary correction.

Interfering Substances To minimize the effect of interfering substances, the protein can be precipitated from the initial test specimen as follows. Add 0.1 volume of 50% trichloroacetic acid to 1 volume of a solution of the test specimen, withdraw the supernatant layer, and dissolve the precipitate in a small volume of 0.5 mol/L sodium hydroxide TS. Use the solution so obtained to prepare the Test Solution.

Comments This test shows minimal difference between equivalent IgG and albumin samples. Addition of the sodium hydroxide and the Biuret Reagent as a combined reagent, insufficient mixing after the addition of the sodium hydroxide, or an extended time between the addition of the sodium hydroxide solution and the addition of the Biuret Reagent will give IgG samples a higher response than albumin samples. The trichloroacetic acid method used to minimize the effects of interfering substances also can be used to determine the protein content in test specimens at concentrations below 500 µg per mL.

Method 6 (Fluorometric method)

This fluorometric method is based on the derivatization of the protein with o-phthalaldehyde (OPA), which reacts with the primary amines of the protein (i.e., NH₂-terminal amino acid and the ε-amino group of the lysine residues). The sensitivity of the test can be increased by hydrolyzing the protein before testing. Hydrolysis makes the ε-amino group of the constituent amino acids of the protein available for reaction with the OPA reagent. The method requires very small quantities of the protein.

Primary amines, such as tris(hydroxymethyl)aminomethane and amino acid buffers, react with OPA and must be avoided or removed. Ammonia at high concentrations will react with OPA as well. The fluorescence obtained when amine reacts with OPA can be unstable. The use of automatic procedures to standardize this procedure may improve the accuracy and precision of the test.

Standard Solutions Unless otherwise specified in the individual monograph, dissolve the reference standard or the reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 10 and 200 µg of protein per mL, the concentrations being evenly spaced.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use the buffer used to prepare the Test Solution and the Standard Solutions.

Reagents and Solutions—

Borate Buffer Dissolve about 61.83 g of boric acid in water, and adjust with potassium hydroxide to a pH of 10.4. Dilute with water to 1000 mL, and mix.

Stock OPA Reagent Dissolve about 120 mg of o-phthalaldehyde in 1.5 mL of methanol, add 100 mL of Borate Buffer, and mix. Add 0.6 mL of polyoxyethylene (23) lauryl ether, and mix. This solution is stable at room temperature for at least 3 weeks.

OPA Reagent To 5 mL of Stock OPA Reagent add 15 µL of 2-mercaptoethanol. Prepare at least 30 minutes prior to use. This reagent is stable for one day.

Procedure Adjust each of the Standard Solutions and the Test Solution to a pH between 8.0 and 10.5. Mix 10 µL of the Test Solution and each of the Standard Solutions with 100 µL of OPA Reagent, and allow to stand at room temperature for 15 minutes. Add 3 mL of 0.5 mol/L sodium hydroxide TS, and mix. Using a suitable fluorometer, determine the fluorescent intensities of solutions from the Standard Solutions and the Test Solution at an excitation wavelength of 340 nm and an emission wavelength between 440 and 455 nm. [Note: The fluorescence of an individual specimen is read only once because irradiation decreases the fluorescent intensity.]

Calculations The relationship of fluorescence to protein concentration is linear. Using the linear regression method, plot the fluorescent intensities of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the fluorescent intensity of the Test Solution, determine the concentration of protein in the test specimen.

Method 7 (Nitrogen method)

This method is based on nitrogen analysis as a means of protein determination. Interference caused by the presence of other nitrogen-containing substances in the test protein can affect the determination of protein by this method. Nitrogen analysis techniques destroy the protein under test but are not limited to protein presentation in an aqueous environment.

Procedure A Determine the nitrogen content of the protein under test as directed elsewhere in the Pharmacopoeia. Commercial instrumentation is available for the Kjeldahl nitrogen assay.

Procedure B Commercial instrumentation is available for nitrogen analysis. Most nitrogen analysis instruments use pyrolysis (i.e., combustion of the sample in oxygen at temperatures approaching 1000°C), which produces nitric oxide (NO) and other oxides of nitrogen (NOₓ) from the nitrogen present in the test protein. Some instruments convert the nitric oxides to nitrogen gas, which is quantified with a thermal conductivity detector. Other instruments mix nitric oxide (NO) with ozone (O₃) to produce excited nitrogen dioxide (NO₂) which emits light when it decays and can be quantified with a chemiluminescence detector. A protein reference standard or reference material that is relatively pure and is similar in composition to the test proteins is used to optimize the injection and pyrolysis parameters and to evaluate consistency in the analysis.

Calculations The protein concentration is calculated by dividing the nitrogen content of the sample by the known nitrogen content of the protein. The known nitrogen content of the protein can be determined from the chemical composition of the protein or by comparison with the nitrogen content of the appropriate reference standard or reference mate-
rial.

1) Example: Minimum Requirements for Biological Products and individual monograph in JP.
2) Purity of the reagent is important.

**G4 Microorganisms**

**Decision of Limit for Bacterial Endotoxins**

The endotoxin limit for injections is to be decided as follows:

\[
Endotoxin\ limit = \frac{K}{M}
\]

where \( K \) is a threshold pyrogenic dose of endotoxin per kg body mass (EU/kg), and \( M \) is equal to the maximum bolus dose of product per kg body mass. When the product is to be injected at frequent intervals or infused continuously, \( M \) is the maximum total dose administered in a single hour period.

\( M \) is expressed in mL/kg for products to be administered by volume, in mg/kg or mEq/kg for products to be administered by mass, and in Unit/kg for products to be administered by biological units. Depending on the administration route, values for \( K \) are set as in the following table.

<table>
<thead>
<tr>
<th>Intended route of administration</th>
<th>( K ) (EU/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>5.0</td>
</tr>
<tr>
<td>Intravenous, for radiopharmaceuticals</td>
<td>2.5</td>
</tr>
<tr>
<td>Intraspinal</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Notes:
1) For products to be administered by mass or by units, the endotoxin limit should be decided based on the labeled amount of the principal drug.
2) Sixty kg should be used as the average body mass of an adult when calculating the maximum adult dose per kg.
3) The pediatric dose per kg body mass should be used when this is higher than the adult dose.
4) The \( K \) values for the intravenous route are applicable to drugs to be administered by any route other than those shown in the table.

**Disinfection and Sterilization Methods**

Disinfection and Sterilization Methods are applied to kill microorganisms in processing equipment/utensils and areas used for drug manufacturing, as well as to perform microbiological tests specified in the monographs, and so differ from “Terminal Sterilization” and “Filtration Method” described in “Terminal Sterilization and Sterilization Indicators”. The killing effect on microorganisms or the estimated level of sterility assurance is greatly variable, so the conditions for disinfection and sterilization treatment must be chosen appropriately for each application. Generally, the following methods are to be used singly or in combination after appropriate optimization of operation procedures and conditions, in accordance with the kind and the degree of the contaminating microorganisms and the nature of the item to which the methods are applied.

The validation of sterilization in accordance with Terminal Sterilization and Sterilization Indicators is required when the methods are applied to the manufacturing processes of drug products.

1. **Disinfection methods**

These methods are used to reduce the number of living microorganisms, but do not always remove or kill all microorganisms present. Generally, disinfection is classified into chemical disinfection with the use of chemical drugs (disinfectants) and physical disinfection with the use of moist heat, ultraviolet light, and other agents.

1.1. **Chemical disinfection**

Microorganisms are killed with chemical drugs. The killing effect and mechanisms of a chemical drug differ depending on the type, applied concentration, action temperature, and action time of the chemical drug used, the degree of contamination on the object to be disinfected, and the species and state (e.g., vegetative bacteria or spore bacteria) of microorganisms.

Therefore, in applying the method, full consideration is required of the sterility and permissible storage period of the prepared chemical drug, the possibility of resistance of microorganisms at the site of application, and the effect of residual chemical drug on the product. In selecting a suitable chemical drug, the following items should be considered in relation to the intended use.

(i) The antimicrobial spectrum
(ii) Action time for killing microorganisms
(iii) Action durability
(iv) Effect of the presence of proteins
(v) Influence on the human body
(vi) Solubility in water
(vii) Influence on the object to be disinfected
(viii) Odor
(ix) Convenience of use
(x) Easy disposability
(xi) Influence on the environment at disposal
(xii) Frequency of occurrence of resistance

1.2. **Physical disinfection**

Microorganisms are killed without a chemical drug.

(i) Steam flow method

Microorganisms are killed by direct application of steam. This method is used for a product which may be denatured by the moist heat method. As a rule, the product is kept in flowing steam at 100°C for 30 – 60 minutes.

(ii) Boiling method

Microorganisms are killed by putting the object in boiling water. This method is used for a product which may be denatured by the moist heat method. As a rule, the product is put in boiling water for 15 minutes or more.

(iii) Intermittent method

Microorganisms are killed by heating for 30 – 60 minutes repeatedly, three to five times, once a day in water at 80 – 100°C or in steam. This method is used for a product which...
may be denatured by the moist heat method. There is another method called the low temperature intermittent method with repeated heating at 60 – 80°C. During the intermission periods between heating or warming, a suitable temperature for the growth of microorganisms of 20°C or higher, must be maintained.

(iv) Ultraviolet method

As a rule, microorganisms are killed by irradiation with ultraviolet rays at a wavelength of around 254 nm. This method is used for products which are resistant to ultraviolet rays, such as smooth-surfaced articles, facilities, and equipment, or water and air. This method does not suffer from the occurrence of resistance, which is observed in chemical disinfection, and shows a killing effect on bacteria, fungi, and viruses. It must be taken into consideration that direct ultraviolet irradiation of the human body can injure the eyes and skin.

2. Sterilization methods

2.1. Heating methods

In these methods, the heating time before the temperature or pressure reaches the prescribed value differs according to the properties of the product, the size of the container, and the conditions. The duration of heating in conducting these methods is counted from the time when all the parts of the product have reached the prescribed temperature.

(i) Moist heat method

Microorganisms are killed in saturated steam at a suitable temperature and pressure. This method is generally used for heat-stable substances, such as glass, porcelain, metal, rubber, plastics, paper, and fiber, as well as heat-stable liquids, such as water, culture media, reagents, test solutions, liquid samples, etc. As a rule, one of the following conditions is used.

115 – 118°C for 30 minutes
121 – 124°C for 15 minutes
126 – 129°C for 10 minutes

(ii) Dry-heat method

Microorganisms are killed in dry-heated air. This method is generally used for heat-stable substances, such as glass, porcelain, metal, as well as heat-stable products, such as mineral oils, fats and oils, powder samples, etc. This method is generally conducted in the way of direct heating by gas or electricity or circulating heated air. As a rule, one of the following conditions is used.

160 – 170°C for 120 minutes
170 – 180°C for 60 minutes
180 – 190°C for 30 minutes

2.2. Irradiation methods

(i) Radiation method

Microorganisms are killed by gamma-rays emitted from a radioisotope or electron beam and bremsstrahlung (X-ray) generated from an electron accelerator. This method is generally used for radiation-resistant substances such as glass, porcelain, metal, rubber, plastics, fiber, etc. The dose is decided according to the material properties, and the degree of contamination of the product to be sterilized. Special consideration is necessary of the possibility of qualitative change of the product after the application of the method.

(ii) Microwave method

Microorganisms are killed by the heat generated by direct microwave irradiation. This method is generally used for microwave-resistant products such as water, culture media, test solutions, etc. As a rule, microwave radiation with a wavelength of around 2450 ± 50 MHz is used.

2.3. Gas methods

Microorganisms are killed by a sterilizing gas. Suitable gases for killing microorganisms include ethylene oxide gas, formaldehyde gas, hydrogen peroxide gas, chlorine dioxide gas, etc. Temperature, humidity, the concentration of gas, and the exposure time differ in accordance with the species of gas used. As sterilizing gases are generally toxic to humans, full consideration is required of the environmental control for the use of gases and the concentration of residual gas. In some of the gas methods, it may be difficult to measure or estimate quantitatively the killing of microorganisms.

2.4. Filtration method

Microorganisms are removed by filtration with a suitable filtering device. This method is generally used for gas, water, or culture media and test solutions containing a substance that is water-soluble and unstable to heat. As a rule, a filter having a pore size of 0.22 µm or smaller is used for the sterilization. However, in this method, a filter with a pore size of 0.45 µm or smaller is permitted to be used.

Media Fill Test (Process Simulation)

The media fill test is one of the process validations employed to evaluate the propriety of the aseptic processing of pharmaceutical products using sterile media, etc. instead of actual products. Therefore, media fill should be conducted under conditions that simulate routine manufacturing procedures, e.g. filling and sealing, operating environment, processing operation, number of personnel involved, etc., and include permissible worst case conditions. Process simulation can be applied to the other aseptic manufacturing processes in addition to aseptic manufacturing processes for finished drug products such as “filling” and “sealing”.

1. Frequency of media fills

1.1. Initial performance qualification

Initial performance qualification should be conducted for each new facility, item of equipment, filling line, and container design (except for multiple sizes of the same container design), etc. As referring to Table 1, a sufficient number of units should be used to simulate aseptic manufacturing process. A minimum of three consecutive separate successful runs should be performed on each separate day.

1.2. Periodic performance requalification

1) As referring to Table 2, a sufficient number of units should be used to simulate aseptic manufacturing process. Media fill run should be conducted at least on semi-annual base for each shift and processing line. All personnel working in the critical processing area should be trained about aseptic processing and participate in a media fill run at least once a year.

2) When filling lines have not been used for over six months, conduct appropriate numbers of media fill runs in the same way as for the initial performance qualification prior to the use of the filling lines.

3) In cases of facility and equipment modification (interchanging parts may not require requalification), major changes in personnel working in critical aseptic processing, anomalies in environmental monitoring results, or a product sterility test showing contaminated products, conduct ap-
Table 1 Initial performance qualification

<table>
<thead>
<tr>
<th>Minimum number of simulations</th>
<th>Number of units filled per simulation</th>
<th>Contaminated units in any of the three simulations</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>&lt; 5000</td>
<td>1 1 1</td>
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<tr>
<td></td>
<td></td>
<td>≥ 1</td>
<td>Investigation, corrective measures, restart validation</td>
</tr>
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<td></td>
<td></td>
<td>5000 – 10000</td>
<td>1 1 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 1</td>
<td>Investigation, consideration of repeat of one media fill</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 1</td>
<td>Investigation, corrective measures, restart validation</td>
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<tr>
<td></td>
<td></td>
<td>&gt; 1</td>
<td>Investigation</td>
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</tbody>
</table>

Table 2 Periodic performance requalification

<table>
<thead>
<tr>
<th>Minimum number of simulations</th>
<th>Number of units filled per simulation</th>
<th>Contaminated units</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Every half year</td>
<td>3</td>
<td>&lt; 5000</td>
<td>1 1 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Investigation, revalidation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5000 – 10000</td>
<td>1 1 1</td>
</tr>
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<td></td>
<td></td>
<td>≥ 1</td>
<td>Investigation, consideration of repeat media fill</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 1</td>
<td>Investigation, corrective measures, revalidation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 1</td>
<td>Investigation</td>
</tr>
</tbody>
</table>

propriate numbers of media fill runs in the same way as for the initial performance qualification prior to the scheduled media fills.

2. Acceptance criteria of media fills

Both in initial performance qualification and periodic performance qualification, the target should be zero growth regardless of number of units filled per simulation. Where contaminated units are found, action shown in Tables 1 and 2 should be taken.

2.1. Investigation of positive units

Where contaminated units are found in media fill, an investigation should be conducted regarding the cause, taking into consideration the following points:

1) Microbial monitoring data
2) Particulate monitoring data
3) Personnel monitoring data (microbial monitoring data on gloves, gowns, etc. at the end of work)
4) Sterilization cycle data for media, commodities, equipment, etc.
5) Calibration data of sterilization equipment
6) Storage conditions of sterile commodities
7) HEPA filter evaluation (integrity tests, velocity, etc.)
8) Pre and post filter integrity test data (including filter housing assembly)
9) Air flow patterns and pressures
10) Unusual events that occurred during the media fill run
11) Characterization of contaminants
12) Hygienic control and training programs
13) Gowning procedures and training programs
14) Aseptic processing technique and training programs
15) Operator’s health status (especially coughing, sneezing, etc., due to respiratory diseases)
16) Other factors that affect sterility

3. Data guidance for media fills

Each media fill run should be fully documented and the following information recorded:

1) Data and time of media fill
2) Identification of filling room and filling line used
3) Container/closure type and size
4) Volume filled per container
5) Filling speed
6) Filter type and integrity test result (in case of filtration)
7) Type of media filled
8) Number of units filled
9) Number of units not incubated and reason
10) Number of units incubated
11) Number of units positive
12) Incubation time and temperature
13) Procedures used to simulate any step of a normal production fill (e.g., mock lyophilization or substitution of vial headspace gas)
14) Microbiological monitoring data obtained during the media fill set-up and run
15) List of personnel who took part in the media fill
16) Growth promotion results of the media (in case of powder fill, an antimicrobial activity test for the powder is necessary)
17) Identification and characterization of the microorganisms from any positive units
18) Product(s) covered by the media fill
19) Investigation of runs with a positive unit or failed runs
20) Management review

4. Media fill procedures

Methods to validate aseptic processing of liquid, powder and freeze-dried products are described. Basically, it is possible to apply media fill procedures for liquid products to other dosage forms.

4.1. Media selection and growth promotion

Soybean-casein digest medium or other suitable media are used. When strains listed in the Microbial Limit Test <4.05> and, if necessary, one or two representative microorganisms which are frequently isolated in environmental monitoring are inoculated under the specified conditions, each strain should show obvious growth.
4.2 Sterile medium preparation
The medium is sterilized according to the pre-validated method.

4.3 Incubation and inspection of media filled units
Leaking or damaged units should be removed and recorded prior to incubation of media filled units. Units should be incubated at 20 – 35°C for not less than 14 days. The use of another temperature range should be justified. If two temperatures are used for incubation, the units are typically incubated for at least 7 days at each temperature starting with the lower temperature. Established temperature should be kept within ±2.5°C. Observe the media filled units for growth of microorganisms on the last day of the incubation. Microorganisms in contaminated units should be followed to identification and characterization. For the identification of contaminants, “Rapid Identification of Microorganisms Based on Molecular Biological Method” shown in General Information or appropriate commercial kit for identification of microorganisms may be applicable.

A. Liquid products
Media fill procedure
Media fill should include normal facility/equipment operations and clean-up routines. Containers, closures, parts of the filling machine, trays, etc. are washed and sterilized according to the standard operating procedures. Media fills should be conducted under processing conditions that include “worst case” conditions, e.g., correction of line stoppage, repair or replacement of filling needles/tubes, replacement of on-line filters, permitted interventions, duration and size of run, number of personnel involved, etc.

It is not necessary to put all worst-case scenarios in a single media fill run, but all worst-case scenarios should be evaluated intentionally. While advanced processing lines are highly automated, often operated at relatively high speeds, and are designed to limit operator intervention, there are processing lines showing frequent human interventions. Although the most accurate simulation model would be the full batch size and duration because it most closely simulates the actual production run, other appropriate models can be justified.

A predetermined volume of medium is filled into sterilized containers at a predetermined filling speed and the containers are sealed. The media are contacted with all interior surfaces in the containers by an appropriate method, and then incubated at the predetermined temperature.

B. Powder products
B.1 Powder selection and antimicrobial activity test
Actual products or placebo powder are used. In general, lactose monohydrate, d-mannitol, polyethylene glycol 6,000, carboxymethyl cellulose salts or media powder, etc. are used as placebo powders. Prior to employing any of the powders, evaluate whether the powder has antimicrobial activity. Media powders are dissolved in water and other powders in liquid medium, and the solutions are inoculated with less than 100 CFU microorganisms of each kind, shown in 4.1, for the growth promotion test. If obvious growth appears in the medium incubated at the predetermined temperature for 5 days, the powder has no antimicrobial activity and is available for the media fill test.

B.2 Sterilization of powders
Powders are bagged in suitable containers (e.g. double heat-sealed polyethylene bags), and are subjected to radiation sterilization.

B.3 Sterility of filling powders
The powders must pass the Sterility Test. However, if the sterilization is fully validated, sterility testing of the powders can be omitted.

B.4 Media fill procedures
Chose a suitable procedure from among the following procedures,
1) Fill sterilized liquid media into containers by suitable methods, and then fill actual products or sterilized placebo powder with the powder filling machine. If sterilized powder media are used as a placebo powder, fill sterilized water instead of sterilized liquid media.
2) Distribute liquid media into containers, and then sterilize them in an autoclave. Remove the containers to the filling area, and then fill actual product or sterilized placebo powder into the containers with the powder filling machine.
3) Fill actual products or sterilized placebo powder into containers with the powder filling machine, and then fill sterilized liquid media into the containers by appropriate methods. If sterilized powder media are used as a placebo powder, fill sterilized water instead of sterilized liquid media.

C. Lyophilized products
In the case of lyophilized products, it may be impossible to conduct a media fill run in the same way as used for actual processing of lyophilized products. The process of freezing and lyophilization of the solution may kill contaminant organisms and change the characteristics of the media too. The use of inert gas as a blanket gas may inhibit the growth of aerobic bacteria and fungi. Therefore, in general, the actual freezing and lyophilization process should be avoided and air is used as the blanket gas. For products manufactured under an anaerobic atmosphere, process simulation should be performed with the use of anaerobic growth media and the inert gas such as nitrogen gas.

Media fill procedures
Use the following method or other methods considered to be equivalent to these methods.
1) After filling of the media into containers by the filling machine, cap the containers loosely and collect them in pre-sterilized trays.
2) After placing the trays in the lyophilizer, close the chamber door, and conduct lyophilization according to the procedures for production operation. Hold them without freezing and boiling-over under weak vacuum for the predetermined time.
3) After the vacuum process, break the vacuum, and seal the stoppers.
4) Contact the media with all product contact surfaces in the containers by appropriate methods, and then cultivate them at the predetermined temperature.

References
Microbial Attributes of Non-sterile Pharmaceutical Products

This chapter is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (• • •). The presence of certain micro-organisms in non-sterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has a potential to adversely affect the health of the patient. Manufacturers have therefore to ensure a low bioburden of finished preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has a potential to adversely affect the health of the patient. Manufacturers have therefore to ensure a low bioburden of finished dosage forms by implementing current guidelines on Good Manufacturing Practice during the manufacture, storage and distribution of pharmaceutical preparations. • This chapter provides guidelines for acceptable limits of viable micro-organisms (bacteria and fungi) existing in raw materials and non-sterile pharmaceutical products. • Microbial examination of non-sterile products is performed according to the methods given in the Microbial Limit Test 4.05 on Microbiological Examination of Non-sterile Products: I. Microbial Enumeration Tests and II. Tests for Specified Micro-organisms. • When these tests are carried out, a microbial control program must be established as an important part of the quality management system of the product. Personnel responsible for conducting the tests should have specialized training in microbiology, biosafety measures and in the interpretation of the testing results.

• 1. Definitions
   (i) Non-sterile pharmaceutical products: Non-sterile drugs shown in monographs of the JP and non-sterile finished dosage forms.
   (ii) Raw materials: All materials, including raw ingredients and excipients, used for the preparation of drugs, except for water and gases.
   (iii) Bioburden: Number and type of viable micro-organisms existing in non-sterile pharmaceutical products.
   (iv) Action levels: Established bioburden levels that require immediate follow-up and corrective action if they are exceeded.
   (v) Alert levels: Established bioburden levels that give early warning of a potential drift from normal bioburden level, but which are not necessary grounds for definitive corrective action, though they may require follow-up investigation.
   (vi) Quality management system: The procedures, operation methods and organizational structure of a manufacturer (including responsibilities, authorities and relationships between these) needed to implement quality management.

2. Scope
   In general, the test for total viable aerobic count is not applied to drugs containing viable micro-organisms as an active ingredient.

3. Sampling plan and frequency of testing
3.1 Sampling methods
   Microbial contaminants are usually not uniformly distributed throughout the batches of non-sterile pharmaceutical products or raw materials. A biased sampling plan, therefore, cannot be used to estimate the real bioburden in the product. A sampling plan which can properly reflect the status of the product batch should be established on the basis of the bioburden data obtained by retrospective validation and/or concurrent validation. In general, a mixture of samples randomly taken from at least different three portions, almost the same amount for each portion, is used for the tests of the product. When the sampling is difficult in a clean area, special care is required during sampling to avoid introducing microbial contamination into the product or affecting the nature of the product bioburden. If it is confirmed that the product bioburden is stable for a certain period, as in the case of non-aqueous or dried products, it is not necessary to do the tests, immediately after the sampling.

3.2 Testing frequency
   The frequency of the tests should be established on the basis of a variety of factors unless otherwise specified. These factors include:
   (i) Dosage forms of non-sterile pharmaceutical products (usage);
   (ii) Manufacturing processes;
   (iii) Manufacturing frequency;
   (iv) Characteristics of raw materials (natural raw material, synthetic compound, etc.);
   (v) Batch sizes;
   (vi) Variations in bioburden estimates (changes in batches, seasonal variations, etc.);
   (vii) Changes affecting the product bioburden (changes in manufacturing process, supplier of raw materials, batch number of raw materials, etc.);
   (viii) Others.

   In general, the tests may be performed at a high frequency during the initial production of a drug to get information on the microbiological attributes of the product or raw materials used for the production. However, this frequency may be reduced as bioburden data are accumulated through retrospective validation and/or concurrent validation. For example, the tests may be performed at a frequency based on time (e.g., weekly, monthly or seasonally), or on alternate batches.

4. Microbial control program
   When the “Microbial Limit Test 4.05” is applied to a non-sterile pharmaceutical product, the methods for the recovery, cultivation and estimation of the bioburden from the product must be validated and a “Microbial control program” covering the items listed below must be prepared.
   (i) Subject pharmaceutical name (product name);
   (ii) Frequency of sampling and testing;
   (iii) Sampling methods (including responsible person, quantity, environment, etc. for sampling);
   (iv) Transfer methods of the samples to the testing area (including storage condition until the tests);
   (v) Treatment of the samples (recovery methods of microbial contaminants);
   (vi) Enumeration of viable micro-organisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);
   (vii) Detection of specified micro-organisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);
   (viii) Estimation of the number of and characterization of microbial contaminants;
   (ix) Establishment of “Microbial acceptance criteria” (including alert level and action level);
5. Microbial acceptance criteria for non-sterile pharmaceutical products

By establishing “Microbial acceptance criteria” for non-sterile pharmaceutical products based upon the total aerobic microbial count (TAMC) and the total combined yeasts/moulds count (TYMC), it is possible to evaluate at the initial processing stage of the product whether the microbiological quality of the raw materials is adequate or not. Furthermore, it is then possible to implement appropriate corrective action as needed to maintain or improve the microbiological quality of the product. The target limits of microbial levels for raw materials (synthetic compounds and minerals) are shown in Table 1.

In general, synthetic compounds have low bioburden levels due to the high temperatures, organic solvents, etc., used in their manufacturing processes. Raw materials originated from plants and animals in general have higher bioburdens than synthetic compounds.

The microbial quality of the water used in the processing of active ingredients or non-sterile pharmaceuticals may have a direct effect on the quality of the finished dosage form. This means it is necessary to keep the level of microbial contaminants in the water as low as possible.

Acceptance criteria for microbiological quality for non-sterile finished dosage forms are shown in Table 2. These microbial limits are based primarily on the type of dosage form, water activity, and so on. For oral liquids and pharmaceutical products having a high water activity, in general, low microbial acceptance criteria are given.

Table 2 includes a list of specified micro-organisms for which acceptance criteria are set. The list is not necessarily

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Total Aerobic Microbial Count (CFU/g or CFU/mL)</th>
<th>Total Combined Yeasts/Moulds Count (CFU/g or CFU/mL)</th>
<th>Specified Micro-organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-aqueous preparations for oral use</td>
<td>$10^3$</td>
<td>$10^2$</td>
<td>Absence of Escherichia coli (1 g or 1 mL)</td>
</tr>
<tr>
<td>Aqueous preparations for oral use</td>
<td>$10^2$</td>
<td>$10^1$</td>
<td>Absence of Escherichia coli (1 g or 1 mL)</td>
</tr>
<tr>
<td>Rectal use</td>
<td>$10^3$</td>
<td>$10^2$</td>
<td>—</td>
</tr>
<tr>
<td>Oromucosal use</td>
<td>$10^2$</td>
<td>$10^1$</td>
<td>Absence of Staphylococcus aureus (1 g or 1 mL)</td>
</tr>
<tr>
<td>Gingival use</td>
<td></td>
<td></td>
<td>Absence of Pseudomonas aeruginosa (1 g or 1 mL)</td>
</tr>
<tr>
<td>Cutaneous use</td>
<td></td>
<td></td>
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<tr>
<td>Nasal use</td>
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<tr>
<td>Auricular use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal use</td>
<td>$10^2$</td>
<td>$10^1$</td>
<td>Absence of Pseudomonas aeruginosa (1 g or 1 mL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Absence of Staphylococcus aureus (1 g or 1 mL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Absence of Candida albicans (1 g or 1 mL)</td>
</tr>
<tr>
<td>Transdermal patches (limits for one patch including adhesive layer and backing)</td>
<td>$10^2$</td>
<td>$10^1$</td>
<td>Absence of Staphylococcus aureus (1 patch)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Absence of Pseudomonas aeruginosa (1 patch)</td>
</tr>
<tr>
<td>Inhalation use (more rigorous requirements apply to liquid preparations for nebulization)</td>
<td>$10^2$</td>
<td>$10^1$</td>
<td>Absence of Staphylococcus aureus (1 g or 1 mL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Absence of Pseudomonas aeruginosa (1 g or 1 mL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Absence of bile-tolerant gram-negative bacteria (1g or 1 mL)</td>
</tr>
</tbody>
</table>
exhaustive and for a given preparation it may be necessary to
test for other micro-organisms depending on the nature of the
starting materials and the manufacturing process.

If it has been shown that none of the prescribed tests will
allow valid enumeration of micro-organisms at the level
prescribed, a validated method with a limit of detection as
close as possible to the indicated acceptance criterion is used.

In addition to the micro-organisms listed in Table 2, the
significance of other micro-organisms recovered should be
evaluated in terms of:

(i) the use of the product: hazard varies according to
the route of administration (eye, nose, respiratory
tract);
(ii) the nature of the product: does the product support
growth, does it have adequate antimicrobial preser-
vation?
(iii) the method of application;
(iv) the intended recipient: risk may differ for neonates,
infants, the debilitated;
(v) use of immunosuppressive agents, corticosteroids;
(vi) presence of disease, wounds, organ damage.

Where warranted, a risk-based assessment of the relevant
factors is conducted by personnel with specialized training in
microbiology and the interpretation of microbiological data.

For raw materials, the assessment takes account of
processing to which the product is subjected, the current
technology of testing and the availability of materials of
the desired quality. Acceptance criteria are based on individual
results or on the average of replicate counts when replicate
counts are performed (e.g. direct plating methods).

When an acceptance criterion for microbiological quality
is prescribed it is interpreted as follows:

$-10^3$ CFU: maximum acceptable count $= 20$,
$-10^4$ CFU: maximum acceptable count $= 200$,
$-10^5$ CFU: maximum acceptable count $= 2000$, and so
forth.

### 6. Acceptance criteria for crude drugs

Target limits of microbial contamination for crude drugs
and crude drug preparations are shown in Table 3. Category
1 includes crude drugs and crude drug preparations which
are used for extraction by boiling water or to which boiling
water is added before use. Category 2 includes crude drugs
which are taken directly without extraction process and
directly consumed crude drug preparations containing pow-
dered crude drugs. In this guideline, enterobacteria and
other gram-negative bacteria, *Escherichia coli*, *Salmonella*,
and *Staphylococcus aureus* are mentioned as specified
micro-organisms, but other micro-organisms such as certain
species of *Bacillus cereus*, *Clostridia*, *Pseudomonas*, *Bur-
kholderia*, *Aspergillus* and *Enterobacter* species are also
necessary to be tested depending on the origin of raw mate-
rials for crude drugs or the preparation method of crude
drug preparations.

### Microbiological Evaluation of
Processing Areas for Sterile
Pharmaceutical Products

This chapter describes the methods for the control and
evaluation of microbial contamination in areas used for the
processing of sterile pharmaceutical products. Such process-
ing areas are classified into critical areas and clean areas ac-
cording to the required levels of air-cleanness. A critical
area is a defined space in which the airborne particulate and
microorganism levels are controlled to meet grade A. The
cleanliness requirements for such a space extend to the sur-
faces of the facilities and equipment which form or are located
within the space, as well as to the supplied raw materials,
chemicals, water, etc. Environmental conditions, such as
temperature, humidity, and air pressure, are also controlled
in this space when required. A clean area is a controlled
space such that the levels of contaminants (particulates and
microorganisms) in air, gases and liquids are maintained
within specified limits, which are less stringent than those of
grade A. When sterile pharmaceutical products are manufac-
tured, the environment, facilities/equipment, and personnel
should be routinely monitored to ensure appropriate
microbiological control in the processing areas. The detec-
tion of microorganisms should be performed under normal
operational conditions, using an appropriate sampling
device, according to an environmental control program es-
blished previously. The sampling, cultivation, counting,
evaluation methods for airborne microorganisms, as
well as those found on surfaces, should also be chosen ap-
propriately, depending on the monitoring purpose, monitor-
ing items, and microorganisms being detected. Sampling
device, measurement methods, media, culture conditions,
frequency of monitoring, and recommended limits for en-
vironmental microorganisms shown in this chapter are for
information only, and are not requirements.

1. Definitions

For the purposes of this chapter, the following definitions
apply.

(i) Processing areas: Areas in which actions such as culti-
vation, extraction/purification, weighing of raw materials,
washing and drying of containers and stoppers, preparation
of solutions, filling, sealing and packaging are performed,
including the gowning area.

(ii) Action levels: Established microbial levels (and type
of microorganisms, if appropriate) that require immediate
follow-up and corrective action if they are exceeded.

(iii) Alert levels: Established microbial levels (and type
of microorganisms, if appropriate) that require immediate
follow-up and corrective action if they are exceeded.
of microorganisms if appropriate) that give early warning of a potential drift from normal operating conditions, but which are not necessarily grounds for definitive corrective action, though they may require follow-up investigation.

(iv) Contaminants: Particulates and microorganisms causing contamination by adhering to surfaces or by being incorporated into materials.

(v) Cleanliness: A quantity which indicates the condition of cleanliness of a monitored item, expressed as mass or number of contaminants contained in a certain volume or area.

(vi) Contamination control: The planning, establishment of systems and implementation activities performed in order to maintain the required cleanliness of a specified space or surface.

(vii) Shift: Scheduled period of work or production, usually less than 12 hours in length, during which operations are conducted by a single defined group of workers.

(viii) Characterization of contaminants: Procedures for classifying contaminants so that they can be differentiated. In routine control, classification to the genus level is sufficient; as required, identification to the species level is performed.

2. Air-cleanliness of processing areas for sterile pharmaceutical products

Airborne particulates in areas used for the processing of pharmaceutical products may act physically as a source of insoluble particles in the products, and biologically as a carrier of microorganisms. So, it is necessary to control strictly the number of particles in the air. The air-cleanliness criteria are shown in Table 1.

2.1. Terminally sterilized products

Solutions should generally be prepared in a grade C environment. Solution preparation may be permitted in a grade D environment if additional measures are taken to minimize contamination. For parenterals, filling should be done in a grade A workstation in a grade B or C environment. The requirements during the preparation and filling of other sterile products are generally similar to those for parenterals.

2.2. Sterile products prepared aseptically after filtration

The handling of starting materials and the preparation of solutions should be done in a grade C environment. These activities may be permitted in a grade D environment if additional measures are taken to minimize contamination, such as the use of closed vessels prior to filtration. After sterile filtration, the product must be handled and filled into containers under grade A aseptic conditions.

2.3. Sterile products prepared aseptically from sterile starting materials

The handling of starting materials and all further processing should be done under grade A conditions.

3. Microbiological environmental monitoring program

Environmental monitoring is especially important in sterility assurance for sterile pharmaceutical products that are manufactured by aseptic processing. The major purpose of environmental monitoring is to predict potential deterioration of the processing environment before it occurs, and to produce high-quality, sterile pharmaceutical products under appropriate contamination control.

3.1. Monitoring of environmental microorganisms

(i) An environmental control program document is prepared for each area used for the processing of sterile pharmaceutical products. The procedures in the document include: 1) items to be monitored, 2) type of microorganisms to be monitored, 3) frequency of monitoring, 4) methods of monitoring, 5) alert and action levels, and 6) actions to be taken when specified levels are exceeded.

(ii) The aseptic processing areas and other processing areas maintained under controlled conditions are monitored on a routine basis. The critical processing areas where sterile products are in contact with environmental air are monitored during every operational shift. The items to be monitored include the air, floor, walls, equipment surfaces, and the gowns and gloves of the personnel. Table 2 shows suggested frequencies of the environmental monitoring.

(iii) The sampling devices used for monitoring environmental microorganisms, as well as the methods and culture media, should be suitable to detect microorganisms that may be present (aerobic bacteria, anaerobic bacteria, molds, yeast, etc.). The cultivation conditions, such as incubation temperature and time, are selected to be appropriate for the specific growth requirements of microorganisms to be detected. Table 3 shows the culture media and cultivation conditions that are generally used in testing for environmental microorganisms.

(iv) The number of microorganisms in the samples is estimated by using the Membrane Filtration, Pour Plating, Spread Plating, or Serial Dilution (Most Probable Number) Methods described in the Microbial Limit Test.

(v) Table 4 shows recommended limits for environmental

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Table 1 Air-cleanliness requirements for processing of sterile pharmaceutical products

<table>
<thead>
<tr>
<th>Air cleanliness</th>
<th>Maximum number of airborne particulates per m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td>at rest</td>
</tr>
<tr>
<td></td>
<td>±0.5 μm</td>
</tr>
<tr>
<td>A (Laminar-airflow zone)</td>
<td>3530</td>
</tr>
<tr>
<td>B (Non laminar-airflow zone)</td>
<td>3530</td>
</tr>
<tr>
<td>C</td>
<td>3530000</td>
</tr>
<tr>
<td>D</td>
<td>3530000</td>
</tr>
</tbody>
</table>

*1 The maximum permitted number of particles in the “in operation” condition corresponds to the standards described under USP <1116> as follows:

Grade A: Class 100 (M3.5); Grade B: Class 10,000 (M5.5); Grade C: Class 100,000 (M6.5);
Grade D: no corresponding standard.

*2 The limit for this area will depend on the nature of the operation carried out there.
Table 2  Suggested frequency of environmental monitoring

<table>
<thead>
<tr>
<th>Processing area</th>
<th>Frequency of monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical area (Grade A)</td>
<td>Each shift</td>
</tr>
<tr>
<td>Clean area adjacent to critical area (Grade B)</td>
<td>Each shift</td>
</tr>
<tr>
<td>Other clean areas (Grade C, D)</td>
<td>Twice a week</td>
</tr>
<tr>
<td>Potential product/container contact areas</td>
<td>Once a week</td>
</tr>
<tr>
<td>Non-product/container contact areas</td>
<td></td>
</tr>
</tbody>
</table>

Table 3  Media and culture conditions

<table>
<thead>
<tr>
<th>Microorganisms to be detected</th>
<th>Media*1</th>
<th>Culture conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobes</td>
<td>Soybean-casein digest agar (or fluid) medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brain-heart infusion agar (or fluid) medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nutrient agar (or fluid) medium</td>
<td></td>
</tr>
<tr>
<td>Yeast and fungi</td>
<td>Soybean-casein digest agar (or fluid) medium</td>
<td></td>
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<tr>
<td></td>
<td>Sabouraud dextrose agar (or fluid) medium</td>
<td></td>
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<tr>
<td></td>
<td>Potato-dextrose agar (or fluid) medium</td>
<td></td>
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<td></td>
<td>Glucose peptone agar (or fluid) medium</td>
<td></td>
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<tr>
<td>Anaerobes*4</td>
<td>Soybean-casein digest agar medium</td>
<td></td>
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<tr>
<td></td>
<td>Fluid cooked meat medium</td>
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<tr>
<td></td>
<td>Reinforced clostridial agar (or fluid) medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thioglycolate medium I (or thioglycolate agar medium) for sterility test</td>
<td></td>
</tr>
</tbody>
</table>

*1 If necessary, antibiotics may be added to media in an appropriate concentration (see Microbial Limit Test). If the existence of disinfectants that may interfere with the test on the surface of the specimen is suspected, add a substance to inactivate them.
*2 When soybean-casein digest agar medium is used for the detection of aerobes, yeast and fungi, incubation at 25 to 30°C for more than 5 days is acceptable.
*3 If a reliable count is obtained in a shorter incubation time than 5 days, this may be adopted.
*4 Generally, anaerobes are not targets for the monitoring. For the detection of anaerobes, agar medium is incubated in an appropriate anaerobic jar.

Table 4  Recommended limits for environmental microorganisms*1

<table>
<thead>
<tr>
<th>Grade</th>
<th>Airborne microorganisms*2 (CFU/m³)</th>
<th>Minimum air sample (m³)</th>
<th>CFU on a surface instruments/facilities (CFU/24–30 cm²)*3</th>
<th>gloves</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt;1</td>
<td>0.5</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>0.5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>0.2</td>
<td>25</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>200</td>
<td>0.2</td>
<td>50</td>
<td>—</td>
</tr>
</tbody>
</table>

*1 Maximum acceptable average numbers of microorganisms under each condition.
*2 These values are by using a slit sampler or equivalent.
*3 Viable microbe cell number per contact plate (5.4 – 6.2 cm in diameter). When swabbing is used in sampling, the number of microorganisms is calculated per 25 cm². For gloves, usually, put their all fingers on the plate.

Microorganisms. The alert and action levels may be adjusted if necessary after sufficient data have been accumulated. The most important point in environmental monitoring is to confirm that an acceptable value of each monitoring item is maintained consistently.

(vi) Microorganisms isolated are characterized if necessary. In addition, analysis of hourly or daily variation of airborne particulate numbers will provide data to assist in the control of the cleanliness of processing areas.

3.2. Evaluation of environmental monitoring data

(i) The data from the environmental monitoring are evaluated on a routine basis for each area and location. The source of any discrepancy should be investigated immediately and the investigation should be documented in a report. After corrective action has been taken, follow-up monitoring should be done to demonstrate that the affected area is once again within specification.

(ii) The report is reviewed and approved by personnel responsible for quality control and distributed to all key personnel associated with the aseptic processing operation.

4. Sampling devices and measuring methodology

Various types of sampling devices and measurement methods are available for the sampling and measurement of...
Microorganisms in the air and on surfaces, and appropriate samplers and measuring methodology are selected according to the purpose of monitoring and the items to be monitored.

### 4.1. Evaluation of airborne microorganisms

#### 4.1.1. Settle plates

Petri dishes of a specified diameter containing a suitable culture medium are placed at the measurement location and the cover is removed there. The plates are exposed for a given time and the microorganisms deposited from the air onto the agar surface are enumerated after incubation. This method is not effective for quantitative monitoring of total airborne microorganisms because it does not detect microorganisms that do not settle onto the surface of the culture media, and the settling velocity of aggregates of microorganisms is affected by air currents and disturbances in airflow. Although the results obtained by the settle plate method are only qualitative or semi-quantitative, this method is suitable for long-term evaluation of possible contamination of products or devices by airborne microorganisms.

#### 4.1.2. Active microbial sampling methods

##### 4.1.2.1. Measuring methods

Methods in which a fixed volume of air is aspirated include filtration-type sampling devices and impact-type sampling devices. With the filter-type sampling devices the desired volume of air can be collected by appropriately changing the air intake rate or the filter size. However, care must be taken to ensure that sterility is maintained while the filter is placed in and removed from the holder. When air sampling devices are used in critical areas, care must be taken to avoid disturbance of the airflow around the products. There are two types of filters; wet-type used gelatin filters and dry-type used membrane filters. With the dry-type filters, static electricity effects can make it impossible to collect quantitatively microorganisms on the filter. When an impact-type sampling device is used, the following points are important: 1) The speed at which the collected air strikes the culture medium surface must be sufficient to capture the microorganisms, but must not have an adverse effect on the collected microorganisms. 2) A sufficient volume of air must be sampled so that even extremely low levels of microbiological contaminants are detected, but the procedure must not cause a significant change in the physical or chemical properties of the culture medium. 3) When the device is used in critical areas, care must be exercised to ensure that the processing of the sterile pharmaceutical products is not adversely affected by the air disturbance.

##### 4.1.2.2. Sampling devices

The most commonly used samplers are as follows: Slit sampler, Andersen sampler, pinhole sampler, centrifugal sampler and filtration-type sampler. Each sampler has specific characteristics. The slit sampler is a device to trap microorganisms in a known volume of air passed through a standardized slit. The air is impacted on a slowly revolving Petri dish containing a nutrient agar. The rotation rate of the Petri dish and the distance from the slit to the agar surface are adjustable and it is possible to estimate the number of microorganisms in the air passed through the device for a period of up to 1 hr. The Andersen sampler consists of a perforated cover and several pieces of Petri dishes containing a nutrient agar, and a known volume of air passed through the perforated cover impacts on the agar medium in the Petri dishes. The sampler is suitable for the determination of the distribution of size ranges of microorganism particulates in the air. The pinhole sampler resembles the slit of the slit sampler, but has pinholes in place of the slit. A known volume of air passed through several pinholes impacts on agar medium in a slowly revolving Petri dish. The centrifugal sampler consists of a propeller that pulls a known volume of air into the device and then propels the air outward to impact on a tangentially placed nutrient agar strip. The sampler is portable and can be used anywhere, but the sampling volume of air is limited.

See above the Measuring methods <4.1.2.1> on the characteristics of the filtration-type sampler.

#### 4.2. Measurement methods for microorganisms on surfaces

##### 4.2.1. Contact plates

Use a contact plate with an appropriate contact surface. The culture medium surface should be brought into contact with the sampling site for several seconds by applying uniform pressure without circular or linear movement. After contact and removal, the plates are covered and, as soon as possible, incubated using appropriate culture conditions. After a contact plate has been used, the site to which the plate was applied must be wiped aseptically to remove any adherent culture medium.

##### 4.2.2. Swabs

A piece of sterilized gauze, absorbent cotton, cotton swab, or other suitable material premoistened with an appropriate rinse fluid is stroked in closely parallel sweeps or slowly rotated over the defined sampling area. After sampling, the swab is agitated in a specified amount of an appropriate sterilized rinse fluid, and the rinse fluid is assayed for viable organisms.

### 5. Test methods for collection performance of a sampling device for airborne microorganisms

The testing of the collection performance of sampling devices for airborne microorganisms is performed in accordance with JIS K 3836 (Testing methods for collection efficiency of airborne microbe samplers) or ISO 14698 – 1 (Cleanrooms and associated controlled environments. Biocontamination control. General principles).

### 6. Growth-promotion test of media and confirmation of antimicrobial substances

This test and confirmation are performed according to “Effectiveness of culture media and confirmation of antimicrobial substances” in the Microbial Limit Test <4.05>.

### 7. Media

#### (i) Soybean-casein digest agar medium or Fluid soybean-casein digest medium

See Microbial Limit Test.

#### (ii) Sabouraud dextrose agar medium or Fluid sabouraud dextrose medium

See Microbial Limit Test. Antibiotic is added if necessary.

#### (iii) Potato-dextrose agar medium or Fluid potato-dextrose medium

See Microbial Limit Test. Antibiotic is added if necessary.

#### (iv) Glucose peptone agar medium or Fluid glucose peptone medium

<table>
<thead>
<tr>
<th>Glucose</th>
<th>20.0 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>2.0 g</td>
</tr>
</tbody>
</table>
Magnesium sulfate heptahydrate 0.5 g
Peptone 5.0 g
Potassium dihydrogen phosphate 1.0 g
Agar 15.0 g
Water 1000 mL
Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 5.6 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracyclin per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium. Antibiotics are added, as appropriate.

*(v) Thioglycolate agar medium or thioglycolate medium*1 for sterility test
See Sterility Test. The agar concentration of Thioglycolate agar medium is about 1.5%.

*(vi) Brain-heart infusion agar medium or Fluid brain-heart infusion medium*2

Bovine brain extract powder*1
An amount equivalent to 200 g of calf brain

Bovine heart extract powder*2
An amount equivalent to 250 g of the material

Peptone 10.0 g
Glucose 2.0 g
Sodium chloride 5.0 g
Disodium hydrogenphosphate dodecahydrate 2.5 g
Agar 15.0 g
Water 1000 mL
Sterilize by heating in an autoclave at 121°C for 15 to 20 min. pH after sterilization: 7.2 – 7.6.

*(vii) Nutrient agar medium or Fluid nutrient medium*3

Meat extract 3.0 g
Peptone 5.0 g
Agar 15.0 g
Water 1000 mL
Sterilize by heating in an autoclave at 121°C for 15 to 20 min. pH after sterilization: 6.6 – 7.0.

*(viii) Fluid cooked meat medium*4

Bovine heart extract powder*2
An amount equivalent to 450 g of the material

Peptone 20.0 g
Glucose 2.0 g
Sodium chloride 5.0 g
Water 1000 mL
Sterilize by heating in an autoclave at 121°C for 15 to 20 min. pH after sterilization: 7.2 – 7.6.

*(ix) Reinforced clostridial agar medium or Fluid reinforced clostridial medium*5

Meat extract 10.0 g
Peptone 10.0 g
Yeast extract 3.0 g
Soluble starch 1.0 g
Glucose 5.0 g
l-Cystein hydrochloride monohydrate 0.5 g
Sodium chloride 5.0 g
Sodium acetate trihydrate 3.0 g
Agar 15.0 g

Preservatives-Effectiveness Tests

The purpose of the Preservatives-Effectiveness Tests is to assess microbiologically the preservative efficacy, either due to the action of product components themselves or any added preservative(s), for multi-dose containers. The efficacy of the preservatives is assessed by direct inoculation and mixing of the test strains in the product, and titration of survival of the test strains with time.

Preservatives must not be used solely to comply with GMP for drugs or to reduce viable aerobic counts. In addition, preservatives themselves are toxic substances. Therefore, preservatives must not be added to products in amounts which might jeopardize the safety of human beings, and consideration must be given to minimizing the amounts of preservative used. These tests are commonly used to verify that products maintain their preservative effectiveness at the design phase of formulation or in the case of periodic monitoring. Although these tests are not performed for lot
release testing, the efficacy of the preservative present in the product packaged in the final containers should be verified throughout the entire dating period.

1. Products and their Categories

The products have been divided into two categories for these tests. Category I products are those made with aqueous bases or vehicles, and Category II products are those made with nonaqueous bases or vehicles. Oil-in-water emulsions are considered Category I products, and water-in-oil emulsions Category II products. Category I is further divided into three subtypes depending on the dosage forms.

- Category IA: Injections and other sterile parenterals
- Category IB: Non-sterile parenterals
- Category IC: Oral products in liquid forms (including syrup products to be dissolved or suspended before use)

Category II: All the dosage forms listed under Category I made with non-aqueous bases or vehicles.

2. Test Microorganisms and Culture Media

The following strains or those considered to be equivalent are used as the test microorganisms.

- *Escherichia coli* ATCC 8739, NBRC 3972
- *Pseudomonas aeruginosa* ATCC 9027, NBRC 13275
- *Staphylococcus aureus* ATCC 6538, NBRC 13276
- *Candida albicans* ATCC 10231, NBRC 1594, JCM 2085
- *Aspergillus brasiliensis* ATCC 16404, NBRC 9455

These test microorganisms are representative of those that might be found in the environment in which the product is manufactured, used or stored, and they are also recognized as opportunistic pathogens. In addition to these strains designated as test microorganisms, it is further recommended to use strains that might contaminate the product and grow on or in it, depending on its characteristics. For the test microorganisms received from coordinated collections of microorganisms, one passage is defined as the transfer of microorganisms from an established culture to fresh medium, and microorganisms subjected to not more than five passages should be used for the tests. Single-strain challenges rather than mixed cultures should be used. The test strains can be harvested by growth on solid agar or liquid media.

Cultures on agar plate media: Inoculate each of the five test strains on the surface of agar plates or agar slants. For growth of bacteria, use Soybean-Casein Digest Agar Medium, and for yeasts and moulds, use Sabouraud Glucose Agar, Glucose-Peptone Agar or Potato Dextrose Agar Medium. Incubate bacterial cultures at 30°C to 35°C for 18 to 24 hours, the culture of *C. albicans* at 20°C to 25°C for 40 to 48 hours and the culture of *A. brasiliensis* at 20°C to 25°C for one week or until good sporulation is obtained. Harvest these cultured cells aseptically using a platinum loop, etc. Suspend the collected cells in sterile physiological saline or in 0.1% Peptone Water and adjust the viable cell count to about 10⁷ microorganisms per mL. In the case of *A. brasiliensis*, suspend the cultured cells in sterile physiological saline or 0.1% Peptone Water containing 0.05 w/v% of polysorbate 80 and adjust the spore count to about 10⁸ per mL. Use these suspensions as the inocula.

Liquid cultures: After culturing each of the four strains except for *A. brasiliensis* in a suitable medium, remove the medium by centrifugation. Wash the cells in sterile physiological saline or 0.1% Peptone Water and resuspend them in the same solution with the viable cell or spore count of the inoculum adjusted to about 10⁸ per mL.

When strains other than the five listed above are cultured, select a culture medium suitable for growth of the strain concerned. The cell suspension may also be prepared by a method suitable for that strain. Use the inoculum suspensions within 24 hours after they have been prepared from the cultivations on agar plate media or in liquid media. Store the inoculum suspensions in a refrigerator if it is not possible to inoculate them into the test specimens within 2 hours. Titrate the viable cell count of the inocula immediately before use, and then calculate the theoretical viable cell count per mL (g) of the product present just after inoculation.

3. Test Procedure

3.1. Category I products

Inject each of the cell suspensions aseptically into five containers containing the product and mix uniformly. When it is difficult to inject the cell suspension into the container aseptically or the volume of the product in each container is too small to be tested, transfer aseptically a sufficient volume of the product into each of alternative sterile containers, and mix the inoculum. When the product is not sterile, incubate additional containers containing the uninoculated product as controls and calculate their viable cell counts (the viable counts of bacteria and those of yeasts and moulds). A sterile syringe, spatula or glass rod may be used to mix the cell suspension uniformly in the product. The volume of the suspension mixed in the product must not exceed 1/100 of the volume of the product. Generally, the cell suspension is inoculated and mixed so that the concentration of viable cells is 10⁸ to 10⁹ cells per mL or per gram of the product. Incubate these inoculated containers at 20°C to 25°C with protection from light, and calculate the viable cell count of 1 mL or 1 g of the product taken at 0, 14 and 28 days subsequent to inoculation. Record any marked changes (e.g., changes in color or the development of a bad odor) when observed in the mixed samples during this time. Such changes should be considered when assessing the preservative efficacy of the product concerned. Express sequential changes in the viable counts as percentages, with the count at the start of the test taken as 100. Titration of the viable cell counts is based, in principle, on the Pour Plate Methods in “Microbial Limit Tests”.

In this case, confirm whether any antimicrobial substance is present in the test specimen. If a confirmed antimicrobial substance needs to be eliminated, incorporate an effective inactivator of the substance in the buffer solution or liquid medium to be used for dilution of the test specimen, as well as in the agar plate count medium. However, it is necessary to confirm that the inactivator has no effect on the growth of the microorganisms. When the occurrence of the preservative or the product itself affects titration of the viable cell count and there is no suitable inactivator available, calculate the viable cell counts by the Membrane Filtration Method in “Microbial Limit Tests”.

3.2. Category II products

The procedures are the same as those described for Category I products, but special procedures and considerations are required for both uniform dispersion of the test microorganism in the product and titration of viable cell counts in the samples.

For semisolid ointment bases, heat the sample to 45°C to 50°C until it becomes oily, add the cell suspension and disperse the inoculum uniformly with a sterile glass rod or spatula. Surfactants may also be added to achieve uniform
dispersion, but it is necessary to confirm that the surfactant added has no effect on survival or growth of the test microorganisms and that it does not potentiate the preservative efficacy of the product. For titration of the viable cell count, a surfactant or emulsifier may be added to disperse the product uniformly in the buffer solution or liquid medium. Sorbitan monooleate, polysorbate 80 or lecithin may be added to improve miscibility between the liquid medium and semisolid ointments or oils in which test microorganisms were inoculated. These agents serve to inactivate or neutralize many of the most commonly used preservatives.

**Table 1** Interpretation criteria by product category

<table>
<thead>
<tr>
<th>Product category</th>
<th>Microorganisms</th>
<th>Interpretation criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 14 days</td>
<td>After 28 days</td>
</tr>
<tr>
<td>Category IA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>0.1% of inoculum count or less</td>
<td>Same or less than level after 14 days</td>
</tr>
<tr>
<td>Yeasts/molds</td>
<td>Same or less than inoculum count</td>
<td>Same or less than inoculum count</td>
</tr>
<tr>
<td>Category IB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>1% of inoculum count or less</td>
<td>Same or less than level after 14 days</td>
</tr>
<tr>
<td>Yeasts/molds</td>
<td>Same or less than inoculum count</td>
<td>Same or less than inoculum count</td>
</tr>
<tr>
<td>Category IC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>10% of inoculum count or less</td>
<td>Same or less than level after 14 days</td>
</tr>
<tr>
<td>Yeasts/molds</td>
<td>Same or less than inoculum count</td>
<td>Same or less than inoculum count</td>
</tr>
<tr>
<td>Category II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>Same or less than inoculum count</td>
<td>Same or less than inoculum count</td>
</tr>
<tr>
<td>Yeasts/molds</td>
<td>Same or less than inoculum count</td>
<td>Same or less than inoculum count</td>
</tr>
</tbody>
</table>

4. **Interpretation**

Interpret the preservative efficacy of the product according to Table 1. When the results described in Table 1 are obtained, the product examined is considered to be effectively preserved. There is a strong possibility of massive microbial contamination having occurred when microorganisms other than the inoculated ones are found in the sterile product to be examined, and caution is required in the test procedures and/or the control of the manufacturing process of the product. When the contamination level in a nonsterile product to be examined exceeds the microbial enumeration limit specified in “Microbial Attributes of Nonsterile Pharmaceutical Products” in General Information, caution is also required in the test procedures and/or the control of the manufacturing process of the product.

5. **Culture Media**

Culture media and buffer solution used for Preservatives-

Effectiveness Tests are described below. Other media may be used if they have similar nutritive ingredients and selective and growth-promoting properties for the microorganisms to be tested.

(i) Soybean Casein Digest Agar Medium  
Casein peptone 15.0 g  
Soybean peptone 5.0 g  
Sodium chloride 5.0 g  
Agar 15.0 g  
Water 1000 mL  
Mix all of the components and sterilize at 121°C for 15–20 minutes in an autoclave. pH after sterilization: 7.1 – 7.3.

(ii) Sabouraud Glucose Agar Medium  
Pepitone (animal tissue and casein) 10.0 g  
Glucose 40.0 g  
Agar 15.0 g  
Water 1000 mL  
Mix all of the components and sterilize at 121°C for 15–20 minutes in an autoclave. pH after sterilization: 5.4 – 5.8.

(iii) Glucose Peptone (GP) Agar Medium  
Glucose 20.0 g  
Yeast extract 2.0 g  
Magnesium sulfate heptahydrate 0.5 g  
Pepitone 5.0 g  
Monobasic potassium phosphate 1.0 g  
Agar 15.0 g  
Water 1000 mL  
Mix all of the components and sterilize at 121°C for 15–20 minutes in an autoclave. pH after sterilization: 5.6 – 5.8.

(iv) Potato Dextrose Agar Medium  
Potato extract 4.0 g  
Glucose 20.0 g  
Agar 15.0 g  
Water 1000 mL  
Mix all of the components and sterilize at 121°C for 15–20 minutes in an autoclave. pH after sterilization: 5.4 – 5.8.

(v) 0.1% Peptone Water  
Pepitone 1.0 g  
Sodium chloride 8.0 g  
Water 1000 mL  
Mix all of the components and sterilize at 121°C for 15–20 minutes in an autoclave. pH after sterilization: 7.2 – 7.4.

**Rapid Counting of Microbes using Fluorescent Staining**

This chapter provides rapid methods using fluorescence staining for the quantitative estimation of viable microorganisms. Incubation on an agar medium has been widely used for quantitative estimation of viable microorganisms, but a number of environmental microorganisms of interest are not easy to grow in culture under usual conditions, thus new microbial detection methods based on fluorescence or luminescence have been developed. In the fluorescence staining method, microorganisms are stained with fluorescent dye, and can easily be detected and counted with various sorts of apparatus, such as a fluorescence microscope or flow cytometer. Methods are available to detect total.
microorganisms, including both dead and viable cells, or to detect only cells with a specified bioactivity by choosing the dye reagent appropriately. Nucleic acid staining reagents, which bind with DNA or RNA, detect all cells containing nucleic acids, whether they are live or dead. This technique is the most fundamental for the fluorescence staining method. On the other hand, fluorescent vital staining methods target the respiratory activity of the microorganism and the activity of esterase, which is present universally in microorganisms. In the microcolony method, microcolonies in the early stage of colony formation are counted. The CFDA-DAPI double staining method and the microcolony method are described below. These methods can give higher counts than the other techniques, because these rapid and accurate techniques provide quantitative estimation of viable microorganisms based on a very specific definition of viability, which may be different from that implicit in other methods. The procedures of these methods described here may be changed as experience with the methods is accumulated. Therefore, other reagents, instruments and apparatus than those described here may also be used if there is a valid reason for so doing.

1. CFDA-DAPI double staining method

Fluorescein diacetate (FDA) reagents are generally used for the detection of microorganisms possessing esterase activity. These reagents are hydrolyzed by intracellular esterase, and the hydrolyzed dye exhibits green fluorescence under blue excitation light (about 490 nm). Modified FDAs such as carboxyfluorescein diacetate (CFDA) are used because of the low stainability of gram-negative bacteria with FDA. The principle of the CFDA-DAPI double staining method, which also employs a nucleic acid staining reagent, 4',6-diamidino-2-phenylindole (DAPI), is as follows. The nonpolar CFDA penetrates into the cells and is hydrolyzed to fluorescent carboxyfluorescein by intracellular esterase. The carboxyfluorescein is accumulated in the living cells due to its polarity, and therefore green fluorescence due to carboxyfluorescein occurs when cells possessing esterase activity are illuminated with blue excitation light. No fluorescent carboxyfluorescein is produced with dead cells, since they are unable to hydrolyze CFDA. On the other hand, DAPI binds preferentially to the adenine and thymine of DNA after penetration into both viable and dead microorganisms, and consequently all of the organisms containing DNA exhibit blue fluorescence under ultraviolet excitation light. Therefore, this double staining method enables to count specifically only live microorganisms possessing esterase activity under blue excitation light, and also to determine the total microbial count (viable and dead microorganisms) under ultraviolet excitation light.

1.1. Apparatus
1.1.1. Fluorescence microscope or fluorescence observation apparatus

Various types of apparatus for counting fluorescently stained microorganisms are available. Appropriate filters are provided, depending on the fluorescent dye reagents used. A fluorescence microscope, laser microscope, flow cytometer, and various other types of apparatus may be used for fluorescence observation.

1.2. Instruments
   (i) Filtering equipment (funnels, suction flasks, suction pumps)
   (ii) Membrane filters (poresize: 0.2 μm); A suitable filter that can trap particles on the surface can be used such as polycarbonate filter, alumina filter, etc.
   (iii) Glass slide
   (iv) Cover glass
   (v) Ocular micrometer for counting (with 10 × 10 grids)

1.3. Procedure

An example of the procedure using fluorescence microscope is described below.

1.3.1. Preparation of samples

Prepare samples by ensuring that microbes are dispersed evenly in the liquid (water or buffer solution).

1.3.2. Filtration

Set a membrane filter (poresize: 0.2 μm) on the funnel of the filtering equipment. Filter an appropriate amount of sample to trap microbes in the sample on the filter.

1.3.3. Staining

Pour sufficient amount of buffer solution for CFDA staining, mixed to provide final concentration of 150 μg/mL of CFDA and 1 μg/mL of DAPI, into the funnel of the filtering equipment and allow staining in room temperature for 3 minutes, then filter the liquid by suction. Pour in sufficient amount of aseptic water, suction filter and remove excess fluorescent reagent left on the filter. Thoroughly dry the filter.

1.3.4. Slide preparation

Put one drop of immersion oil for fluorescence microscope on the glass slide. Place the air dried filter over it, with the filtering side on the top. Then put one drop of immersion oil for fluorescence microscope on the surface of the filter, place a cover glass. Put another drop of immersion oil for fluorescence microscope on the cover glass when using an oilimmersion objective lens.

1.3.5. Counting

Observe and count under fluorescence microscope, with 1000 magnification. In case of CFDA-DAPI double staining method, count the microorganisms (with esterase activity) exhibiting green fluorescence under the blue excitation light first to avoid color fading by the ultraviolet light, then count the microorganisms (with DNA) exhibiting blue fluorescence under the ultraviolet excitation light in the same microscopic field. Count the organisms exhibiting fluorescence on more than 20 randomly selected fields among 100 grids observed through an ocular micrometer of the microscope, and calculate the total number of organisms using the following formula. The area of the microscopic field should be previously determined with the ocular and objective micrometers. The amount of the sample to be filtered must be adjusted so that the cell number per field is between 10 and 100. It might be necessary to reprepare the sample in certain instances. (In such case that the average count number is not more than 2 organisms per field, or where more than 5 fields are found which have no organism per field, it is assumed that the microorganism count is below the detection limit.)

\[
\text{Number of microbes (cells/mL)} = \frac{(\text{average number of microbes per visual field}) \times (\text{area of filtration})}{(\text{amount of sample filtered}) \times (\text{area of one microscopic field})}
\]

1.4. Reagents and test solutions

   (i) Aseptic water: Filter water through a membrane filter with 0.2 μm pore size to remove particles, then sterilize it by heating in an autoclave at 121°C for 15 minutes. Water for injection may be used.
2.2. Instruments

Other types of apparatus may be used for fluorescence microscopy. A fluorescence microscope, laser microscope and various types of counting apparatus are available. Various nucleic acid staining reagents can be used for staining microorganisms, then observed and counted under fluorescence microscope or other suitable systems. By this method, even colonies which are undetectable with the naked eye can be identified, so viable microorganisms can be counted rapidly and with high precision. This method enables to count the number of proliferative microorganisms, with short incubation time. In this method, the organisms are trapped on a membrane filter, the filter is incubated on a medium for a short time, and the microcolonies are counted. By this method, even colonies which are undetectable with the naked eye can be identified, so viable organisms can be counted rapidly and with high precision.

2.3. Microcolony method

Microcolonies, which are in early stages of colony formation, are fluorescently stained, then observed and counted under fluorescence microscope or other suitable systems. This method enables to count the number of proliferative microorganisms, with short incubation time. In this method, the organisms are trapped on a membrane filter, the filter is incubated on a medium for a short time, and the microcolonies are counted. By this method, even colonies which are undetectable with the naked eye can be identified, so viable organisms can be counted rapidly and with high precision.

2.3.1. Preparation of samples

Prepare samples by ensuring that microbes are dispersed evenly in the liquid (water or buffer solution).

2.3.2. Filtration

Set a membrane filter (pore size: 0.2 μm) on the funnel of the filtering equipment. Filter an appropriate amount of sample to trap microbes in the sample on the filter.

2.3.3. Incubation

Remove the filter from the filtering equipment and place it with filtering side facing up on a culture medium avoiding formation of air bubbles between the filter and the medium. Incubate at a suitable temperature for appropriate hours in a dark place. It should be noted that the appropriate incubation conditions (such as medium, incubation temperature and/or incubation time) are different, depending on the sample.

2.3.4. Fixation

Soak filter paper with an appropriate amount of neutral buffered formaldehyde test solution, then place the filter that has been removed from the culture medium on top with filtering side up, and allow to remain at room temperature for more than 30 minutes to fix the microcolonies.

2.3.5. Staining

Soak filter paper with an appropriate amount of staining solution (such as 1 μg/mL of DAPI, 2% polyoxyethylene sorbitan monolaurate), then place the filter on top with filtering side up, and then leave at room temperature, light shielded for 10 minutes to stain microcolonies. Wash the filter by placing it with the filtering side facing up on top of a filter paper soaked with aseptic water for 1 minute. Thoroughly air dry the filter.

2.3.6. Slide preparation

Put one drop of immersion oil for fluorescence microscope on the slide glass. Place an air dried filter over it, with the filtering side on the top. Then, put one drop of immersion oil for fluorescence microscope on top, place a cover glass.

2.3.7. Counting

Count the organisms exhibiting fluorescence on more than 20 randomly selected fields among the 100 grids observed through an ocular micrometer of the microscope with 400 or 200 magnification, and calculate the total number of organisms using the following formula. The area of the microscopic field should be previously determined with the ocular and objective micrometers. In such case that the average count number is not more than 2 microcolonies per field, or where more than 5 fields are found which have no microcolony per field, it is assumed that the microorganism count is below the detection limit.

\[
\text{Number of microcolonies (cells/mL)} = \frac{\text{(average number of microcolonies per visual field) \times (area of one microscopic field)}}{\text{(amount of sample filtered) \times (area of filtration)}}
\]

2.4. Reagents and test solutions

(i) Aseptic water: Filter water through a membrane filter with 0.2 μm pore size to remove particles, and sterilize it by heating in an autoclave at 121°C for 15 minutes. Water for injection may be used.

(ii) Staining solution: Dissolve 10 mg of DAPI in 100 mL of aseptic water. Dilute the solution 10 times with aseptic water and filter through a membrane filter with pore size of 0.2 μm. Store at 4°C in light shielded condition. Dissolve polyoxyethylene sorbitan monolaurate to the final concentration of 2%, when using.

(iii) Neutral buffered formaldehyde solution (4w/v% formaldehyde solution; neutrally buffered).

(iv) Immersion oil for fluorescence microscope
Rapid Identification of Microorganisms Based on Molecular Biological Method

This chapter describes the methods for the identification or estimation of microorganisms (bacteria and fungi), found in in-process control tests or lot release tests of pharmaceutical products, at the species or genus level based on their DNA sequence homology. The identification of isolates found in the sterility test or aseptic processing can be helpful for investigating the causes of contamination. Furthermore, information on microorganisms found in raw materials used for pharmaceutical products, processing areas of pharmaceutical products, and so on is useful in designing measures to control the microbiological quality of drugs. For the identification of microorganisms, phenotypic analysis is widely used, based on morphological, physiological, and biochemical features and analysis of components. Commercial kits based on differences in phenotype patterns have been used for the identification of microorganisms, but are not always applicable to microorganisms found in raw materials used for pharmaceutical products and in processing areas of pharmaceutical products. In general, the identification of microorganisms based on phenotypic analysis needs special knowledge and judgment is often subjective. It is considered that the evolutionary history of microorganisms (bacteria and fungi) is memorized in their ribosomal RNAs (rRNAs), so that systematic classification and identification of microorganisms in recent years have been based on the analysis of these sequences. This chapter presents a rapid method to identify or estimate microorganisms based on partial sequences of divergent regions of the 16S rRNA gene for bacteria and of the internal transcribed spacer 1 (ITS1) region located between 18S rRNA and 5.8S rRNA for fungi, followed by comparison of the sequences with those in the database. Methods described in this chapter do not take the place of usual other methods for the identification, and can be modified based on the examiner’s experience, and on the available equipment or materials. Other gene regions besides those mentioned in this chapter can be used if appropriate.

1. Apparatuses
   - (i) DNA sequencer
   - Various types of sequencers used a gel board or capillary can be used.
   - (ii) DNA amplifier
   - To amplify target DNA and label amplified (PCR) products with sequencing reagents.

2. Procedures
   The following procedures are described as an example.

2.1 Preparation of template DNA
   - It is important to use a pure cultivated bacterium or fungus for identification. In the case of colony samples, colonies are picked up with a sterilized toothpick (in the case of fungi, a small fragment of colony sample is picked up), and suspended in 0.3 mL of DNA releasing solution in a 1.5 mL centrifuge tube. In the case of culture fluid, a 0.5 mL portion of fluid is put in a 1.5 mL centrifuge tube and centrifuged at 10,000 rpm for 10 min. After removal of the supernatant, the pellet is suspended in 0.3 mL of DNA releasing solution, and then heated at 100°C for 10 min. In general, PCR can be run for bacteria and yeasts heated in DNA releasing solution. For fungi, DNA extraction from culture fluid is better because some of colony samples can disturb PCR reaction.

2.2 PCR
   - Add 2 μL of template DNA in PCR reaction solution. Use 10F/800R primers (or 800F/1500R primers in the case to analyze also a latter part of 16S rRNA) for bacteria and ITS1F/ITS1R primers for fungi, and then perform 30 amplification cycles at 94°C for 30 sec, 55°C for 60 sec, and 72°C for 60 sec. DNA fragments are amplified about 800 bp in the case of bacteria and about 150 – 470 bp depending on the strain in the case of fungi. Include a negative control (water instead of the test solution) in the PCR.

2.3 Confirmation of PCR products
   - Mix 5 μL of PCR product with 1 μL of loading buffer solution, place it in a 1.5 w/v% agarose gel well, and carry out electrophoresis with TAE buffer solution (1-fold concentration). Carry out the electrophoresis together with appropriate DNA size markers. After the electrophoresis, observe PCR products on a trans-illuminator (312 nm) and confirm the presence of a single band of the targeted size. If multiple bands are observed, cut the targeted band out of the gel, and extract DNA by using appropriate commercial DNA extraction kit.

2.4 Purification of PCR products
   - Remove unincorporated PCR primers and deoxynucleoside triphosphates (dNTP) from PCR products by using appropriate purification methods.

2.5 Quantification of purified DNA
   - When purified DNA is measured by spectrophotometer, calculate 1 OD260 nm as 50 μg/mL.

2.6 Labeling of PCR products with sequencing reagents
   - Use an appropriate fluorescence-labeled sequencing reagent suitable for the available DNA sequencer or its program and label the PCR products according to the instructions provided with the reagent.

2.7 Purification of sequencing reagent-labeled PCR products
   - Transfer the product in 75 μL of diluted ethanol (7 in 10) into a 1.5 mL centrifuge tube, keep in an ice bath for 20 min, and centrifugate at 15,000 rpm for 20 min. After removal of supernatant, add 250 μL of diluted ethanol (7 in 10) to the precipitate and centrifugate at 15,000 rpm for 5 min. Remove the supernatant and dry the precipitate.

2.8 DNA homology analysis
   - Place sequencing reagent-labeled PCR products in the DNA sequencer and read the nucleotide sequences of the PCR products. Compare the partial nucleotide sequence with those in the BLAST database.

3. Judgment
   - If sequencing data show over 90% identity with a sequence in the database, in general, judgment may be made as follows.
     - (i) In the case of bacteria, compare the nucleotides in the product obtained with the 10F primer (the 800F primer when 800F/1500R primers are used) with the BLAST database. Higher ranked species are judged as identified species or closely related species.
     - (ii) In the case of fungi, compare sequencing data for the product obtained with the ITS1F primer with the...
4. Reagents, Test Solutions

(i) 0.5 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS: Dissolve 18.6 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 100 mL.

(ii) 1 mol/L Tris buffer solution, pH 8.0: Dissolve 24.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in a suitable amount of water, adjust the pH to 8.0 with 0.2 mol/L hydrochloric acid TS, and add water to make 200 mL.

(iii) TE buffer solution: Mix 1.0 mL of 1 mol/L tris buffer solution, pH 8.0 and 0.2 mL of 0.5 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, and add water to make 100 mL.

(iv) DNA releasing solution: Divide TE buffer solution containing 1 vol% of polyoxyethylene (10) octylphenyl ether into small amounts and store frozen until use.

(v) PCR reaction solution

10-fold buffer solution* 5 μL
dNTP mixture** 4 μL
10 μmol/L Sense primer 1 μL
10 μmol/L Anti-sense primer 1 μL
Heat-resistant DNA polymerase (1 U/μL) 1 μL
Water 36 μL

* Being composed of 100 mmol/L 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride, pH 8.4, 500 mmol/L potassium chloride, 20 mmol/L magnesium chloride and 0.1 g/L gelatin.

** A solution containing 2.5 mmol/L each of dGTP (sodium 2'-deoxyguanosine 5'-triphosphate), dATP (sodium 2'-deoxycytidine 5'-triphosphate), dCTP (sodium 2'-deoxythymidine 5'-triphosphate) and dTTP (sodium 2'-deoxythymidine 5'-triphosphate). Adequate products containing these components as described above may be used.

(vi) Sequencing reagent: There are many kinds of sequencing methods, such as the dye-primer method for labeling of dNTP terminators, primer, the dye-terminator method for labeling of dNTP terminators, and so on. Use an appropriate sequencing reagent kit for the apparatus and program to be used.

(vii) 50-Fold concentrated TAE buffer solution: Dissolve 242 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 57.1 mL of acetic acid (100) and 100 mL of 0.5 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, and add water to make 1000 mL.

(viii) 1-Fold concentrated TAE buffer solution: Dilute 50-fold concentrated TAE buffer solution (1 in 50) prepared before use is referred to as 1-fold concentrated TAE buffer solution.

(ix) Agarose gel: Mix 1.5 g of agarose, 2.0 mL of 50-fold concentrated TAE buffer solution, 10 μL of a solution of ethidium bromide (3,8-diamo-5-ethyl-6-phenylparan-thridinium bromide) (1 in 100) and 100 mL of water. After dissolving the materials by heating, cool the solution to about 60℃, and prepare gels.

(x) Loading buffer solution (6-fold concentrated): Dissolve 0.25 g of bromophenol blue, 0.25 g of xylene cyanol FF and 1.63 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 50 mL of water, and add 30 mL of glycerol and water to make 100 mL.

(xi) PCR primers

Sterility Assurance for Terminally Sterilized Pharmaceutical Products

As indicated in the “Terminal Sterilization and Sterilization Indicators”, the pharmaceuticals to which terminal sterilization can be applied, generally must be sterilized so that a sterility assurance level of 10^-6 or less is obtained. The sterility assurance level of 10^-6 or less can be proven by using a sterilization process validation based on physical and microbiological methods, but cannot be proven by sterility tests of the sterilized products. This chapter deals with the necessary requirements for the appropriate management of the important control points of the sterilization process for the parametric release of products, without performing sterility tests on products which have been subjected to terminal sterilization (in the case of radiation sterilization, called dosimetric release). Parametric release is a method that can be applied in cases where the sterilization system is clearly defined, important control points are clearly specified, and the sterilization system process can be validated by microbiological methods using appropriate biological indicators.

1. Definitions

The definitions of the terminology used in this chapter are provided below.

1.1. Terminal sterilization

A process whereby a product is sterilized in its final container or packaging, and which permits the measurement and evaluation of quantifiable microbial lethality.

1.2. Validation

A documented procedure for obtaining, recording and interpreting the results needed to show that a process will consistently yield a product complying with predetermined specifications.

1.3. Periodic re-validation

Validation that is regularly performed to reconfirm that a process is consistently yielding a product complying with predetermined specifications. It should confirm that variables and the acceptable ranges are permissible to yield a product consistently of the required quality.

1.4. Facility/equipment qualification

This is to provide evidence that the manufacturing facilities/equipment, measuring equipment, and manufacture environment control facilities, etc. have been properly selected, correctly installed, and are operated in conformity with the specifications at the time of installation and during operation.
1.5. Operation qualification

This is to provide evidence to confirm physically, chemically and microbiologically that equipment, operated in accordance with its operational instructions, operates as specified and affords a product meeting the specifications.

1.6. Support system for sterilization process

This refers to the facility/equipment that is associated with the sterilization devices, such as the preconditioning and aeration for ethylene oxide sterilization, the steam supply equipment for moist heat sterilization, and the loading devices for radiation sterilization.

1.7. Quality system

The procedures, resources and organizational structure of a manufacturer (responsibilities, authorities and relationships between these) required to implement quality management.

1.8. Change control system

A system designed to evaluate all of the changes that may affect the quality of the pharmaceutical product, in order to ensure that the process is continuously controlled.

1.9. $F_0$ value

Assume a value of 10°C for the Z value defined as the number of degrees of temperature required for a 10-fold change in the D value. The $F_0$ value indicates the time (minutes) required to give the equivalent lethality at $T_b$ of the sterilization heat obtained by integrating the lethality rate ($L$) over an entire heating cycle.

$$L = \log^{-1} \left( \frac{T_b - T_0}{Z} \right) = 10^{\frac{T_b - T_0}{Z}}$$

$T_0$ = Temperature inside the chamber or inside the product to be sterilized
$T_b$ = Reference temperature (121°C)
$F_0 = \int_{T_0}^{T_b} Ldt$

1.10. Control device

A general term for the devices and measurement equipment, including the equipment for controlling, measuring and recording the physical parameters that can be measured (temperature, humidity, pressure, time, radiation dose, etc.).

1.11. Parametric release

A release procedure based on an evaluation of the production records and critical parameters of the sterilization process (temperature, humidity, pressure, time, radiation dose, etc.) based on the results of validation, in lieu of release based on testing results of the final product.

2. Sterilization Validation

2.1. Subject of the Implementation

A manufacturer of sterile pharmaceuticals (hereafter, “manufacturer”) must establish a quality system, implement product sterilization validation for the categories below as a general rule, and continuously control the sterilization process based on the results of the sterilization validation.

a) Sterilization process
b) Sterilization process support system

2.2. Documenting Sterilization Validation Procedure

2.2.1. The manufacturer must prepare a “Sterilization Validation Procedure” defining the items listed below regarding the procedures for managing the sterilization process.

a) Details related to the range of duties of the persons responsible for the validation, as well as the extent of their authority
b) Details related to the implementation period for the sterilization validation
c) Details related to the creation, modification, and approval of the sterilization validation plan documents
d) Details related to the reporting, evaluation, and approval of the sterilization validation implementation results
e) Details related to the storage of documentation concerning the sterilization validation
f) Other required matters

2.2.2. The sterilization validation procedure must list the names of the enactors, the date of enactment, and when there are revisions, must also list the reviewers, date of revisions, revised sections and reasons for the revisions.

2.2.3. The manufacturer must properly store and maintain the sterilization validation procedure after clarifying the procedures related to alterations and deletions of the contents of the sterilization validation procedure.

2.3. Persons Responsible for the Validation

The manufacturer must assign persons to be responsible for the sterilization validation. The responsible parties must perform each of the duties listed below according to the sterilization validation procedure.

2.3.1. For products that are to be produced according to the sterilization validation procedure, a written sterilization validation implementation plan must be prepared. The implementation plan will specify the following points based on a consideration of the implementation details of the sterilization validation.

a) Subject pharmaceutical name (product name)
b) Purpose of the applicable sterilization validation
c) Expected results
d) Verification methods (including inspection results and evaluation methods)
e) Period of verification implementation
f) Names of persons performing the sterilization validation (persons-in-charge)
g) Names of the persons who created the plan, creation date, and in the event of revisions, the names of the revisers, date of the revisions, revised sections, and reasons for revision.
h) Technical requirements for the applicable sterilization validation
i) Other required matters for the applicable sterilization validation

2.3.2. The following sterilization validation is implemented according to the plan defining the items above.

a) When the manufacturing license and additional (modification) licenses for product production are obtained, implementation items for the sterilization validation to be executed are:

1) Product qualification
2) Facility/equipment qualification
   1) Installation qualification
   2) Operation qualification
3) Performance qualification
   1) Physical performance qualification
   2) Microbiological performance qualification
b) Sterilization validation to be executed until it is time to renew the manufacturing license.
1. Re-validation when there are changes
2. Periodic re-validation (The items implemented, etc. must be determined based on a consideration of relevant factors such as the sterilization method.)

2.3.3. Evaluate the results of the sterilization validation and verify that sterility is assured.

2.3.4. Make a written report of the results of the sterilization validation to the manufacturer’s authorized person.

2.3.5. Perform the day-to-day management of the sterilization process.

3. Microorganism Control Program

When parametric release is adopted, it is important to control the bioburden in the raw materials of the product, the containers and stoppers, and in the product before sterilization. The bioburden is measured with a previously specified method and frequency, and when required, surveys of the characteristics of the isolated microorganisms are made to investigate their resistance to the applicable sterilization method. Refer to the “Microbiological Evaluation of Process Areas for Sterile Pharmaceutical Products” regarding the method for evaluating the environmental microorganisms in the processing areas of pharmaceutical products.

4. Sterilization Indicators

Biological indicators (BI), chemical indicators (CI), and dosimeters are among the means used to monitor a sterilization process and as indices of sterility (refer to Terminal Sterilization and Sterilization Indicators). When using sterilization indicators it is important to consider environmental and human safety, and to take all necessary precautions. The BI used for sterilization validation and daily process control must be defined in the specification, and recorded in writing. When BI are used for daily process control it must be verified that the loading pattern on the form, product, or simulated product has a resistance equal to or greater than that used for the microbiological performance qualification.

5. Establishment of a Change Control System

Changes which have a large effect on the sterile quality, such as changes in sterilization equipment, loading pattern, and sterilization conditions, correspond to changes of the parametric release conditions for the relevant pharmaceutical product. A change control system must be defined in the sterilization validation procedure; and when there are changes in the causes of variation that have been previously specified, there must be an investigation of the causes of variation and of acceptable conditions to verify that the pharmaceutical product is guaranteed always to conform to the quality standards. Furthermore, before modifications are made to a sterilization process that has been validated, it is mandatory to obtain approval for the implementation of the modifications in question from the appropriate authorized person.

6. Release Procedure

A release procedure must be created to clarify the conditions required for shipment based on parametric release of terminally sterilized products. The following points must be evaluated and recorded when a product is released.

Depending on the sterilization method, some of these items may be omitted or modified.

a) Batch record
b) Microorganism evaluation data of production environment
c) Bioburden data for the raw materials and product before sterilization
d) Data related to the sterilization indicators
e) Data on the maintenance management of the sterilization process and sterilization process support systems
f) Data on the management of sterilization parameters
g) Data on the calibrations of measurement equipment
h) Re-validation data
i) Other

7. Critical Control Points

The important control points for each sterilization method are presented.

7.1. Moist heat sterilization

Moist heat sterilization is a method for killing microorganisms in which saturated water vapor is generated or introduced into a sterilization chamber at the appropriate temperature and pressure, and the chamber is then heated for a certain period of time. It is roughly classified into saturated vapor sterilization, in which the target microorganisms are directly exposed to the saturated vapor, and unsaturated vapor sterilization, in which the fluid inside a container, such as an ampule, is subjected to moist heat energy or high-frequency energy from the outside.

7.1.1. Important control points

A process control procedure must be created, specifying the process parameters that affect the sterile quality of the pharmaceutical product, and the permissible range of variation for each parameter. The important control points for the moist heat sterilization are indicated below.

a) Heating history (usually indicated by $F_0$ value)
b) Temperature
c) Pressure
d) Time
e) Product loading format/loading density
f) Other necessary matters

7.1.2. Utilities

The utilities and control devices required for moist heat sterilization determine the quality and precision.

a) Quality of the vapor used
b) Quality of the air introduced into the sterilization chamber to restore pressure, etc.
c) Quality of the water used for cooling
d) Precision of the temperature control devices
e) Precision of the pressure control devices
f) Precision of the time control devices

7.2. Ethylene oxide gas sterilization

Ethylene oxide gas allows sterilization at low temperatures, so there is typically little injury to the substance being sterilized; however, since the gas is toxic it must be handled with extreme caution. The sterilization process consists of preconditioning, a sterilization cycle, and aeration. The preconditioning is performed before the sterilization cycle to process the product so that temperature and relative humidity in the room or container are within the range in the specifications. The sterilization cycle indicates the stage at which the actual sterilization is performed, and consists of removal of the air, conditioning (when used), injection of the sterilization gas, maintenance of the sterilization conditions, removal of the sterilization gas, and replacement of the air. The aeration is the process of eliminating the residual ethylene oxide gas from the product, either inside the steril...
zation chamber or in a separate location.

7.2.1. Important control points

The important control points for the ethylene oxide gas sterilization are indicated below.

7.2.1.1. Preconditioning (when performed)

a) Time, temperature, humidity
b) Product loading pattern/loading density
c) Sterilization loading temperature and/or humidity
d) Time from the end of preconditioning until the start of the sterilization
e) Other necessary matters

7.2.1.2. Conditioning

a) If pressure reduction is performed, the pressure achieved and required time
b) Reduced pressure maintenance period
c) Time, temperature, pressure, humidity
d) Sterilization loading temperature and humidity
e) Other necessary matters

7.2.1.3. Sterilization cycle

a) Pressure increase, injection time, and final pressure for the injection of the sterilization gas
b) Concentration of the ethylene oxide gas (it is desirable to analyze directly the gas concentration inside the sterilization chamber, but the following alternatives are acceptable if direct analysis is difficult)
i) Mass of gas used
ii) Volume of gas used
iii) Conversion calculation using the initial low pressure level and the gas injection pressure
c) Temperature within the sterilization chamber
d) Temperature of the loaded products to be sterilized
e) Effect time (exposure time)
f) Product loading pattern/loading density
g) BI placement points and cultivation results
h) Other necessary matters

7.2.1.4. Aeration

a) Time, temperature
b) Loaded sterilized substance temperature
c) Pressure variation in the sterilization chamber and/or the aeration room
d) Rate of change of the air or other gases in the aeration room
e) Other necessary matters

7.2.2. Utilities

The utilities and control devices required for ethylene oxide sterilization determine the quality and precision.

a) Quality of the ethylene oxide gas
b) Quality of the injected vapor or water
c) Quality of the replacement air after the completion of sterilization
d) Quality of the BI
e) Precision of the temperature control devices
f) Precision of the pressure control devices
g) Precision of the humidity control devices
h) Precision of the time control devices
i) Other

7.3. Irradiation Sterilization

Irradiation sterilization refers to methods of killing microorganisms through exposure to ionizing radiation. The types of ionizing radiation used are gamma-rays (γ-rays) emitted from a radioisotope such as 60Co or 137Cs, or electron beams and bremsstrahlung (X-ray) generated from an electron accelerator. In the case of γ-rays, the cells are killed by secondarily generated electrons, while in the case of the electron beam, the cells are killed by the electrons generated directly from the electron accelerator. For this reason, the processing time for electron beam sterilization is generally shorter than that for γ-ray sterilization; but, since the penetration of the γ-rays is better than that of the electron beam, there must be appropriate consideration of the density and thickness of the substance being sterilized when choosing between these methods. For an irradiation sterilization process, the control procedures primarily make use of dosimeters and measure the absorbed dose in the substance being sterilized. This is called dosimetric release.

7.3.1. Important control points

The important control points for the irradiation sterilization are indicated below.

7.3.1.1. γ-ray radiation

a) Irradiation time (timer setting or conveyor speed)
b) Absorbed dose
c) Product loading pattern
d) Other necessary matters

7.3.1.2. Electron beam and X-ray radiation

a) Electron beam characteristics (average electron beam current, electron energy, scan width)
b) Conveyor speed
c) Absorbed dose
d) Product loading pattern
e) Other necessary matters

7.3.2. Utilities

A traceable calibration, performed according to national standards, must be performed for the radiation devices and dose measurement systems. This calibration must be performed as specified in a written plan in order to verify that the equipment is kept within the required range of accuracy.

7.3.2.1. Required calibration items for gamma-radiation equipment

a) Cycle time or conveyor speed
b) Weighing device
c) Dose measurement system
d) Other

7.3.2.2. Required calibration items for electron-beam and X-ray radiation equipment

a) Electron beam characteristics
b) Conveyor speed
c) Weighing device
d) Dose measurement system
e) Other

References

1) Validation Standards, PAB Notification No.158, Ministry of Health and Welfare 1995
2) Sterilization Validation Standards, PMSB/IGD Notification No.1, Ministry of Health and Welfare 1997
4) ISO 9000 series, International Standards for Quality Assurance
5) ISO 11134 Industrial moist heat sterilization
6) ISO 11135 Ethylene oxide sterilization
7) ISO 11137 Radiation sterilization
8) ISO 11138 Biological indicators
9) ISO 11140 Chemical indicators
10) ISO 11173-1 Microbiological Methods Part 1: Estima-
Terminal Sterilization and Sterilization Indicators

Sterilization is a process whereby the killing or removal of all forms of viable microorganisms in substances is accomplished. It is achieved by terminal sterilization or a filtration method. For substances to which terminal sterilization can be applied, an appropriate sterilization method should be selected in accordance with the properties of the product, including the packaging, after full consideration of the advantages and disadvantages of each sterilization method, from among the heat method, irradiation method, and gas method. After installation of the sterilizer (including design and development of the sterilization process), validation is required to confirm that the sterilization process is properly performing its designed function, under conditions of loading and unloading of the product, on the basis of sufficient scientific evidence. After the process has been validated and the sterilization of the product commenced, the process must be controlled correctly, and qualification tests of the equipment and procedures must be performed regularly.

The bioburden per product, prior to terminal sterilization, must be evaluated periodically or on the basis of batches. Refer to the ISO standard (ISO 11137-1) relevant to bioburden estimation. For a substance to which terminal sterilization can be applied, generally use sterilization conditions such that a sterility assurance level of less than 10^{-6} can be obtained. The propriety of the sterilization should be judged by employing an appropriate sterilization process control, with the use of a suitable sterilization indicator, and if necessary, based on the result of the sterility test. The filtration procedure is used for the sterilization of a liquid product, to which terminal sterilization can not be applied. Concerning the disinfection and/or sterilization necessary for processing equipment and areas of pharmaceutical products, and performing microbiological tests specified in the monographs, see Disinfection and Sterilization Methods.

1. Definitions

The definitions of the terms used in this text are as follows:

(i) Terminal sterilization: A process whereby a product is sterilized in its final container or packaging, and which permits the measurement and evaluation of quantifiable microbial lethality.

(ii) Product: A generic term used to describe raw materials, intermediate products, and finished products, to be sterilized.

(iii) Bioburden: Numbers and types of viable microorganisms in a product to be sterilized.

(iv) Sterility assurance level (SAL): Probability of a viable microorganism being present in a product unit after exposure to the proper sterilization process, expressed as 10^{-n}.

(v) Integrity test: A non-destructive test which is used to predict the functional performance of a filter instead of the microorganism challenge test.

(vi) D value: The value which shows the exposure time (decimal reduction time) or absorbed dose (decimal reduction dose) required to cause a 1-logarithm or 90% reduction in the population of test microorganisms under stated exposure conditions.

(vii) Sterilization indicator: Indicators used to monitor the sterilization process, or as an index of sterility, including biological indicators (BI), chemical indicators (CI), dosimeters and the like.

2. Sterilization

2.1. Heat Method

In the heat method, microorganisms are killed by heating.

2.1.1. Moist heat method

Microorganisms are killed in saturated steam under pressure. In this method, factors which may affect the sterilization include temperature, steam pressure and exposure time. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature, steam pressure and exposure time, and they should be included in the specifications of the sterilizer.

2.1.2. Dry-heat method

Microorganisms are killed in dry heated air. This method is usually conducted in a batch-type dry heat sterilizer or a tunnel-type dry heat sterilizer. In this method, factors which may affect the sterilization include temperature and exposure time, and they should be included in the specifications of the sterilizer.

2.2. Irradiation method

Microorganisms are directly killed by ionizing radiation, or by the heat generated by microwave radiation.

2.2.1. Radiation method

Ionizing radiations which may be used are gamma (\(\gamma\)) rays emitted from a radioisotope such as cobalt 60, an electron beam and bremsstrahlung (X rays) generated from an electron accelerator. Although any procedure can be applied to thermally unstable products with no radioactivity residue, it is necessary to consider the possibility of material degradation. Although a 25 kGy dose is traditionally used as a sterilization dose, there are some ways to calculate the dose as follows: the bioburden of the substance to be sterilized is measured and the sterilization dose is calculated based on the mean bioburden and the standard resistance distribution (Method 1 in ISO 11137), the dose is calculated based on the bioburden and D value of the most resistant microorganisms (Log method) (see 5.3.). In the case of the radiation sterilization procedure, factors which may affect the sterilization include dose (absorbed dose) and exposure time. Therefore, in \(\gamma\) ray sterilization process control, it is required to determine the dose (the absorbed dose) at appropriate intervals and to monitor continuously the exposure time in terms of the operating parameters (the conveyor speed, the cycle time). The dose control mechanism should be included in the specifications of the sterilizer. In the case of electron beam or bremsstrahlung irradiation, it is required to monitor the acceleration voltage, the beam current and beam scanning width besides the above-mentioned items.

2.2.2. Microwave method

Microorganisms are killed by the heat generated by micro-
wave radiation, usually at the frequency of 2450 ± 50 MHz. This method is applied to liquids or water-rich products in sealed containers. Since a glass or plastic container may be destroyed or deformed due to the rise of the inner pressure, the containers must be certified to be able to withstand the heat and the inner pressure generated during microwave sterilization. Leakage of electromagnetic radiation must be at a sufficiently low level to cause no harm to humans and no interference with radio communications and the like. In this method, factors which may affect the sterilization include temperature, processing time and microwave output power. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature, gas concentration (pressure), humidity and exposure time. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature, time and the microwave output power, and they should be included in the specifications of the sterilizer.

2.3. Gas method

Ethylene oxide (EO) is widely used as a sterilization gas. Since EO gas has an explosive nature, a 10 - 30% mixture with carbon dioxide is commonly used. Also, as EO gas is a strong alkylating agent, it cannot be applied to the products which are likely to react with or absorb it. Furthermore, because EO gas is toxic, the residual concentration of EO gas and other secondarily generated toxic gases in products sterilized with EO gas must be reduced to less than the safe levels thereof by means of aeration and the like before the product is shipped. In this method, factors which may affect the sterilization include temperature, gas concentration (pressure), humidity and exposure time. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature, gas concentration (pressure), humidity and exposure time, and they should be included in the specifications of the sterilizer.

3. Filtration method

Microorganisms are removed by using a sterilizing filter made of an appropriate material. However, this method is not intended for microorganisms smaller than bacteria. Generally, a sterilizing filter challenged with more than 10^7 microorganisms of a strain of Brevundimonas diminuta (ATCC 19146, NBRC 14213, JCM 2428), cultured under the appropriate conditions, per square centimeter of effective filter area should provide a sterile effluent. In this method, factors which may affect the sterilization include pressure, flow rate, filter unit characteristics and the like. In routine filtration process control, it is required to perform integrity tests of the sterilizing filter after each filtration process (also prior to the filtration process, if necessary).

4. Sterilization Indicators

4.1. Biological indicator (BI)

A BI is prepared from specific microorganisms resistant to the specified sterilization process and is used to develop and/or validate a sterilization process. The dry type BI is classified into two kinds. In one, bacterial spores are added to a carrier such as filter paper, glass or plastic and then the carriers are dried and packaged. In the other, bacterial spores are added to representative units of the product to be sterilized or to simulated products. Packaging materials of the BI should show good heat penetration in dry heat sterilization and good gas or steam penetration in ethylene oxide and moist heat sterilizations. It should be confirmed that any carrier does not affect the D value of the spores. In the case of a liquid product, the spores may be suspended in the same solution as the product or in a solution showing an equivalent effect in the sterilization of biological indicator. However, when the spores are suspended in liquid, it is necessary to ensure that the resistance characteristics of the spores are not affected due to germination.

Typical examples of biological indicator

<table>
<thead>
<tr>
<th>Sterilization Method</th>
<th>Representative Microorganisms</th>
<th>Strain Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moist heat method</td>
<td>Geobacillus stearothermophilus</td>
<td>ATCC 7953, NBRC 13737, JCM 9488</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATCC 12980, NBRC 12550, JCM 2501</td>
</tr>
<tr>
<td>Dry heat method</td>
<td>Bacillus atrophaeus</td>
<td>ATCC 9372, NBRC 13721</td>
</tr>
<tr>
<td>Gas method</td>
<td>Bacillus atrophaeus</td>
<td>ATCC 9372, NBRC 13721</td>
</tr>
</tbody>
</table>

* In addition to these microorganisms, other microorganisms with the greatest resistance to the sterilization procedure concerned, found in the bioburden, can be used as the biological indicator.

4.4.2. Setting up procedure of BI

4.1.2.1. In the case of dry materials

A Dry type BI is placed at predetermined cold spots in the product to be sterilized or a suitable product showing an equivalent effect in the sterilization. The BIs are usually primary packaged in the same way as the product, including a secondary packaging, if applicable.

4.1.2.2. In the case of wet materials

Spores are suspended as the BI in the same solution as the product or in an appropriate similar solution, and should be placed at cold spots in the sterilizer.

4.1.3. Culture conditions

Soybean casein digest medium is generally used. General culture conditions are at 55 – 60°C for 7 days in the case of G. stearothermophilus and at 30 – 35°C for 7 days in the case of B. atrophaeus.

4.2. Chemical indicator (CI)

CI is an indicator which shows a color change of a substance applied to a paper slip, etc. as a result of physical and/or chemical change due to exposure to heat, gas or radiation. The CI can be classified into three types. The first is employed to identify whether or not sterilization has already been implemented, the second is employed to control the sterilization process (for example, its color changes after sterilization for a sufficient time), and the third is the Bowie
& Dick type used to evaluate the effectiveness of air removal during the pre-vacuum phase of the pre-vacuum sterilization cycle.

4.3. Dosimeter

In the radiation (γ-ray) method, the sterilization effect depends on the absorbed radiation dose, so the sterilization process control is mainly performed by measuring the dose. A dosimeter is installed at a position corresponding to the minimum dose region of an exposed container or a position where the dose is in a known relation to that in the above region. Measurement should be done for each radiation batch. If there are many containers in the same batch, dosimeters should be employed so that more than one dosimeter is always installed at the effective radiation section of the irradiation chamber. It should be noted that dosimeters may be affected by environmental conditions (temperature, humidity, ultraviolet light, time until reading, etc.) before and during irradiation. Practical dosimeters for γ-ray and bremsstrahlung sterilization include the dyed polymethylmethacrylate dosimeter, clear polymethylmethacrylate dosimeter, ceric-cerous sulfate dosimeter, alanine-EPR dosimeter and the like. A dosimeter for gamma radiation can not generally be used for sterilization process control with an electron beam of less than 3 MeV energy. Dosimeters for electron beam sterilization include the cellulose acetate dosimeter, radioluminescent film dosimeter and the like. A practical dosimeter must be calibrated against an appropriate national or international standard dosimetry system.

5. Determination of sterilization conditions using microorganism as an indicator

Taking account of the characteristics upon the sterilization concerned, bioburden, etc. of a product to be sterilized, chose a suitable method from the followings and determine the conditions.

5.1. Half-cycle method

In this method, a sterilization time of twice as long as that required to inactive all of 10^6 counts of BI placed in the product is used, regardless of bioburden count in the product being sterilized or the resistance of the objective microorganisms to the sterilization.

5.2. Overkill method

In this method, a sterilization condition giving a sterility assurance level of not more than 10^{-6} counts is used, regardless of bioburden count in the product being sterilized or the resistance of the objective microorganisms to the sterilization. Generally, a sterilization condition providing 12 logarithmic reduction (12D) of a known count of BI of more than 1.0 D value is used.

5.3. Combination of BI and bioburden

Generally, a count of mean bioburden added three times of its standard deviation obtained by an extensive bioburden estimation is considered as the maximum bioburden count, and the sterilization time (or radiation dose) is calculated with the bioburden count based on an objective sterility assurance level. When this procedure is used, it is required to determine the resistance of the bioburden to the sterilization as well as the bioburden count in the product being sterilized. If a more resistant microorganism than the BI spore is found in the bioburden estimation, it should be used as the BI.

$$\text{Sterilization time (or radiation dose)} = D \times \log \frac{N_0}{N}$$

5.4. Absolute bioburden method

The sterilization conditions are determined by employing the D value of the most resistant microorganism found in the product or environment by the resistant estimation and being based on the bioburden count in the product. Generally, a count of mean bioburden added three times of its standard deviation obtained by an extensive bioburden estimation is employed as the bioburden count. When this procedure is used, it is required to make frequent counting and resistance determination of microorganisms in daily bioburden estimation.

6. References

(i) ISO 11134 Industrial moist heat sterilization
(ii) ISO 11135 Ethylene oxide sterilization
(iii) ISO 11137 Radiation sterilization
(iv) ISO 11138 Biological indicators
(v) ISO 11140 Chemical indicators
(vi) ISO 11173 Microbiological methods

Part 1: Estimation of population of microorganisms on products

G5 Crude Drugs

Aristolochic Acid

Aristolochic acid, which occurs in plants of Aristolochia-ceae, is suspected to cause renal damage. It is also reported to be oncogenic (see References). Aristolochic acid toxicity will not be a problem if crude drugs of the origin and parts designated in the JP are used, but there may be differences in crude drug nomenclature between different countries, and it is known that crude drug preparations not meeting the specifications of the JP are circulating in some countries. Consequently, when crude drugs or their preparations are used, it is important that the materials should not include any plant containing aristolochic acid.

Since Supplement I to JP14, the test for aristolochic acid I was added to the Purity under Asiasarum Root, which consists of the rhizome and root. Because the aerial part of the plant may contain aristolochic acid and may have been improperly contaminated in Asiasarum Root. It is considered that Akebia Stem, Sinomenium Stem and Saussurea Root do not contain aristolochic acid, unless plants of origin other than that designated in the JP are used. However, contamination of aristolochic acid might occur, as mentioned above. In this case, the test described in the Purity under Asiasarum Root is useful for checking the presence of aristolochic acid.

References:

Drug & Medical Device Safety Information (No.161) (July, 2000)
New England Journal of Medicine (June 8, 2000).
Mutation Research 515, 63 – 72 (2002).
Purity Tests on Crude Drugs Using Genetic Information

The first step in the quality assurance of natural products is the use of raw materials from the right part of the right origin (the right source). Therefore, it is clearly stated in Article 4 of the General Rules For Crude Drugs that the source of a crude drug is the approval or rejection criteria. There are various methods for differentiating the sources of crude drugs, such as morphological methods, organoleptic tests, and chemical methods, and appropriate methods for each are described in the individual monographs. Morphological methods, organoleptic tests, and chemical methods are discrimination methods for species that are based on the phenotypic characteristics of the crude drugs. On the other hand, together with recent progress in molecular biology techniques and the accumulation of genetic information on plants, differentiating methods of crude drugs based on genotypes have been established. Unlike morphological and other methods that are based on phenotypic characteristics, the genotypic methods are not affected by environmental factors. Also, the methods have several advantages, such as specialized expertise and skill for classification are not needed, and objective results are easily obtained.

The evolution of living organisms is accomplished by genetic mutation, and differences among the nucleotide sequences of genes of closely related species reflect the strain relationships between the species. Based on this theory, in recent years methods that classify species phylogenetically using the nucleotide sequence of rDNA that codes for ribosomal RNA (rRNA) on the nuclear genome have been adopted. In the same way, the sequence of this rDNA is also most often used in the classification of higher plants based on the genotype. In particular, it is very easy to classify closely related species using the intergenic transcriber space (ITS) region of the rDNA region, since by comparison with the coded gene region nucleotide substitution is more often undertaken. Furthermore, since the genes on the nuclear genome originate from the parents’ genoms, there is an advantage that interspecies hybrids can be detected. Higher plants also have mitochondrial genes and chloroplastic genes. Although the genes on these genomes are also often used for classification, interspecies hybrids cannot be confirmed because the genes are normally unparental inheritance.

The two methods presented here have been developed based on the reported identification methods of Atractylodes Lancea Rhizome and Atractylodes Rhizome\(^1\) utilizing the gene sequence of the ITS of rDNA. Inter-laboratory validation study for purity test of Atractylodes Rhizome targeted for Atractylodes Lancea Rhizome have been completed. The plant sources for Atractylodes Lancea Rhizome stipulated in the individual monographs are Atractylodes lancea De Candolle or A. chinensis Koidzumi (Compositae), while those for Atractylodes Rhizome are A. japonica Koidzumi ex Kitamura or A. ovata De Candolle (Compositae). The approval or rejection of the source of Atractylodes Lancea Rhizome is, in principle, determined by the description of the crude drug, including microscopy, while that of Atractylodes Rhizome is determined by the description of the crude drug, including microscopy, together with color reaction, which is an identification test. In the above scientific paper, it was shown that these 4 plant species can be clearly classified by comparing the nucleotide sequences of the ITS mentioned above, and that the species can be easily classified without performing sequence analysis by performing PCR using a species specific primer set or by using a restriction enzyme which recognizes species specific sequence.

In validation studies, the simplicity of the test is given maximum consideration. We examined methods that observe PCR amplification bands using species specific primer sets (Mutant Allele Specific Amplification: Method 1) and that observe DNA fragments produced by restriction enzyme treatment of the PCR products, which are prepared using a primer set common to each plant source (PCR-Restriction Fragment Length Polymorphism: Method 2), and do not involve nucleotide sequence analyses. In these methods based on PCR, an extremely small amount of template DNA is amplified to billions to hundred-billions times. Therefore, when using them as identification tests for powdered crude drugs, the target DNA fragment can be observed even if the vast majority of the crude drug for analysis is not appropriate plant species and there is only a minute amount of powder from a crude drug derived from a suitable plant. Consequently, in identification tests, either a cut or a whole crude drug must be used, as long as one is careful to avoid contamination by powder originating from other crude drugs.) On the other hand, when used as a purity test, the form of the crude drug is irrelevant as long as the gene amplification is performed properly and the target gene is not polymorphic, so if DNA fragments of an inappropriate plant are confirmed in the purity test, regardless of the form of the crude drug, it becomes clear there is contamination by an inappropriate crude drug.

The methods shown here are reference information and at the present stage results obtained using the methods do not affect the approval or rejection of the crude drug in each monograph. Furthermore, by performing the sequence analysis outlined in the previous paper, it goes without saying that more accurate decision concerning the source species can be made.

1. DNA Amplification Equipment

DNA amplification equipment is used to amplify the DNA which is extracted from a crude drug and then purified. Since there are slight differences in the methods of temperature control, and so on depending on the equipment used, there may be differences in the intensity, etc. of the PCR amplification bands even if PCR is carried out under the stipulated conditions. Therefore, when judging results based solely on the presence or absence of PCR amplification bands as in 3. Methods 1, confirm that only proper amplification bands are obtained when performing PCR using DNA obtained from samples confirmed beforehand to be the source species. If proper amplification bands are not obtained, the PCR temperature conditions should be slightly adjusted. This equipment can be used for the restriction enzyme treatment in 4. Method 2.

2. General precautions

Crude drugs are different from fresh plants in that they are dried products and a certain amount of time has passed since they were harvested. Therefore, in many cases the DNA has undergone fragmentation. Furthermore, various substances that can block or interfere with the PCR reaction...
may be present in the plant. For these reasons, the extraction and purification of template DNA is the process that should receive the greatest amount of attention. In the case of Atractylodes crude drugs, the periderm should be removed using a clean scalpel or other clean instrument before pulverizing the sample because very often there are inhibitory substances present in the periderm.

3. Method 1 (Mutant Allele Specific Amplification Method)

Generally, this method is referred to as Mutant Allele Specific Amplification (MASA) or Amplification Refractory Mutation System (ARMS), and it provides nucleotide sequence information of sample-derived template DNA, based upon the presence or absence of DNA amplification in PCR using a species specific primer set.

3.1. Procedure

The following is an example procedure.

3.1.1. Preparation of template DNA

There are various methods with which to extract and purify DNA from the samples. It is recommended that commercially available DNA extraction kits be used when considering their advantages of not using any noxious reagents and not requiring any complicated purification procedures. In this case, attention should be paid to the final amount (concentration) of DNA obtained, and the initial amount of initial sample and the volume of liquid to elute the DNA need to be controlled. When extraction and purification are performed using silica gel membrane type kits stipulated in notifications related to inspection methods of the foods produced by recombinant DNA techniques, it is appropriate to use 200 mg of sample, 1 mL of AP1 buffer solution, 2 μL of RNase A, and 325 μL of AP2 buffer solution. Also, the most important things are that the supernatant loaded on the first column is clear and that there is no need to load 1 mL unreasonably. Furthermore, 50 μL is an appropriate volume used in the final elution of the DNA, and normally the initial eluate is used as the DNA sample stock solution.

3.1.2. Confirmation of purity of DNA in DNA sample stock solution and assay of DNA

The purity of the DNA in the stock solution can be confirmed by the OD_{260 nm}/OD_{280 nm} ratio using a spectrophotometer. A ratio of 1.5 indicates that the DNA has been adequately purified. The amount of DNA is calculated using 1 OD_{260 nm} = 50 μg/mL. The measurement mentioned above is performed using appropriately diluted DNA sample stock solution. Based on the results obtained, dilute with water to the concentration needed for the subsequent PCR reactions, dispense the solution into micro tubes as the sample DNA solution, and if necessary store frozen at not over −20°C. The dispensed DNA sample is used immediately after thawing and any remaining solution should be discarded and not refrozen. If the concentration of the DNA sample stock solution does not reach the concentration stipulated in PCR, it is used as a DNA sample solution.

3.1.3. PCR

When a commercially available PCR enzyme mentioned in the above notification is used, it is appropriate that 25 μL of reaction mixture consisting of 2.5 μL of the PCR buffer solution containing magnesium, dNTP (0.2 mmol/L), 5′ and 3′ primer (0.4 mmol/L), Taq DNA polymerase (1.25 units), and 5 μL of 10 ng/μL sample DNA solution (50 ng of DNA) is prepared on ice. Among them, the PCR buffer solution and dNTP are provided as adjuncts to the enzyme. When conducting purity tests on Atractylodes Lancea Rhizome in Atractylodes Rhizome, the primer sets used are C and D (C is positive with A. lancea, D is positive with A. chinensis) as described in the paper mentioned above (J. Nat. Med. 60, 149 – 156, 2006), however, when a combination of primer A and B is used, it is possible to confirm the source species of each of the respective specimens. In order to confirm that the DNA has been extracted correctly, the reaction solution containing the positive control primer (Pf and Pr) as shown below should be prepared. In addition, the negative control solutions which are not containing DNA sample or either of the primer sets should be prepared and simultaneously conduct PCR.

\[
\text{Pf: } 5'\text{-CAT TGT CGA AGC CTG CAC AGC A-3'} \\
\text{Pr: } 5'\text{-CGA TGC GTG AGC CCA GAT ATC C-3'}
\]

The PCR reaction is performed under the following conditions: starting the reaction at 95°C for 10 minutes, 30 cycles of 0.5 minutes at 95°C and 0.75 minutes at 68°C (69°C only when using the primer set C), terminate reaction at 72°C for 7 minutes, and store at 4°C. The resulting reaction mixture is used for the following process as PCR amplification reaction solution.

3.1.4. Agarose gel electrophoresis and detection of PCR products

After completion of the PCR reaction, mix 5 μL of the PCR amplification reaction solution with an appropriate volume of gel loading buffer solution, add the mixture to the wells of 2 w/v% agarose gel, and then perform electrophoresis using 1-fold TAE buffer solution (refer to General Information, Rapid Identification of Microorganisms Based on Molecular Biological Method). Run in parallel an appropriate DNA molecular mass standard. Electrophoresis is terminated when the bromophenol blue dye in the gel loading buffer has advanced to a point corresponding to 1/2 to 2/3 the length of the gel.

Stain the gel after electrophoresis when not using gel stained in advance with ethidium bromide. Place the gel that has undergone electrophoresis and staining in a gel image analyzer, irradiate with ultraviolet light (312 nm), and detected its electrophoresis pattern. Compare this to the DNA molecular mass standard and determine the absence or presence of the target amplification band.

3.2. Judgement

Confirm at first that a 305 bp band is found with the reaction solution to which the positive control primer set has been added, and confirm there are no bands in a solution with no primer sets and a solution with no sample DNA solution. Next, if a 226 bp band is confirmed when the primer set C is added or if a 200 bp band is confirmed when the primer set D is added, the sample is judged to be Atractylodes Lancea Rhizome (in the case of cut crude drug, contamination of Atractylodes Lancea Rhizome is observed) and it is rejected. The sample is judged not to be Atractylodes Lancea Rhizome (in the case of cut crude drug, there is no contamination of Atractylodes Lancea Rhizome) and the purity test is acceptable if a 305 bp band is confirmed with the positive control primer set, bands are not observed in reaction solution without primer and reaction solution without DNA sample solution, and a 226 bp band is not observed with the primer set C and a 200 bp band is not observed with the primer set D. If a band is not observed with
the positive control primer, it is to be concluded that the DNA extraction failed and the procedure should be started over again from the DNA extraction step. If bands are confirmed in reaction solutions without primer sets or without DNA sample solution, it should be assumed that there was an error in the PCR procedure and therefore the procedure should be repeated again from the step 3.1.3. PCR.

4. Method 2 (PCR-Restriction Fragment Length Polymorphism)

Generally, this method is referred to as PCR-Restriction Fragment Length Polymorphism (RFLP), and it provides nucleotide sequence information of sample-derived template DNA, based upon the DNA fragment pattern produced by restriction enzyme treatment of the PCR products, which are amplified by using a primer set common to the DNA sequence of the objective plant.

The test is performed with 25 samples randomly taken from a lot, and each sample is designated with a number from 1 to 25. Differentiation of the sources is performed by individual PCR-RFLP measurement of the samples, and decision of the acceptability of the purity is dependent on how many nonconforming samples are present in the first 20 samples, taken in numerical order, for which judgement is possible as described below.

4.1. Procedure

The following is an example procedure.

4.1.1. Preparation of template DNA

There are various methods with which to extract and purify DNA from the samples. It is recommended that commercially available DNA extraction kits be used, when considering their advantages of not using noxious reagents and not requiring complicated purification procedures. Recently, PCR reagents that inhibit the effect of PCR enzyme-inhibiting substances present in samples have become commercially available, and by using these reagents, it is possible to prepare the template DNA from the sample simply by incubating the sample with the DNA extraction reagent. Here, a recommended DNA preparing procedure using such PCR reagents is described for the convenience of experimenters.

Cut 20 mg of the sample into small pieces with a clean knife, add 400 μL of the DNA extraction reagent, and incubate at 55°C overnight (16-18 hours). Then heat at 95°C for 5 minutes to inactivate the enzyme in the reagent. Centrifuge to precipitate the sample, and use 50 μL of the supernatant liquid as the template DNA solution. The DNA solution prepared in this method can not be used for concentration measurement based on OD 260 nm, because it contains many foreign substances affecting OD 260 value from the sample.

The composition of the DNA extraction reagent is as follows:

- 2-Amino-2-hydroxymethyl-1,3-propanediol-hydrochloric acid (pH 8.0) 5 mmol/L
- Ethylenediamine tetraacetate 20 mmol/L
- Sodium chloride 400 mmol/L
- Sodium dodecyl sulfate 200 μg/mL
- Proteinase K 0.3%

4.1.2. PCR

In the method using the PCR enzyme and PCR reagent as described, the reaction mixture is prepared on an ice bath in a total volume of 20 μL of a solution containing 10.0 μL of 2-fold concentrated PCR reagent, 5'- and 3'-primers (0.5 μmol/L), Taq DNA polymerase (0.5 units) and 0.5 μL of template DNA solution.

The PCR reaction is performed under the following conditions: 95°C for 10 minutes, 40 cycles of 95°C for 0.5 minute, 65°C for 0.25 minute, and 72°C for 0.25 minute and 72°C for 7 minutes. Store the solution at 4°C, and use this solution as the PCR amplified solution. A negative control (containing water instead of the template DNA solution) must be included in the procedure.

The sequence of each primer is as follows:

5'-primer: 5'-GGC ACA ACA CGT GCC AAG GAA AA-3'
3'-primer: 5'-CGA TGC GTG AGC CGA GAT ATC C-3'

4.1.3. Restriction enzyme treatment

The treatment is performed on individual reaction solutions using two enzymes, Fau I and Msp I. In the case of Fau I, to an appropriate amount of the reaction solution, composed of a reaction buffer containing 1.0 unit of enzyme, add 3.0 μL of PCR products while cooling in an ice bath to make 15.0 μL. In the case of Msp I, to an appropriate amount of the reaction solution, composed of a reaction buffer containing 20.0 units of enzyme, add 3.0 μL of PCR products while cooling in an ice bath to make 15.0 μL. Incubate these solutions at the temperature recommended by the manufacturer for 2 hours, and then inactivate the enzyme by heating at 72°C for 10 minutes. The negative control of the PCR reaction is also treated in the same manner.

4.1.4. Agarose gel electrophoresis and detection of DNA fragments

After the restriction enzyme treatment, mix the total amount of the reaction solution and an appropriate amount of the gel loading buffer solution, place it in a 4 w/v% agarose gel well, and carry out electrophoresis with 1-fold concentrated TAE buffer solution (see “Rapid Identification of Microorganisms Based on Molecular Biological Methods” under General Information). Carry out the electrophoresis together with appropriate DNA molecular mass standard. Stop the electrophoresis when the bromophenol blue included in the loading buffer solution has moved about 2 cm from the well. The 4 w/v% agarose gel is sticky, difficult to prepare and hard to handle, so that it is better to use a commercially available precast gel.

After the electrophoresis, stain the gel, if it is not already stained, with ethidium bromide, and observe the gel on an illuminating device under ultraviolet light (312 nm) to confirm the electrophoretic pattern.

4.2. Judgement

4.2.1. Judgment of each sample

Confirms that no band is obtained with the negative control of the PCR, other than the primer dimer (about 40 bp) band. A sample treated with Fau I, showing bands of about 80 bp and 60 bp, or that treated with Msp I, showing bands of about 90 bp and 50 bp, is judged as Atractylodes Lancea Rhizome. A sample not showing any band other than a band at about 140 bp and the primer dimer band is judged as Atractylodes Rhizome. If a sample does not show any band other than the primer dimer band, it is considered that PCR products were not obtained, and judgement is impossible for the sample.

4.2.2. Judgment of the purity

Judgement of the purity is based on the result of the judgment of each sample. If there is no sample that is judged as Atractylodes Lancea Rhizome among 20 samples taken in
On the Scientific Names of Crude Drugs Listed in the JP

The notation system of the scientific names for the original plants and animals of crude drugs listed in JP is not the same as the taxonomic system used in the literature. The reason for this is that the JP is not an academic text, but an ordinance. The relationship between the scientific names used in the JP and those generally used taxonomically is indicated in the following table, to avoid misunderstanding by JP users owing to differences in the notation system.

<table>
<thead>
<tr>
<th>Crude Drug</th>
<th>Scientific names used in the JP</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia</td>
<td>Acacia senegal Willdenow</td>
<td>Leguminosae</td>
</tr>
<tr>
<td>アラビアゴム</td>
<td>= Acacia senegal (L.) Willd.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other species of the same genus</td>
<td></td>
</tr>
<tr>
<td>Achyranthes Root</td>
<td>Achyranthes fauriei Leveille et Vaniot</td>
<td>Amaranthaceae</td>
</tr>
<tr>
<td>ゴシツ</td>
<td>= Achyranthes fauriei H. Lev. &amp; Vaniot</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Achyranthes bidentata Blume</td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>Gelidium elegans Kuetzing</td>
<td>Gelidiaceae</td>
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<td>Other species of the same genus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other red algae</td>
<td></td>
</tr>
<tr>
<td>Akebia Stem</td>
<td>Akebia quinata Decaisne</td>
<td>Lardizabalaceae</td>
</tr>
<tr>
<td>モクツウ</td>
<td>= Akebia quinata (Therb. ex Houtt.) Deene.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Akebia trifoliata Koidzumi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>= Akebia trifoliata (Thunb.) Koidz.</td>
<td></td>
</tr>
<tr>
<td>Alisma Rhizome</td>
<td>Alisma orientale Juzepczuk</td>
<td>Alismataceae</td>
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<td></td>
<td>Alisma plantago-aquatica L. var. orientale Sam.</td>
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<tr>
<td>Crude Drugs</td>
<td>General Information</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>---------------------</td>
<td></td>
</tr>
</tbody>
</table>
| **Aloe** アロエ | *Aloe ferox* Miller  = *Aloe ferox* Mill.  
Hybrid between *Aloe ferox* Miller and *Aloe africana* Miller  
*Aloe africana* Miller  = *Aloe africana* Mill.  
Hybrid between *Aloe ferox* Miller and *Aloe spicata* Baker |
| **Alpinia Officinarum Rhizome** リョウキョウ | *Alpinia officinarum* Hance  
Zingiberaceae |
| **Amomum Seed** シュクシャ | *Amomum xanthioides* Wall.  = *Amomum xanthioides* Wall.  
*Amomum villosum* Lour. var. *xanthioides* (Wall.) T. L. Wu & Senjen  
Zingiberaceae |
| **Anemarrhena Rhizome** チモ | *Anemarrhena asphodeloides* Bunge  
Liliaceae |
| **Angelica Dahurica Root** ピャクシ | *Angelica dahurica* Bentham et Hooker filius ex Franchet et Savatier  
= *Angelica dahurica* (Hoffm.) Bent. & Hook. f. ex Franch. & Sav.  
Umbelliferae |
| **Apricot Kernel** キョウニン | *Prunus armeniaca* Linné  
= *Prunus armeniaca* L.  
*Prunus armeniaca* Linné var. *ansu* Maximowicz  
= *Prunus armeniaca* L. var. *ansu* Maxim. |
| **Aralia Rhizome** ドクカツ | *Aralia cordata* Thunberg  
= *Aralia cordata* Thunb.  
Araliaceae |
| **Areca** ビンロウジ | *Areca catechu* Linné  
= *Areca catechu* L.  
Palmae |
| **Artemisia Capillaris Flower** インチンコウ | *Artemisia capillaris* Thunberg  
= *Artemisia capillaris* Thunb.  
Compositae |
| **Asiasarum Root** サイシン | *Asiasarum sieboldii* F. Maekawa  
= *Asiasarum sieboldii* (Miq.) F. Maek.  
*Asarum sieboldii* Miq.  
*Asarum sieboldii* Miq. var. *seoulense* Nakai  
*Asiasarum heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa  
= *Asiasarum heterotropoides* (F. Schmidt) F. Maek. var. *mandshuricum* (Maxim.) F. Maek.  
*Asarum heterotropoides* F. Schmidt var. *mandshuricum* (Maxim.) Kitag.  
Aristolochiaceae |
| **Asparagus Tuber** テンモンドウ | *Asparagus cochinchinensis* Merrill  
= *Asparagus cochinchinensis* (Lour.) Merr.  
Liliaceae |
| **Astragalus Root** オウギ | *Astragalus membranaceus* Bunge  
= *Astragalus membranaceus* (Fisch.) Bunge  
*Astragalus mongholicus* Bunge  
*Astragalus membranaceus* (Fisch.) Bunge var. *mongholicus* (Bunge) Hsiao  
Leguminosae |
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<th>Scientific Name and Synonyms</th>
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<td>Atractylodes Lancea Rhizome</td>
<td>Atractylodes lancea De Candolle = Atractylodes lancea (Thunb.) DC.</td>
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<td>Atractylodes chinensis Koidzumi = Atractylodes chinensis (DC.) Koidz.</td>
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<td>Atractylodes macrocephala Koidzumi = Atractylodes macrocephala Koidz.</td>
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<td>* Atractylodes ovata De Candolle = Atractylodes ovata (Thunb.) DC.</td>
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<td>Bearberry Leaf</td>
<td>Arctostaphylos uva-ursi Sprengel = Arctostaphylos uva-ursi (L.) Spreng.</td>
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<td>Atropa belladonna L.</td>
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<td>Benincasa cerifera Savi</td>
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<td>Oryza sativa L.</td>
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<td>Cinnamon Oil</td>
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<td>Coptis chinensis Franchet = Coptis chinensis Franch.</td>
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<td>Coptis deltoidea C. Y. Cheng et Hsiao</td>
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<td>Coptis teeta</td>
<td>Coptis teeta Wall</td>
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<td>Cornus Fruit</td>
<td>Cornus officinalis Siebold et Zuccarini = Cornus officinalis Siebold &amp; Zucc.</td>
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<td>= Crataegus pinnatifida Bunge var. major N. E. Brown</td>
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<td>= Dioscorea batatas Decaisne = Dioscorea batatas Decne.</td>
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<td>= Dioscorea opposita Thunb.</td>
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<td>Ephedra Herb</td>
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<td>Ephedra equisetina Bunge</td>
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| Epimedium Herb | Epimedium pubescens Maximowicz = Epimedium pubescens Maxim. |
| Epimedium brevicornu Maximowicz = Epimedium brevicornu Maxim. |
| Epimedium wushanense T. S. Ying |
| Epimedium sagittatum Maximowicz = Epimedium sagittatum (Siebold & Zucc.) Maxim. |
| Epimedium koreanum Nakai |
| Epimedium grandiflorum Morren var. thunbergianum Nakai = Epimedium grandiflorum Morr. var. thunbergianum (Miq.) Nakai |
| Epimedium sempervirens Nakai |

| Eucommia Bark | Eucommia ulmoides Oliver = Eucommia ulmoides Oliv. | Eucommiaceae |

| Euodia Fruit | Euodia ruticarpa Hooker filius et Thomson = Euodia ruticarpa (A. Juss.) Hook. f. & Thomson |
| * Evodia rutaecarpa Bentham = Evodia rutaecarpa (A. Juss.) Benth. |
| Tetradium ruticarpum (A. Juss.) Hartley |
| Euodia officinalis Dode |
| * Evodia officinalis Dode |
| Evodia rutaecarpa (A. Juss.) Benth. var. officinalis (Dode) Huang |
| Euodia bodinieri Dode |
| * Evodia bodinieri Dode |
| Evodia rutaecarpa (A. Juss.) Benth. var. bodinieri (Dode) Huang |

| Fennel | Foeniculum vulgare Miller = Foeniculum vulgare Mill. | Umbelliferae |

| Fennel Oil | Foeniculum vulgare Miller = Foeniculum vulgare Mill. | Umbelliferae |
| Illicium verum Hooker filius = Illicium verum Hook. f. | Illiciaceae |

| Forsythia Fruit | Forsythia suspensa Vahl = Forsythia suspensa (Thunb.) Vahl |
| Forsythia viridissima Lindley = Forsythia viridissima Lindl. | Oleaceae |

| Fritillaria Bulb | Fritillaria verticillata Willdenow var. thunbergii Baker = Fritillaria verticillata Willd. var. thunbergii (Miq.) Baker |
| Fritillaria thunbergii Miq. | Liliaceae |

| Gambir | Uncaria gambir Roxburgh = Uncaria gambir (Hunter) Roxb. | Rubiaceae |

| Gardenia Fruit | Gardenia jasminoides Ellis |
| Gardenia jasminoides Ellis f. longicarpa Z. W. Xie & Okada | Rubiaceae |

| Gastrodia Tuber | Gastrodia elata Blume | Orchidaceae |

<p>| Gentian | Gentiana lutea Linnè = Gentiana lutea L. | Gentianaceae |</p>
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<td><em>Zingiber officinale</em> Roscoe</td>
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<td><em>Panax ginseng</em> C. A. Meyer</td>
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<td><em>Glehnia littoralis</em> Fr. Schmidt ex Miqu.</td>
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| **Rehmannia Root** | *Rehmannia glutinosa* Liboschitz var. *purpurea* Makino  
= *Rehmannia glutinosa* Libosch. var. *purpurea* Makino | *Scrophulariaceae* |
| **Rhubarb** | *Rheum palatum* Linné  
= *Rheum palatum* L. | *Polygonaceae* |
| | *Rheum tanguticum* Maximowicz  
= *Rheum tanguticum* Maxim. |  |
| | *Rheum officinale* Baillon  
= *Rheum officinale* Baill. |  |
| | *Rheum coreanum* Nakai |  |
| | Hybrid between above species |  |
| **Rose Fruit** | *Rosa multiflora* Thunberg  
= *Rosa multiflora* Thunb. | *Rosaceae* |
| **Rosin** | Several plants of *Pinus* genus | *Pinaceae* |
| **Royal Jelly** | *Apis mellifera* Linné  
= *Apis mellifera* L. | *Apidae* |
| | *Apis cerana* Fabricius |  |
| **Safflower** | *Carthamus tinctorius* Linné  
= *Carthamus tinctorius* L. | *Compositae* |
| **Saffron** | *Crocus sativus* Linné  
= *Crocus sativus* L. | *Iridaceae* |
| **Saposhnikovia Root and Rhizome** | *Saposhnikovia divaricata* Schischkin  
= *Saposhnikovia divaricata* (Turcz.) Schischk. | *Umbelliferae* |
| **Sappan Wood** | *Caesalpinia sappan* Linné  
= *Caesalpinia sappan* L. | *Leguminosae* |
| **Saussurea Root** | *Saussurea lappa* Clarke  
= *Saussurea lappa* (Decne.) C. B. Clarke | *Compositae* |
| | Aucklandia lappa Decne. |  |
| **Schisandra Fruit** | *Schisandra chinensis* Bailon  
= *Schisandra chinensis* (Turcz.) Baill. | *Schisandraceae* |
| **Schizonepeta Spike** | *Schizonepeta tenuifolia* Briquet  
= *Schizonepeta tenuifolia* Briq. | *Labiatae* |
| **Scopolia Rhizome** | *Scopolia japonica* Maximowicz  
= *Scopolia japonica* Maxim. | *Solanaceae* |
| | *Scopolia carniiolica* Jacquin  
= *Scopolia carniiolica* Jacq. |  |
| | *Scopolia parviflora* Nakai  
= *Scopolia parviflora* (Dunn) Nakai |  |
<p>| <strong>Scutellaria Root</strong> | <em>Scutellaria baicalensis</em> Georgi | <em>Labiatae</em> |</p>
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When “Other species of the same genus” is included as its original plants the scientific name is not written in Monograph, however, it is written in this table.

Reference
**G6 Drug Formulation**

**Tablet Friability Test**

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The Tablet Friability Test is a method to determine the friability of compressed uncoated tablets. The test procedure presented in this chapter is generally applicable to most compressed tablets. Measurement of tablets friability supplements other physical strength measurement, such as tablet crushing strength.

Use a drum, with an internal diameter between 283 and 291 mm and a depth between 36 and 40 mm, of transparent synthetic polymer with polished internal surfaces, and subject to minimum static build-up (see figure for a typical apparatus). One side of the drum is removable. The tablets are tumbled at each turn of the drum by a curved projection with an inside radius between 75.5 and 85.5 mm that extends from the middle of the drum to the outer wall. The outer diameter of the central ring is between 24.5 and 25.5 mm. The drum is attached to the horizontal axis of a device that rotates at 25 ± 1 rpm. Thus, at each turn the tablets roll or slide and fall onto the drum wall or onto each other.

For tablets with a unit mass equal to or less than 650 mg, take a sample of whole tablets corresponding as near as possible to 6.5 g. For tablets with a unit mass of more than 650 mg, take a sample of 10 whole tablets. The tablets should be carefully dedusted prior to testing. Accurately weigh the tablet sample, and place the tablets in the drum. Rotate the drum 100 times, and remove the tablets. Remove any loose dust from the tablets as before, and accurately weigh.

Generally, the test is run once. If obviously cracked, cleaved, or broken tablets are present in the tablet sample after tumbling, the sample fails the test. If the results are difficult to interpret or if the mass loss is greater than the targeted value, the test should be repeated twice and the mean of the three tests determined. A maximum mean mass loss from the three samples of not more than 1.0% is considered acceptable for most products.

If tablet size or shape causes irregular tumbling, adjust the drum base so that the base forms an angle of about 10° with the horizontal and the tablets no longer bind together when lying next to each other, which prevents them from falling freely.

Effervescent tablets and chewable tablets may have different specifications as far as friability is concerned. In the case of hygroscopic tablets, an appropriate humidity-controlled environment is required for testing.

Drums with dual scooping projections, or apparatus with more than one drum, for the running of multiple samples at one time, are also permitted.

**G7 Containers and Package**

**Plastic Containers for Pharmaceutical Products**

Various kinds of plastics are used as the materials for manufacturing containers for pharmaceutical products. Such plastics should not have the properties to deteriorate the efficacy, safety or stability of the pharmaceutical products to be packed in the container. In selecting a suitable plastic container, it is desirable to have sufficient information on the manufacturing processes of the plastic container including that on the substances added. Since each plastic has specific properties and a wide variety of pharmaceutical products may be packed in plastic containers, the compatibility of plastic containers with pharmaceutical products should be judged for each combination of container and the specific pharmaceutical product to be contained therein. Such judgement should be performed through a verification that the container for the pharmaceutical preparation can comply with the essential requirements for the container, i.e., the design specifications, based on the data from the experiments on the prototype products of the container and/or information from scientific documentation, etc. In addition, such compatibility must be ensured based upon an appropriate quality assurance system.

Furthermore, in introducing a plastic container, it is desirable that proper disposal method after use is taken into consideration.

1. **Essential Requirements in Designing Plastic Containers for Pharmaceutical Products**

The plastic material used for the container should be of high quality. Therefore, recycled plastic materials, which are of unknown constitution, must not be used.

The leachables or migrants from the container should not deteriorate the efficacy or stability of the pharmaceutical products contained therein. In addition, the possible toxic hazards due to the leachables or migrants should not exceed a given level. Furthermore, the amounts of leachable or migratable chemical substances, such as monomers and additives, from the containers to the pharmaceutical products contained therein must be sufficiently small from the viewpoint of safety.

The container should have a certain level of physical properties such as hardness, flexibility, shock resistance, tensile strength, tear strength, bending strength, heat resistance
and the like, in accordance with its intended usage.

The quality of the pharmaceutical products packed in the container must not deteriorate during storage. For example, in the case of pharmaceutical products which are unstable to light, the container should provide a sufficient level of light shielding. In the case of pharmaceutical products which are easily oxidized, the container material should not allow the permeation of oxygen. In the case of aqueous pharmaceutical products and pharmaceutical products that must be kept dry, the container material should not allow the permeation of water vapor. In addition, it is necessary to pay attention to the permeability of solvents other than water through the container. The concentration of the pharmaceuticals must not be decreased by more than a certain level due to the adsorption of the pharmaceuticals on the surface of the container, the migration of the pharmaceuticals into the inside of the material of the container, or the loss of pharmaceuticals through the container. Also, the pharmaceutical products contained therein must not be degraded by an interaction with the material of the container.

The container should not be deformed, should not deteriorate and should not be degraded by the pharmaceutical products contained therein. Unacceptable loss of function of the container should not result from a possible high temperature or low temperature or their repetitions encountered during storage or transportation.

The container should be of a required level of transparency, when it is necessary to examine foreign insoluble matter and/or turbidity of the pharmaceutical products by visual observation.

In the case of pharmaceutical products which must be sterilized, it is required to satisfy the above-mentioned essential requirements of the container after the sterilization, if there is a possibility that the quality of the container may change after the sterilization. There should not be any residue or generation of new toxic substances of more than certain risk level after the sterilization. In addition, the container should not have any inappropriate structure and/or material that might result in any bacterial contamination of the pharmaceutical products contained therein during storage and transportation after sterilization.

2. Toxicity Evaluation of Container at Design Phase

For design verification, the toxicity of the container should be evaluated. For the toxicity evaluation, it is desirable to select appropriate test methods and acceptance criteria for the evaluation, and to explain the rationale for the selection clearly. The tests should be conducted using samples of the whole or a part of the prototype products of the container. If the container consists of plural parts of different materials, each part should be tested separately. Such materials as laminates, composites, and the like are regarded as a single material. To test containers made of such materials, it is recommended to expose the inner surface of the container, which is in contact with the pharmaceutical products contained therein, to the extraction media used in the tests as far as possible.

The tests required for the toxicity evaluation of the container are different depending upon the tissue to which the container material is in contact. The container material should not allow the permeation of oxygen. In the case of aqueous pharmaceutical products and pharmaceutical products that must be kept dry, the container material should not allow the permeation of water vapor. In addition, it is necessary to pay attention to the permeability of solvents other than water through the container. The concentration of the pharmaceuticals must not be decreased by more than a certain level due to the adsorption of the pharmaceuticals on the surface of the container, the migration of the pharmaceuticals into the inside of the material of the container, or the loss of pharmaceuticals through the container. Also, the pharmaceutical products contained therein must not be degraded by an interaction with the material of the container.

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2.1. Examplification of Standard Tests

(i) Guidelines for Basic Biological Tests of Medical Devices and Materials (PAB Notification, YAKU-KI NO.99, June 27, 1995), Principles and selection of tests
(ii) ISO 10993-1: Biological evaluation of medical device—Evaluation and testing

2.1.1. Selection of Tests

(i) Guidelines for Basic Biological Tests of Medical Devices and Materials, I. Cytotoxicity Test 10. Cytotoxicity test using extract of medical device or material
(ii) ISO 10993-5: Biological evaluation of medical devices—Tests for cytotoxicity : in vitro methods
(iii) USP 24 <88> Biological reactivity tests, in vivo

2.1.2. Acute Toxicity Test

(i) ASTM F750-82: Standard practice for evaluating material extracts by systemic injection in the mice
(ii) BS5736: Part 3 Method of test for systemic toxicity; assessment of acute toxicity of extracts from medical devices
(iii) USP 24 <88> Biological reactivity tests, in vivo

2.1.3. Cytotoxicity Test

(i) Guidelines for Basic Biological Tests of Medical Devices and Materials, I. Cytotoxicity Test 10. Cytotoxicity test using extract of medical device or material
(ii) ISO 10993-5: Biological evaluation of medical devices—Tests for cytotoxicity : in vitro methods
(iii) USP 24 <88> Biological reactivity tests, in vitro

2.1.4. Hemolysis Test

(i) Guidelines for Basic Biological Tests of Medical Devices and Materials, VII. Hemolysis Test
(ii) ISO 10993-4: Biological evaluation of medical devices—Selection of tests for interaction with blood. Annex D
(iii) ASTM F756-82: Standard practice for assessment of hemolytic properties of materials

2.1.5. Sensitization Test

(i) Guidelines for Basic Biological Tests of Medical Devices and Materials, II. Sensitization Test
(ii) ISO 10993-10: Biological evaluation of medical devices—Tests for irritation and sensitization

3. Test Results to be Recorded per Production Unit

At the commercial production phase, it is required to establish acceptance criteria on at least the test items listed below and to record the test results of each production unit of plastic containers for pharmaceutical products. In addition, it is desirable to explain the rationale for setting the acceptance criteria clearly. However, these requirements should not be applied to orally administered preparations except for liquid preparations.

(i) Combustion Tests: Residue of ignition, heavy metals. If necessary, the amounts of specified metals (lead, cadmium, etc.)
(ii) Extraction Tests: pH, ultraviolet absorption spectra, potassium permanganate-reducing substances, foaming, non-volatile residue
(iii) Cytotoxicity Test
(iv) Any other tests necessary for the specific container for aqueous infusions.

test and hemolysis test

(ii) preparations in contact with skin or mucous membranes:
Cytotoxicity test and sensitization test

(iii) liquid orally administered preparations:
Cytotoxicity test
G8 Water

Quality Control of Water for Pharmaceutical Use

Water used for manufacturing pharmaceutical products and for cleaning their containers and equipments used in the manufacture of the products is referred to as “pharmaceutical water”. For assuring the quality of pharmaceutical water consistently, it is important to verify through appropriate process validation of water processing system that water with the quality suitable for its intended use is produced and supplied, and to keep the quality of produced water through routine works for controlling the water processing system.

1. Types of Pharmaceutical Water
   1.1. Water

   The specification for “Water” is prescribed in the Japanese Pharmacopoeia (JP) monograph. It is required for Water to meet the Quality Standards for Drinking Water provided under the Article 4 of the Japanese Water Supply Law. In the case that Water is produced at individual facilities using well water or industrial water as source water, it is necessary for produced water to meet the Quality Standards for Drinking Water and an additional requirement for ammonium of “not more than 0.05 mg/L”. Furthermore, when Water is to be used after storing for a period of time, it is necessary to prevent microbial proliferation.

   Water is used as source water for Purified Water and Water for Injection. It is also used for manufacturing intermediates of active pharmaceutical ingredients (APIs), and for pre-washing of the equipments used in the manufacture of pharmaceutical products.

   1.2. Purified Water

   The specifications for “Purified Water” and “Purified Water in Containers” are prescribed in the JP monographs. Purified Water is prepared by distillation, ion-exchange, reverse osmosis (RO), ultrafiltration (UF) capable of removing substances with molecular masses of not less than approximately 6000, or a combination of these processes from Water, after applying some adequate pretreatments if necessary. For the production of Purified Water, appropriate control of microorganisms is required. Particularly, in the case that Purified Water is prepared by ion-exchange, RO or UF, it is necessary to apply the treatments adequate for preventing microbial proliferation, or to sanitize the system periodically.

   When Purified Water is treated with chemical agents for sterilizing, preventing microbial proliferation, or maintaining the endotoxin level within an appropriate control range, a specification suitable for the intended use of treated water should be established individually, and a process control for keeping the quality of treated water in compliance with the specification thus established should be performed.

   “Purified Water in Containers” is prepared from Purified Water by introducing it in a tight container.

   1.3. Sterile Purified Water

   The specification for “Sterile Purified Water in Containers” (its alternative name is Sterile Purified Water) is prescribed in the JP monograph.

   Sterile Purified Water in Container is prepared from Purified Water by 1) introducing it into a hermetic container, sealing up the container, then sterilizing the product, or 2) making it sterile by using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

   Plastic containers for aqueous injections may be used in place of hermetic containers.

1.4. Water for Injection

   The specifications for “Water for Injection” and “Sterile Water for Injection in Containers” are prescribed in the JP monographs. Water for Injection is prepared by distillation or reverse osmosis and/or ultrafiltration (RO/UF), either from Water after applying some adequate pretreatments such as ion exchange, RO, etc., or from Purified Water.

   In the case of water processing systems based on distillation, it is necessary to take care for avoiding contamination of produced water by the impurities accompanied with the entrain.

   In the case of water processing system based on RO/UF, it is required to provide water with equivalent quality to that prepared by distillation consistently, based on substantial process validation through long-term operation and elaborate routine control of the system. It is essential to ensure consistent production of water suitable for Water for Injection by the entire water processing system including pretreatment facilities, in any systems based on RO/UF. For the water supplied to the system, it is also required to keep the quality suitable as source water through adequate validation and routine control on the water.

   For the water processing system based on RO/UF, routine control should be performed by analyzing water specimens, monitoring some quality attributes using in-line apparatus and checking the volume of water passed through the system. In addition, it is recommended to carry out periodical appearance observation and air-leak test on the membranes being currently used. It is also recommended to establish protocols for keeping the performance of membrane modules within appropriate control ranges and for estimating the timing to exchange the modules, through diagnosis on the degree of deterioration based on the results of tensile strength test on the used membrane modules, and visual observation on those modules whether any leakages of membranes have occurred or not, and to what extent they have occurred. Furthermore, it is desirable to establish the frequency of membrane exchange considering with its actual condition of use.

   In the case that Water for Injection is stored in the water processing system temporarily, a stringent control for microorganisms and endotoxins should be taken. An acceptable criterion of lower than 0.25 EU/mL for endotoxins is specified in the JP monograph of Water for Injection.

   “Sterile Water for Injection in Container” is prepared from Water for Injection by 1) introducing it into a hermetic container, sealing up the container, then sterilizing the product, or 2) making it sterile by using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

   Plastic containers for aqueous injections may be used in place of hermetic containers.
2. Reverse Osmosis and/or Ultrafiltration (RO/UF)

RO/UF are the methods for refining water by using membrane modules based on either reverse osmosis or ultrafiltration, or the modules combining them, and used as the alternative methods for distillation in the production of Purified Water or Water for Injection.

When Water for Injection is produced by RO/UF, a water processing system equipped with pretreatment facilities, facilities for producing Water for Injection and facilities for supplying Water for Injection is usually used. The pretreatment facilities are used to remove solid particles, dissolved salts and colloids in source water, and placed before the facilities for producing Water for Injection so as to reduce the load on the facilities for producing Water for Injection. They consist of apparatus properly selected from aggregation apparatus, precipitation-separation apparatus, filtration apparatus, chlorine sterilization apparatus, oxidation-reduction apparatus, residual chlorine-removing apparatus, precise filtration apparatus, reverse osmosis apparatus, ultrafiltration apparatus, ion exchange apparatus, etc., depending on the quality of source water. The facilities for producing Water for Injection consist of apparatus for supplying pretreated water, sterilization apparatus with ultraviolet rays, heat exchange apparatus, membrane modules, apparatus for cleaning and sterilizing the facilities, etc. The facilities for supplying Water for Injection consist of a reservoir tank for storing Water for Injection in the facilities temporarily, pipe lines, heat exchange apparatus, a pump for circulating Water for Injection in the facilities, pressure control apparatus, etc.

In the case that Water for Injection is stored in the water processing system temporarily, it should usually be circulated in a loop consisting of a reservoir tank and pipe lines at a temperature not lower than 80°C for preventing microbial proliferation.

When Purified Water is produced by RO/UF, basic composition of water processing system is almost the same as that for Water for Injection described above.

When RO/UF is utilized for preparing pharmaceutical water, it is necessary to select the most appropriate combination of membrane modules in consideration of the quality of source water and the quality of produced water required for its intended use. When the ultrafiltration membrane is used to prepare Purified Water or Water for Injection, membrane modules capable of removing microorganisms and substances with molecular masses not less than approximately 6000 should be used.

3. Selection of Pharmaceutical Water

Depending on the intended use of pharmaceutical water, the water suitable for assuring the quality of final products without causing any trouble during their manufacturing processes, should be selected from the above 4 types (1.1. ~ 1.4.) of pharmaceutical water specified in the JP. Table 1 exemplifies a protocol for such selection (in the case of pharmaceutical water used for the manufacture of drug products).

Sterile Purified Water in Containers or Water for Injection (or Sterile Water for Injection in Containers) may be used in place of Purified Water or Purified Water in Containers.

3.1 Drug Products

For the manufacture of sterile drug products, for which contamination with microorganisms or endotoxins is not permissible, Water for Injection (or Sterile Water for Injection in Containers) should be used. For the manufacture of ophthalmics and eye ointments, Purified Water (or Purified Water in Containers) can also be used.

For the manufacture of non-sterile drug products, water with a quality not lower than that of Purified Water (or Purified Water in Containers) should be used. However, out of the non-sterile drug products such as liquids, ointments, suspensions, emulsions, suppositories and aerosols, for those which require care against microbiological contamination, Purified Water (or Purified Water in Containers) adequately controlled from microbiological viewpoints should be used in consideration of the possible impacts of preservatives formulated in the drug products. For the manufacture of products containing crude drugs, it is recommended to select adequate type of water considering with viable counts of the crude drugs used for manufacturing the product and microbial limit required for the product.

Water used for pre-washing of containers or equipment surfaces that comes in direct contact with the drug products should have the quality not lower than that of Water. Water used for final rinsing should have an equivalent quality to that of water used for manufacturing drug products.

3.2. Active Pharmaceutical Ingredient (API)

Water used for manufacturing active pharmaceutical ingredient (API) should be selected in consideration of the characteristics of drug product for which the API is to be used, and its manufacturing process, so that the quality of the final drug product is assured.

Water used for manufacturing API or for cleaning containers or equipment surfaces that comes in direct contact with the raw materials or API intermediates, should have the quality not lower than that of Water adequately controlled from the chemical and microbiological viewpoints, even if the water is used at an earlier stage of synthetic or extraction process in the manufacture of API. Water used in the final purification process should have the quality equal to or higher than that of Purified Water (or Purified Water in Containers). Water used for final rinsing of containers or equipment surfaces that comes in direct contact with the APIs should have an equivalent quality to that of water used for manufacturing the APIs.

For manufacturing sterile API, Sterile Purified Water in Containers or Water for Injection (or Sterile Water for Injection in Containers) should be used. Similarly, for manufacturing APIs used for drug products where endotoxin control is required and there are no subsequent processes capable of removing endotoxins, Water for Injection (or Sterile Water for Injection in Containers), or Purified Water (or Purified Water in Containers) for which endotoxins are controlled at a low level, should be used.

4. Quality Control of Pharmaceutical Water

4.1. Outline

Verification that water with the quality required for its intended use has been produced by the pharmaceutical water processing system through substantial validation studies at an earlier stage of its operation, is the prerequisite for conducting quality control on pharmaceutical water in a routine and periodical manner. If this prerequisite is fulfilled, the following methods are applicable for quality control of pharmaceutical water.
Table 1  An Exemplified Protocol for Selecting Pharmaceutical Water  
(Water Used in the Manufacture of Drug Products or APIs)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Class of Pharmaceutical Water</th>
<th>Application</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Product</td>
<td>Water for Injection or Sterile Water for Injection in Containers</td>
<td>Injections, Ophthalmics, Eye Ointments</td>
<td>For ophthalmics and eye ointments for which precautions should be taken against microbial contamination, Purified Water or Purified Water in Containers kept its viable counts at low levels through sterilization, UF filtration, etc. should be used.</td>
</tr>
<tr>
<td>Purified Water</td>
<td>Water for Injection or Sterile Water for Injection in Containers</td>
<td>Ophthalmics, Eye Ointment</td>
<td>For liquids, solutions, ointments, suspensions, emulsions, suppositories, aerosols and so on for which precautions should be taken against microbial contamination, Purified Water or Purified Water in Containers adequately controlled from microbiological viewpoints should be used.</td>
</tr>
<tr>
<td></td>
<td>Purified Water in Containers</td>
<td>Aerosols, Liquids and Solutions, Extracts, Elixirs, Capsules, Granules, Pills, Suspensions and Emulsions, Suppositories, Powders, Spirits, Tablets, Syrups, Infusions and Decoctions, Plasters, Tinctures, Troches, Ointments, Cataplasms, Aromatic Waters, Liniments, Lemonades, Fluidextracts, Lotions, and Pharmaceutical preparations of percutaneous absorption type</td>
<td></td>
</tr>
<tr>
<td>Active Pharmaceutical Ingredient (API)</td>
<td>Water for Injection or Sterile Water for Injection in Containers</td>
<td>Sterile APIs, and APIs rendered sterile in the formulation process</td>
<td>In the manufacture of APIs to be rendered sterile in the formulation process and have no subsequent processes capable of removing endotoxins, Purified Water or Purified Water in Containers controlled endotoxins at a low level should be used.</td>
</tr>
<tr>
<td>Purified Water</td>
<td>Purified Water in Containers</td>
<td>APIs, APIs rendered sterile in the formulation process, and API intermediates</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>API Intermediates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For routine control, it is very useful to control quality of produced water based on the monitoring of electrical conductivity (conductivity) and total organic carbon (TOC). In addition, items to be monitored periodically, such as some specified impurities, viable counts, endotoxins, insoluble particulate matters, etc., should be determined according to the intended use of pharmaceutical water. The frequency of measurement should be determined considering with the variation in the quality of water to be monitored.

The following are points to consider in controlling the quality of produced water from microbiological and physicochemical (conductivity and TOC) viewpoints. It is necessary to monitor other items if necessary, and to confirm that they meet the specifications established individually.

4.2. Sampling

Monitoring should be conducted at an adequate frequency to ensure that the pharmaceutical water processing system is well-controlled and that water with acceptable quality is continuously produced and supplied. Specimens should be collected at the representative locations in the facilities for producing and supplying water, with particular care so that collected specimens reflect the operating condition of the pharmaceutical water processing system. An adequate protocol for the control of microorganisms at the sampling site should be established considering with the situation around the site.

Sampling frequency should be established based on the data from validation studies on the system. For microbiological monitoring, it is adequate to use the water specimens for the test within 2 hours after sampling. In the case that it is not possible to test within 2 hours, the specimens should be kept at 2 – 8°C and be used for the test within 12 hours.

4.3. Alert and Action Levels

In producing pharmaceutical water using a water processing system, microbiological and physicochemical monitoring is usually carried out to assure that water with required qual-
The main purpose of microbiological monitoring program for pharmaceutical water processing system is to foresee any microbiological quality deterioration of the produced water, and to prevent any adverse effects on the quality of pharmaceutical products. Consequently, detecting all of the microorganisms present in the water to be monitored may not be necessary. However it is required to adopt a monitoring technique able to detect a wide range of microorganisms, including slow growing microorganisms. The followings indicate incubation-based microbiological monitoring techniques for pharmaceutical water processing systems. To adopt a rapid microorganism detection technique, it is necessary to confirm in advance that the microbial counts obtained by such techniques are equivalent to those obtained by the incubation-based monitoring techniques.

4.4.1. Media and Incubation Conditions
There are many mesophilic bacteria of heterotrophic type that are adapted to poor nutrient water environments. Heterotrophic bacteria may form bio-films in many pharmaceutical water processing systems, and to cause quality deterioration of the produced water. Therefore, it is useful to monitor microbiological quality of water by using the R2A Agar Medium, which is excellent for growing bacteria of oligotrophic type. On the other hand, in routine microbiological monitoring, an approach by using the Standard Agar Medium, prescribed in the Quality Standards for Drinking Water provided under the Article 4 of the Japanese Water Supply Law, is widely employed. In this approach, the trend in microbiological change of water processing system is estimated from the number of viable microorganisms capable of proliferating at 30–35°C in the Standard Agar Medium in a comparatively short period of time.

Table 2 shows examples of measurement methods, minimum sample sizes, media, and incubation periods for estimating viable counts.

The media shown in Table 2 are as follows.

(i) Standard Agar Medium
Casein peptone 5.0 g
Yeast extract 2.5 g
Glucose 1.0 g
Agar 15.0 g
Water 1000 mL
Mix all the ingredients, and sterilize by heating in an autoclave at 121°C for 15–20 minutes. pH after sterilization: 6.9–7.1.

(ii) R2A Agar Medium
Peptone (casein and animal tissue) 0.5 g
Water constant mass (after drying) slightly soluble in ethanol (99.5) and in acetone. Yellow crystalline powder. Freely soluble in water, and incubating at 20 – 25°C 1 mL of the diluted fluid onto the R2A Agar Medium any necessary correction. For purification, use the strains listed below or other strains and incubating at 20 – 25°C for 48 hours, sufficient proliferation of the inoculated strain must be observed.

Staphylococcus aureus: ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276

Pseudomonas aeruginosa: ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275

Colon bacillus (Escherichia coli): ATCC 8739, NCIMB 8545, CIP 53.126 or NBRC 3972

4.4.3. Action Levels for Microorganisms for Pharmaceutical Water Processing System

The following action levels are considered appropriate and generally applicable to pharmaceutical water processing systems.

Action Levels for viable counts in various types of pharmaceutical water

Water: 100 CFU/mL (Acceptance criterion prescribed in the Quality Standards for Drinking Water provided under the Article 4 of the Water Supply Law)

Purified Water: 100 CFU/mL**

Water for Injection: 10 CFU/100 mL**

(*Viable counts obtained using the Standard Agar Medium. **Viable counts obtained using the R2A Agar Medium)

Although the action level for Purified Water shown above is set at the same level as that for Water, in near future, an adequate action level would be set for Purified Water in consideration of the current level of technologies. Therefore, for the time being, it is recommended for each facility to perform a higher level of microbiological control of water processing system based on the action level established individually.

When actual counts in validation studies or routine control exceed the above action levels, it is necessary to isolate and identify the microorganisms present in the water, and to sanitize or disinfect the affected system.

4.5. Physicochemical Monitoring

Physicochemical monitoring of a pharmaceutical water processing system is usually performed using conductivity and TOC as the indicators for water quality. By monitoring conductivity, total amounts of inorganic salts present in the water can be estimated, and by monitoring TOC, total amount of organic compounds present in the water can be estimated. Normally, the Conductivity Measurements (2.51) and the Test for Total Organic Carbon (2.59) specified in the General Tests, Processes and Apparatus of the JP should be applied to these physicochemical monitoring. However, since tests for monitoring are performed in the situations different from those for judging pass/fail to the acceptance criteria prescribed in the monographs, supplements necessary to cover the situations to which the JP general tests cannot be applied, are described below.

To adopt the monitoring using conductivity and TOC as the indicators for inorganic and organic impurities at individual facility, appropriate alert and action levels, and countermeasures against unexpected apparatus failures should be established for each indicator.

4.5.1. Monitoring of Conductivity as the Indicator for Inorganic Impurities

Measurement of conductivity for monitoring is usually
conducted continuously using an in-line apparatus with a flow-through type or pipe-insertion type cell. Alternatively, offline batch testing may be performed using a dip type cell with water specimens taken at point-of-use sites or other appropriate locations of the pharmaceutical water processing system. For the operation control of a pharmaceutical water processing system, guides for judging whether it is adequate to continue the operation of the system or not, based on the results from monitoring of conductivity, are shown below, both for the cases of monitoring at the standard temperature (20°C) by applying Conductivity Measurements §2.51 of the JP and monitoring at temperatures other than 20°C by applying <645> WATER CONDUCTIVITY of the United States Pharmacopeia (USP) with some modifications.

4.5.1. Monitoring of Conductivity by applying the Conductivity Measurements §2.51 of the JP

The Conductivity Measurements §2.51 of the JP principally requires to measure the conductivity at the standard temperature (20°C). However, measurement at a temperature within a range of 15 – 30°C may also be acceptable, when the results are corrected using the equation prescribed in the Conductivity Measurements §2.51. In this case, the recommended allowable conductivity (action level) for Purified Water and Water for Injection is as follows.

- Action Level 1.0 μS·cm⁻¹ (20°C)

Since the above allowable conductivity is established for in-line monitoring, an alternative action level may be used for the monitoring based on offline batch testing.

4.5.1.2. Monitoring of Conductivity by applying the <645> WATER CONDUCTIVITY of the USP with some modifications

Usually, it is somewhat difficult to control the temperature exactly in in-line conductivity monitoring. Therefore, the following approach can be applied for the monitoring at temperatures other than the standard temperature (20°C) of the JP. This approach is based on the Stages 1 and 2 of the three-stage approach described in “<645> WATER CONDUCTIVITY” of the USP.

Stage 1 (In-line Measurement)

(i) Determine the temperature and the conductivity of the water specimens using a non-temperature-compensated conductivity reading.

(ii) From the Table 3, find the temperature value equal to or just lower than the measured temperature. Adopt the corresponding conductivity value on this table as the allowable conductivity at the measured temperature.

(iii) If the observed conductivity is not greater than the allowable conductivity adopted above, the water tested meets the requirement for monitoring conductivity. If the observed conductivity exceeds the allowable conductivity, proceed with Stage 2.

Stage 2 (Off-line Measurement)

(i) Measure the conductivity of the water specimen, by transferring it into a container and agitating it vigorously in order to attain equilibrium between the water specimen and the atmosphere on absorbing/desorbing carbon dioxide.

(ii) Transfer a sufficient amount of water to be tested into a suitable container, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing the conductivity periodically. When the change in conductivity, due to the uptake of atmospheric carbon dioxide, becomes not greater than 0.1 μS·cm⁻¹ per 5 minutes, adopt the observed value as the conductivity (25°C) of the water specimen.

(iii) If the conductivity of the water specimen at 25°C obtained above is not greater than 2.1 μS·cm⁻¹, the water tested meets the requirement for monitoring conductivity. If the observed value exceeds 2.1 μS·cm⁻¹, it should be judged that the water tested does not meet the requirement for monitoring conductivity.

4.5.2. Monitoring of TOC as the Indicator for Organic Impurities

The acceptance criterion of TOC is specified as “not greater than 0.50 mg/L (500 ppb)” in the monographs of Purified Water and Water for Injection. However it is recommended for each facility preparing pharmaceutical water to conduct operation control of pharmaceutical water processing system through TOC monitoring on produced water based on its own alert and action levels for TOC determined individually. The followings are the recommended action levels for TOC.

- Action Level: ≦ 300 ppb (in-line) ≦ 400 ppb (off-line)

The Quality Standards for Drinking Water provided under the Article 4 of the Japanese Water Supply Law require that TOC should be “not greater than 3 mg/L (3 ppm)”. Taking the above recommended action levels into consideration, it is also recommended for each facility to conduct quality control of source water through TOC monitoring based on its own alert and action levels for TOC determined individually.

The JP specifies the Test for Total Organic Carbon §2.59, and normally, TOC measurement should be conducted using an apparatus which meets the requirements described in the JP method. However, if a TOC apparatus conforms to the apparatus suitability test requirements described in “<643> TOTAL ORGANIC CARBON” of the USP, or those described in the “Methods of Analysis 2.2.44. TOTAL ORGANIC CARBON” of the JP, it is considered as meeting the requirements for TOC monitoring.
ORGANIC CARBON IN WATER FOR PHARMACEUTICAL USE of the European Pharmacopoeia (EP), the apparatus can be used for the monitoring of pharmaceutical water processing system, if sufficiently pure water not contaminated with ionic organic substances, or organic substances having nitrogen, sulfur, phosphorus or halogen atoms in their chemical structures, is used as the source water supplied to the system.

A TOC apparatus, characterized by calculating the amount of organic carbon from the difference in conductivity before and after the decomposition of organic substances without separating carbon dioxide from the sample solution, may be influenced negatively or positively, when applied to the water specimens containing ionic organic substances, or organic substances having nitrogen, sulfur, phosphorus or halogen atoms in their chemical structures. Therefore, the apparatus used for TOC monitoring should be selected appropriately in consideration of the purity of the water to be monitored and the contamination risk in the case of apparatus failure.

4.6. Storage of Water for Injection

In storing Water for Injection temporarily, adequate measures able to prevent microbial proliferation stringently, such as circulating it in a loop at a high temperature must be taken, and an appropriate storage time should also be established based on the validation studies, in consideration of the risks of contamination and quality deterioration.

5. Points to Consider for Assuring the Quality of Pharmaceutical Water in Containers

There are some specific points to consider for assuring the quality of pharmaceutical water in containers (Purified Water in Containers, Sterile Purified Water in Containers and Sterile Water for Injection in Containers), which are available as commercially products.

5.1. Methods for Preparing Sterile Pharmaceutical Water in Containers and Their Sterilization Validation

The following 2 different preparation methods are described in the monographs of Sterile Purified Water in Containers and Sterile Water for Injection in Containers.

(i) Introduce Purified Water (or Water for Injection) into a hermetic container, seal up the container, then sterilize the product.

(ii) Make Purified Water (or Water for Injection) sterile by using a suitable method, introduce the sterilized water into a sterile hermetic container by applying aseptic manipulation, then seal up the container.

For assuring the sterility of pharmaceutical water products, only the validation of final sterilization process is required in the case of preparation method (i), whereas validations of all the processes are indispensable in the case of preparation method (ii), since the latter is based on the idea to assure the sterility of pharmaceutical water products by “aseptically” introducing Purified Water (or Water for Injection) treated in advance with filtration sterilization, etc. into a sterile hermetic container, and sealing it up.

5.2. Deterioration of Water Quality during the Storage in Containers

5.2.1. Conductivity (as the indicator for inorganic impurities)

The conductivity of pharmaceutical water in containers may increase to some higher levels due to the absorption of carbon dioxide from the atmosphere at the time of its preparation and that passed through plastic layer of the containers during storage, and also due to ionic substances released from the containers, even if the conductivity of Purified Water or Water for Injection used for its production is maintained at the level not more than 1.0 $\mu$S cm$^{-1}$. Particularly in the cases of pharmaceutical water products packed in small scale glass containers, it is necessary to pay attention to the change of conductivity during storage.

5.2.2. Potassium Permanganate-reducing Substances or Total Organic Carbon (TOC) (as the indicator for organic impurities)

JP specifies the classical test of potassium permanganate-reducing substances in the monographs of Purified Water in Containers, Sterile Purified Water in Containers and Sterile Water for Injection in Containers for controlling organic impurities in pharmaceutical water in containers. It forms a remarkable contrast to the specifications of Purified Water and Water for Injection, in which JP requires to control organic impurities in pharmaceutical water in bulk based on the test of TOC (acceptance criterion: not more than 0.5 mg/L (500 ppb)). This is because that it is considered difficult to establish the specification of pharmaceutical water in containers for organic impurities based on the test of TOC from the facts that there were many cases of remarkable increases in TOC values after storage of water in containers. Particularly in the cases of pharmaceutical water products packed in small scale plastic containers, it is necessary to pay attention to the increase of materials released from containers during storage.

The test of potassium permanganate-reducing substances is retained in the specifications of pharmaceutical water in containers, not as the most suitable method for the test of organic impurities present in the water in containers, but as a counter measure for performing the test of the water in containers with the same test method despite of the material (glass, polyethylene or polypropylene, etc) and the size (0.5 – 2000 mL) of the containers, and the duration of storage. Therefore, it is recommended to adopt the test of TOC as the alternative for the test of potassium permanganate-reducing substances, and to perform quality control of pharmaceutical water in containers based on TOC measurements under the responsibility of each manufacturer, if possible.

In such cases, it is recommended to adopt the following values as the levels preferable to attain.

For products containing not more than 10 mL of water:

- TOC not greater than 1500 ppb
- For products containing more than 10 mL of water:
  - TOC not greater than 1000 ppb

As for the pharmaceutical water packed in the plastic containers made of polyethylene, polypropylene, etc., in addition to the concern for the release of materials such as monomer, oligomers, plasticizers, etc. from plastics, it is necessary to pay attention to the storage environment of the products to avoid the contaminations with low molecular volatile organics such as ethanol, or low molecular air pollutants such as nitrogen oxides, since these plastics have the properties of permeating various gases.

5.2.3. Microbial Limit (Total Aerobic Viable Counts)

For Purified Water in Containers, it is not required to assure the sterility, but it is necessary to produce it by using sanitary or aseptic processes in order to meet the acceptance criterion of “10$^2$ CFU/mL” for total aerobic viable counts.
throughout the period of their storage. It is also necessary to take special care against microbial contamination during its circulation. In addition, it is recommended to use them as soon as possible after opening their seals.

The acceptance criterion of "10^2 CFU/mL" for total aerobic viable counts of Purified Water in Containers is at the same level as the action level for viable counts in the production of Purified Water (in bulk). However, different from the case of microbiological monitoring of Purified Water, Soybean-Casein Digest Agar Medium is used for the test of total aerobic viable counts of Purified Water in Containers to detect microorganisms contaminated from the surroundings during its storage and circulation.

5.3. Points to consider in the case that commercially available products of pharmaceutical water in containers are used for the manufacture of pharmaceutical products

It is allowable to use commercially available products of pharmaceutical water in containers (Purified Water in Containers, Sterile Purified Water in Containers or Sterile Water for Injection in Containers) for the manufacture of pharmaceutical products and products for clinical trial, and for the tests of pharmaceutical products. In such cases, it is necessary to consider the following points.

(i) When such products are used for manufacturing pharmaceutical products, it is recommended to use them soon after confirming their compliances to the requirements of JP monograph from the test results at the time of its receipt or those offered from the supplier of the products.

(ii) In the case that such products are used for manufacturing pharmaceutical products, it is necessary to validate the process in which the water was used as a part of process validation of pharmaceutical products. In the case that they are used for manufacturing products for clinical trial, it is necessary to confirm that the water doesn’t give any adverse effects on the quality of the products.

(iii) The products of sterile pharmaceutical water in containers should be used only once after opening their seals, and it must be avoided to use them again after storage.

(iv) It is recommended to prepare a standard operation practice (SOP) adequate for its intended use, considering that the contamination and quality deterioration of the water due to human and laboratory environmental origins might go on rapidly immediately after opening the product seal.

Water to be used in the Tests of Drugs

The water to be used in the tests of drugs is defined as “the water suitable for performing the relevant test” in the paragraph 20 under General Notices of the JP. Therefore, it is necessary to confirm that the water to be used in a test of a drug is suitable for the purpose of the relevant test before its use.

Unless otherwise specified in the individual test method, Purified Water, Purified Water in Containers or the water produced by an appropriate process, such as ion exchange or ultrafiltration, may be used for these purposes. Water produced for these purposes at other individual facilities may also be used.

The water for tests specified in General Tests in the JP is as follows:

- Water for ammonium limit test: <1.02 Ammonium Limit Test (Standard Ammonium Solution)
- Water for bacterial endotoxins test: <4.01 Bacterial Endotoxins Test
- Water for particulate matter test (for injections): <6.07 Insoluble Particulate Matter Test for Injections
- Water for particulate matter test (for ophthalmic solutions): <6.00 Insoluble Particulate Matter Test for Ophthalmic Solutions
- Water for particulate matter test (for plastic containers): <7.02 Test Methods for Plastic Containers

The water for tests specified in General Information in the JP is as follows:

- Water for aluminum test: Test for Trace Amounts of Aluminum in Trans Parenteral Nutrition (TPN) Solutions
- Water for ICP analysis: Inductively Coupled Plasma Emission Spectral Analysis

The term “water” described in the text concerning tests of drugs means “the water to be used in the tests of drugs” as defined in the paragraph 20 under General Notices.

G9 Others

International Harmonization Implemented in the Japanese Pharmacopoeia Sixteenth Edition

Items for which harmonization has been agreed among the European Pharmacopoeia, the United States Pharmacopoeia and the Japanese Pharmacopoeia are implemented in the Japanese Pharmacopoeia Sixteenth Edition (JP 16). They are shown in the tables below.

The column headed Harmonized items shows the harmonized items written in the Pharmacopoeial Harmonization Agreement Document, and the column headed JP 16 shows the items as they appear in JP 16. In the Remarks column, notes on any differences between JP 16 and the agreement are shown as occasion demands.

The date on which the agreement has been signed is shown on the top of each table. In the case where the harmonized items have been revised and/or corrected, this is indicated in parenthesis.
### Harmonized items

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### Oct. 2007

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Sample preparation
Outgassing
Adsorbate
Quantity of sample

Method 1: The dynamic flow method
Method 2: The volumetric method

Reference materials

Figure 1 Schematic diagram of the dynamic flow method apparatus
Figure 2 Schematic diagram of the volumetric method apparatus

May 2007

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June 2004 (Method 1)/May 2007 (Rev. 1) (Method 2)

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Sieving methods

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2) Air entrainment methods
air jet and sonic sifter sieving

Interpretation

Table 1 Size of standard sieve series in range of interest

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(3) Assay  
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Photometric techniques

(1) Turbidimetric techniques  
(2) Chromogenic technique  
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Reagents, test solutions

Amoebocyte lysate  
Lysate TS  
Water for bacterial endotoxins test (BET)

Table 1  
Table 2  
Table 3  
Table 4

Reagents, Test Solutions

Prescribed in §9.41


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2 General procedures  
3 Enumeration methods  
4 Growth promotion test, suitability of the counting method and negative controls  
3.1. Preparation of test strains  
3.2. Negative control  
3.3. Growth promotion of the media  
3.4. Suitability of the counting method in the presence of product |
II. Microbiological Examination of Non-Sterile Products: Tests for Specified Micro-organisms

1. Introduction

2. General procedures

3. Growth promoting and inhibitory properties of the media, suitability of the test and negative controls

   3-1 Preparation of test strains
   3-2 Negative control
   3-3 Growth promotion and inhibitory properties of the media
   3-4 Suitability of the test method

4. Testing of products

   4-1 Bile-tolerant gram-negative bacteria
   4-2 *Escherichia coli*
   4-3 *Salmonella*
   4-4 *Pseudomonas aeruginosa*
   4-5 *Staphylococcus aureus*
   4-6 *Clostridia*
   4-7 *Candida albicans*

5. Recommended solutions and culture media

   Table II-1 Growth promoting, inhibitory and indicative properties of media

   Table II-2 Interpretation of results

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</table>
Media for the test may be prepared as described below, or equivalent commercial media may be used provided that they comply with the growth promotion test.

Fluid thioglycollate medium
Soya-bean casein digest medium

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined

Sterility
Growth promotion test of aerobes, anaerobes and fungi
Method suitability test
Membrane filtration
Direct inoculation

Test for sterility of the product to be examined

The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined

Membrane filtration
Aqueous solutions
Soluble solids
Oils and oily solutions
Ointments and creams

Direct inoculation of the culture medium
Oily liquids
Ointments and creams
Catgut and other surgical sutures for veterinary use

Observation and interpretation of results
Application of the test to parenteral preparations, ophthalmic and other non-injectable preparations required to comply with the test for sterility

Minimum number of items to be tested

| Table 1. Strains of the test microorganisms suitable for use in the growth promotion test and the method suitability test |
| Table 2. Minimum quantity to be used for each medium |
| Table 3. Minimum number of items to be tested |

(i) Fluid thioglycollate medium
(ii) Soya-bean casein digest medium

3. Suitability of the culture medium

3.1. Sterility
3.2. Growth promotion test of aerobes, anaerobes and fungi
4. Method suitability test
(i) Membrane filtration
(ii) Direct inoculation

5. Test for sterility of the product to be examined

5.1. Membrane filtration
(i) Aqueous solutions
(ii) Soluble solids
(iii) Oils and oily solutions
(iv) Ointments and creams

5.2. Direct inoculation of the culture medium
(i) Oily liquids
(ii) Ointments and creams

6. Observation and interpretation of results
7. Application of the test to parenteral preparations, ophthalmic and other non-injectable preparations required to comply with the test for sterility

8. Minimum number of items to be tested

Table 1. Strains of the test microorganisms suitable for use in the growth promotion test and the method suitability test
Table 2. Minimum quantity to be used for each medium
Table 3. Minimum number of items to be tested

Non-harmonized item: The large volume preparation is specified as not less than 100 mL labeled.
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<th>Remarks</th>
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<tr>
<td><strong>Uniformity of Dosage Units</strong>&lt;br&gt;(Introduction)</td>
<td><strong>6.02 Uniformity of Dosage Units</strong>&lt;br&gt;(Introduction)</td>
<td>JP’s particular description: Additional explanation on Liquids. Additional explanation for the part not containing drug substance.</td>
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<td>Liquid dosage forms</td>
<td>(ii) Liquid dosage forms</td>
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<td>1.1. Calculation of acceptance value</td>
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<td>Mass variation</td>
<td>2. Mass variation</td>
<td>JP’s particular description: Assuming that the concentration of drug substance is uniform in each lot.</td>
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<td>Uncoated or film-coated tablets</td>
<td>(i) Uncoated or film-coated tablets</td>
<td>The phrase “in conditions of normal use. If necessary, compute the equivalent volume after determining the density.” is deleted.</td>
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<td>(iv) Solid dosage forms other than tablets and capsules</td>
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<td>(v) Liquid dosage forms</td>
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<td>2.1. Calculation of acceptance value</td>
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<td>Criteria</td>
<td>3. Criteria</td>
<td>JP’s particular description: Addition of “(divided forms, lyophilized forms)” and “(true solution)”. The phrases “at time of manufacture” and “For purposes of this Pharmacopoeia” are deleted.</td>
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<td>Table 1 Application of content uniformity (CU) and mass variation (MV) test for dosage forms</td>
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June 2004 (Rev.1)

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<td><strong>Test for Extractable Volume of Parenteral Preparations</strong>&lt;br&gt;(Introduction)</td>
<td><strong>6.05 Test for Extractable Volume of Parenteral Preparations</strong>&lt;br&gt;(Introduction)</td>
<td>JP’s particular description: Explanation on this Test</td>
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June 2004 (Rev.1)

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<td><strong>Particulate Matter in Injectables</strong>&lt;br&gt;(Introduction)</td>
<td><strong>6.07 Insoluble Particulate Matter Test for Injections</strong>&lt;br&gt;(Introduction)</td>
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Method 1.
Light obscuration particle count test

1. Method 1. Light obscuration particle count test

1.1. Apparatus
1.1.1. Calibration
1.1.1.2. Electronic method
1.1.1.3. Automated method
1.1.2. Sample volume accuracy
1.1.3. Sample flow rate
1.1.4. Sensor
1.1.4.1. Sensor resolution (Particle size resolution of apparatus)
1.1.4.2. Particle counting accuracy
1.1.4.3. Threshold accuracy

1.1.1.1. Manual method
1.1.1.2. Electronic method
1.1.3. Sample flow rate

Method 2.
Microscopic particle count test


2.1. Apparatus
2.2. General precautions
2.3. Method
2.4. Evaluation

General precautions
1.2. General precautions
1.3. Method
1.4. Evaluation

Method 3.
Evaluation

1. Circular diameter graticule
Fig. 1 Circular diameter graticule

Oct. 2007 (Rev.1)

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<td>(iii) Auxiliary tube</td>
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<td>2.1. Immediate-release preparations</td>
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<td>JP’s particular description: Setting a test for Granules, Dry Syrups and Pills. Allowing use of water as the test fluid. Setting each intervals of the immersion. Making a definition of complete disintegration.</td>
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### Dissolution

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<td>6.10 Dissolution Test</td>
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<tr>
<td>Apparatus 1 (Basket apparatus)</td>
<td>1. Apparatus</td>
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<td>(iii) Time</td>
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**Interpretation**

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**Acceptance Tables**

- Acceptance Table 1
- Acceptance Table 2
- Acceptance Table 3
- Acceptance Table 4

**Figures**

- Fig. 1 Apparatus 1, Basket stirring element
- Fig. 2 Apparatus 2, Paddle stirring element
- Fig. 2a Alternative sinker
### JP XVI

#### General Information / Others

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Nov. 2008

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July 2003 (Rev. 1)

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### Harmonized items JP 16 Remarks

#### Methylcellulose
- **Definition**
- **Labeling**
- **Identification (1)**
- **Identification (2)**
- **Identification (3)**
- **Identification (4)**
- **Identification (5)**
- **Viscosity**
- **Method 1**
- **Method 2**
- **pH**
- **Heavy metals**
- **Loss on drying**
- **Residue on ignition**
- **Assay**

#### Anhydrous Dibasic Calcium Phosphate
- **Definition**
- **Identification (1)**
- **Identification (2)**
- **Acid-insoluble substances**
- **Chloride**
- **Sulfate**
- **Carbonate**
- **Barium**
- **Loss on ignition**
- **Assay**

#### Dibasic Calcium Phosphate
- **Definition**
- **Identification (1)**
- **Identification (2)**
- **Acid-insoluble substances**
- **Chloride**
- **Sulfate**
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**Powder Flow**

(Introduction)

Angel of repose

Basic methods for angel of repose

Variations in angel of repose

Angel of repose general scale of flowability

Experimental considerations for angle of repose

Recommended procedure for angle of repose

Compressibility index and Hausner ratio

Basic methods for compressibility index and Hausner ratio

Experimental considerations for the compressibility index and Hausner ratio

Recommended procedure for compressibility index and Hausner ratio

Flow through an orifice

Basic methods for flow through an orifice

Variations in methods for flow through an orifice

General scale of flowability for flow through an orifice

Experimental considerations for flow through an orifice

Recommended procedure for flow through an orifice

Shear cell methods

Basic methods for shear cell

Recommendations for shear cell

Table 1 Flow properties and corresponding angle of repose

Table 2 Scale of flowability

(Introduction)

1. Angel of repose

1.1 Basic methods for angel of repose

1.2 Variations in angel of repose

1.3 Angel of repose general scale of flowability

1.4 Experimental considerations for angle of repose

1.5 Recommended procedure for angle of repose

2. Compressibility index and Hausner ratio

2.1 Basic methods for compressibility index and Hausner ratio

2.2 Experimental considerations for the compressibility index and Hausner ratio

2.3 Recommended procedure for compressibility index and Hausner ratio

3. Flow through an orifice

3.1 Basic methods for flow through an orifice

3.2 Variations in methods for flow through an orifice

3.3 General scale of flowability for flow through an orifice

3.4 Experimental considerations for flow through an orifice

3.5 Recommended procedure for flow through an orifice

4. Shear cell methods

4.1 Basic methods for shear cell

4.2 Recommendations for shear cell

Table 1 Flow properties and corresponding angle of repose

Table 2 Scale of flowability
### Amino Acid Analysis

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Methodologies of amino acid analysis

**Method 1-Postcolumn ninhydrin detection general principle**

**Method 2-Postcolumn OPA fluorometric detection general principle**

**Method 3-Precolumn PITC derivatization general principle**

**Method 4-Precolumn AQC derivatization general principle**

**Method 5-Precolumn OPA derivatization general principle**

**Method 6-Precolumn DABS-Cl derivatization general principle**

**Method 7-Precolumn FMOC-Cl derivatization general principle**

**Method 8-Precolumn NBD-F derivatization general principle**

**Data calculation and analysis**

**Calculations**

- Amino acid mole percent
- Unknown protein samples
- Known protein samples

---

**Harmonized items**

**General Information**

**Capillary Electrophoresis**

- **Apparatus**
- **Capillary zone electrophoresis**
- **Optimisation**
- **Instrumental parameters**
  - **Voltage**
  - **Polarity**
  - **Temperature**
  - **Capillary**
- **Electrolytic solution parameters**
  - **Buffer type and concentration**
  - **Buffer pH**
  - **Organic solvents**
  - **Additives for chiral separations**
- **Capillary gel electrophoresis**
- **Characteristics of gels**
- **Capillary isoelectric focusing**
- **Loading step**
  - loading in one step

**JP XVI**

**Remarks**

1. **Capillary Zone Electrophoresis**
   - **Optimization**
   - **Instrumental parameters**
     - (1) Voltage
     - (2) Polarity
     - (3) Temperature
     - (4) Capillary
   - **Electrolytic solution parameters**
     - (1) Buffer type and concentration
     - (2) Buffer pH
     - (3) Organic solvents
     - (4) Additives for chiral separations
2. **Capillary Gel Electrophoresis**
   - **Characteristics of Gels**
3. **Capillary Isoelectric Focusing**
   - (1) Loading step
   - (i) loading in one step
### Micellar Electrokinetic Chromatography (MEKC)

Optimisation

**Instrumental parameters**
- Voltage
- Temperature
- Capillary

**Electrolytic solution parameters**
- Surfactant type and concentration
- Buffer pH
- Organic solvents
- Additives for chiral separations
- Other additives

**Quantification**
- Calculations

**System Suitability**
- Apparent number of theoretical plates
- Resolution
- Symmetry factor
- Signal-to-noise ratio

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<td>4.5. Isocratic selection</td>
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<td>4.6. Other parameters</td>
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<td>Analysis and identification of peptides</td>
<td>4.7. Validation</td>
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<td>Table 1. Examples of cleavage agents.</td>
<td>5. Analysis and identification of peptides</td>
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<tr>
<td>Table 2. Techniques used for the separation of peptides.</td>
<td></td>
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</tr>
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</table>
### Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

**General Information**

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

**Remarks**

- Characteristics of polyacrylamide gels
- Denaturing polyacrylamide gel electrophoresis
- Reducing conditions
- Non-reducing conditions
- Characteristics of discontinuous buffer system gel electrophoresis
- Preparing vertical discontinuous buffer SDS polyacrylamide gels
- Assembling of the gel moulding cassette
- Preparation of the gel
- Mounting the gel in the electrophoresis apparatus and electrophoretic separation
- Detection of protein in gels
- Coomassie staining
- Silver staining
- Drying of stained SDS polyacrylamide gels
- Molecular-mass determination
- Validation of the test
- Quantification of impurities
- Reagents, test solutions
  - Blocking solution
  - Coomassie staining solution
  - Destaining solution
  - Developer solution
  - Fixing solution
  - Silver nitrate reagent
  - Trichloroacetic acid reagent
- Table 1 – Preparation of resolving gel
- Table 2 – Preparation of stacking gel

---

### Total Protein Assay

**General Information**

Total Protein Assay

**Remarks**

- Method 1
- Standard solution

---

- 1. Characteristics of Polyacrylamide Gels
- 2. Polyacrylamide Gel Electrophoresis under Denaturing Conditions
- 2.1. Reducing conditions
- 2.2. Non-reducing conditions
- 3. Characteristics of Discontinuous Buffer System Gel Electrophoresis
- 4. Preparing Vertical Discontinuous Buffer SDS-Polyacrylamide Gels
- 4.1. Assembling of the gel moulding cassette
- 4.2. Preparation of the gel
- 4.3. Mounting the gel in the electrophoresis apparatus and electrophoretic separation
- 5. Detection of Proteins in Gels
- 5.1. Coomassie staining
- 5.2. Silver staining
- 6. Drying of Stained SDS-Polyacrylamide Gels
- 7. Molecular-Mass Determination
- 8. Suitability of the Test (Validation)
- 9. Quantification of Impurities
- 10. Test Solutions
  - Blocking TS
  - Coomassie staining TS
  - Destaining TS
  - Developer TS
  - Fixing TS
  - Silver nitrate TS for silver staining
  - Trichloroacetic acid TS for fixing
  - Table 1. Preparation of resolving gel
  - Table 2. Preparation of stacking gel

- Method 1 (UV method)
- Standard Solution
<table>
<thead>
<tr>
<th>Test solution</th>
<th>Procedure</th>
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<td>Light-scattering Calculations</td>
<td>Light-Scattering Calculations</td>
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**Method 2**
- Standard solutions
- Test solution
- Blank
- Reagents and solutions
  - Copper sulfate reagent
  - SDS Solution
  - Sodium hydroxide solution
  - Alkaline copper reagent
  - Diluted Folin-Ciocalteu's phenol reagent
- Procedure
- Calculations
- Interfering substances
  - Sodium deoxycholate reagent
  - Trichloroacetic acid reagent
- Procedure

**Method 3**
- Standard solutions
- Test solution
- Blank
- Coomassie reagent
- Procedure
- Calculations

**Method 4**
- Standard solutions
- Test solution
- Blank
- Reagents
  - BCA Reagent
  - Copper sulfate reagent
  - Copper-BCA reagent
- Procedure
- Calculations

**Method 5**
- Standard solutions
- Test solution
- Blank
- Biuret reagent
- Procedure
- Calculations
- Interfering substances

Explanatory footnote “Example: the Minimum Requirements for Biological Products and individual monograph of JP” is added.
### Method 6 (Fluorometric method)

**Standard solutions**

- Test solution
- Blank

**Reagents**

- Borate buffer
- Stock OPA reagent
- OPA reagent

**Procedure**

- Procedure

**Calculations**

- Calculations

### Method 7 (Nitrogen method)

**Procedure A**

- Procedure A

**Procedure B**

- Procedure B

**Calculations**

- Calculations

---

**Nov. 2005**

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<td>3. Sampling plan and frequency of testing</td>
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<td>4. Microbial control program</td>
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**Feb. 2004**

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<td>General Information Tablet Friability Test</td>
<td>JP’s particular description</td>
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In 1961 it was decided that the atomic weights of the elements would be based on values relative to the mass number of 12 (no fractions) for carbon (\(^{12}\text{C}\)). Ever since, there has been a marked improvement in the quality and quantity of data on the nuclide masses and isotope ratios of the elements using physical methods such as mass spectrometry. The Commission on Isotope Abundances and Atomic Weights (CIAAW) of the International Union of Pure and Applied Chemistry (IUPAC) collected and examined newly measured data and publishes a new atomic weight table every two years (in the odd years). Based on this table, in April of each year the Atomic Weight Subcommittee of the Chemical Society of Japan also publishes an atomic weight table. The numbers of the following Atomic Weight Table (2010) is based on the numbers published by the IUPAC in 2007\(^1\). For a more detailed explanation, the user is referred to a report\(^2\) and a review\(^3\) published by the CIAAW.

The atomic weight values of each of the elements shown in the atomic weight tables are, as stated in the preface to the table, for elements that originate on Earth and are present in substances that exist naturally. Atomic weights are, with the exception of single nuclide elements (elements consisting of one stable nuclide), not natural constants like the speed of light, but rather change depending on a variety of factors, such as the method of treatment or the origin of the substance containing that element. This is because the atomic weight is dependent on the relative frequency (isotope ratio) of the stable nuclides comprising each of the respective elements. Due to advancements in measurement techniques, the isotopic frequencies of each of the elements are not necessarily constant, and fluctuate due to a variety of processes that occur on the Earth. We have come to learn that this is reflected in the atomic weights. The result of this is that differences have arisen in the accuracy of the atomic weights between elements. The figures in parentheses that follow the atomic weight values in the atomic weight tables represent the uncertainty with respect to the last digit in the atomic weight. For example, in the case of hydrogen, 1.00794(7) means 1.00794 ± 0.00007.

The atomic weight of a single nuclide element is the most accurate and the precision is also high. This is because it is not necessary to consider the isotope ratio since single nuclide elements do not possess a multiple number of stable isotopes. The atomic weights of such elements are determined based on the mass\(^5\) of each nuclide determined by physical techniques, taking into consideration the uncertainty with constant criteria.

Among the elements, the majority of samples gathered on Earth exhibit a constant isotope composition, however, some specific samples have isotope compositions that are different to these. These kinds of elements are indicated by a “g”, which means the value in the atomic weight table cannot be used as is, depending on the sample, as the atomic weight of these elements. In relation to this, oxygen for example exists in a number of forms on Earth, such as in air, salt water, fresh water, and in rocks, and because the isotope compositions fluctuate among these substances, oxygen is not an element for which only one value can be used. Thus, an “r” is attached to an element for which a precise atomic weight cannot be given, no matter how much progress is made in techniques for measuring the isotope composition. On the other hand, it is also possible, depending on the element, to use an isotope that has undergone artificial fractionation as a reagent. Typical elements that are representative include hydrogen, lithium, boron, and uranium. This type of element is identified by an “m”, and particularly in cases where the atomic weight is a problem, it is necessary to be careful by referring to the label of the reagent.

Standard Atomic Weights 2010

(Scaled to $A_r(\text{C}) = 12$, where $^{12}\text{C}$ is a neutral atom in its nuclear and electronic ground state)

The atomic weights of many elements are not invariant but depend on the origin and treatment of the material. The standard values of $A_r(E)$ and the uncertainties (in parentheses, following the last significant figure to which they are attributed) apply to elements of natural terrestrial origin. The footnotes to this table elaborate the types of variation which may occur for individual elements and which may be larger than the listed uncertainties of values of $A_r(E)$. Names of elements with atomic number 112 to 118 are provisional.

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<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
<th>Atomic Number</th>
<th>Atomic Weight</th>
<th>Footnotes</th>
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<tr>
<td>Hydrogen</td>
<td>H</td>
<td>1</td>
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<td>4.002602(2)</td>
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<td>10.811(7)</td>
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<td>Cobalt</td>
<td>Co</td>
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<td>Zinc</td>
<td>Zn</td>
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<td>Br</td>
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<td>Strontium</td>
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<td>Rhodium</td>
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</table>

Footnotes:
- $g$: ground state
- $m$: metastable
- $r$: resonant
- $u$: unassigned
- $A_r$: atomic weight
- $E$: terrestrial origin
- $\pm$: uncertainty
- $^*$: provisional

Names of elements with atomic number 112 to 118 are provisional.
<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
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<th>Atomic Weight</th>
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<td>Uuo</td>
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* : Element has no stable isotopes.
† : Commercially available Li materials have atomic weights that range between 6.939 and 6.996; if a more accurate value is required, it must be determined for the specific material.
g : Geological specimens are known in which the element has an isotopic composition outside the limits for normal material. The difference between the atomic weight of the element in such specimens and that given in the table may exceed the stated uncertainty.
m : Modified isotopic compositions may be found in commercially available material because it has been subjected to an undisclosed or inadvertent isotopic fractionation. Substantial deviations in atomic weight of the element from that given in the table can occur.

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